GROWTH RESPONSES WITHIN THE GENUS *CYPERUS* EXPOSED TO ALUMINIUM AND IRON IN HYDROPONICS

By

OLUTOYOSI OLAIDE AYENI

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Supervisor: Prof L Kambizi

Co-supervisors: Prof C Laubscher and Prof O Fatoki

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ABSTRACT

Generally, aluminium (Al) is required as a micronutrient by plants. The metabolism of Al within the plant can exert a number of effects within the plant. These include: interfering with cell division in both root tips and lateral roots, increasing cell wall rigidity, maintaining the correct cellular redox state, as well as the various other physiological and growth responses. Al is one of the most abundant elements in the earth's crust and becomes toxic in many plants when the concentration is greater than 2-3 ppm, where the soil has a pH<5.5. Iron (Fe) is an equally important element, and the toxicity of this metal possesses constraints primarily on wetland plants growing in acidic soils that have high reducible iron content. The impact of metal toxicity (Al and Fe) requires an understanding of many aspects related to Al and Fe uptake, transport and distribution by plants in wetland ecosystems. In this study, three species of *Cyperus* viz. *Cyperus alternifolius*, *Cyperus prolifer* and *Cyperus textilis* were used to carry out phytotoxicity tests to monitor xenobiotic substances.

A study of comparative morphological and physiological involving the three macrophytes (*C. alternifolius*, *C. prolifer* and *C. textilis*) was carried out hydroponically in a greenhouse. For the morphological and biochemical investigation, shoots and roots were collected from plants and is subjected to four varying concentrations of Al and Fe. The aims were to: compare morphological response (wet/dry and relative leaf growth rate) variance among the three selected species after exposure to Al and Fe metal contamination, determine the concentrations of Al and Fe in the test plants and, determine the impact of the two metals on two physiological indices viz. photosynthesis and the chlorophyll content.

The concentrations of the metals were analysed by using the highly sensitive inductively coupled plasma-mass spectrophotometry (ICP-MS) method. The rate of photosynthesis was measured using the Infra-Red Gas Analyzer (IRGA). The chlorophyll content of leaves was determined by using a portable version of an imaging-PAM chlorophyll fluorometer (PAM-MINI) Waltz, Effeltrich (Germany) which was connected to a computer with data acquisition software.

Results showed that prolonged exposure of the test plants to both Al and Fe reduced the chlorophyll content significantly ($P \leq 0.05$) and this was accompanied by a decrease in the photosynthetic rate. The toxicity of both Al and Fe on the physiological parameters was influenced by the identity of plant species tested, the growth stage of the plant, the length of the exposure time to both Al and Fe as well as by the concentration of the metals. It was apparent from the results that all of the species investigated were suitable for use in phytoremediation. The most suitable was *C. alternifolius* as it showed the highest accumulation of the metals in its tissues.

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DEDICATION

For my daughters (Tolu, Orejesu and Tofunmi)

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CHAPTER ONE

GENERAL INTRODUCTION AND BACKGROUND

1.1 General introduction

Human activities in wetland areas such as marshes and swamps can be deleterious to the environment (Uwem *et al*., 2016:150). The term heavy metal (HM) refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Environmental pollutants, such as heavy metals (HM) pose significant risks to both ecosystem and human health (Nouri *et al.,* 2008:1337). It is apparent that wetland ecosystems are under increasing threat (Nouri *et al.,* 2008:43). The existence of functioning wetland ecosystems is critical for the survival of all living species, including humans. However, the degradation of wetland ecosystems is ubiquitous and increasing. It is recognised globally that the identification and protection of wetland ecosystems is urgent and essential. As many industrial processes are located near the water bodies into which industry effluents are often discharged, pollution of water bodies by a number of organic and inorganic materials is increasing (Gleick, 1998:23; Gensemer & Playle, 1999:315). One of the most hazardous classes of pollutant adversely affecting fragile wetland ecosystems is the heavy metals (Wuana *et al*., 2010:486).

Metals are natural elements and over millennia, many have been extracted from the earth by human activities such as mining. These metals are subsequently used for various industrial processes and products. Metal pollutants are often toxic and commonly present in industrial and household wastewaters which are frequently discharged into the environment. Such industries include electroplating, metal finishing operations, electronic-circuit production, steel and non-ferrous processes, mining and fine-chemical and pharmaceutical production. Metals comprise a major category of globally-distributed chemical pollutants. All metals are non-degradable and as they often adversely affect many forms of life (Ahalya *et al.,* 2005:71).

Appendix A, B and C, the terms used throughout this study are defined. While, Table 1.1 lists examples of common wetland plants. *Cyperus* forms an integral genus in these ecosystems. For this study, three *Cyperus* species were selected as examples of wetland plants and were investigated as natural suitable candidates for phytoremediation of selected metals in wetland systems. Two metals were investigated, viz. Al and Fe.

Table 1.1: Examples of wetland plants (McCutcheon & Schnoor, 2003:29)

Wetland plants	Common names
Cyperus prolifer	*Miniature papyrus*
Cyperus alternifolius	*Umbrella flatsedge*
Cyperus textilis	Mat sedge
Carex spp.	Sedges
Myriophylum aquaticum	Parrot feather
Phragmites spp.	Reeds
Celtis occidentalis	Backberry
Elaeagnus augustifolia	Russian olive
Taxodium distichum	Bald cypress
Typha spp.	Cattails
Andropogon sp.	Bluesterm grasses
Betula nigra	River birch
Scirpus spp.	Bulrushes
Spartina spp.	Cordgrasses
Bouteloua spp.	Grama grasses
Coronill avaria	Crownvetch
Festuca arundinacea	Tall fescue
Trifolium pretense	Red clover
Solida gorigida	Stiff goldenrod
Panicum virgatum	Switchgrass
Nelian thusannus	Common sunflower
Agropyron spp.	Wheat grasses
Trifolium repens	White clove
Festuc arubra	Red fescue
Lolium pp.	Rye grasses
Trifolium hybridum	Alsike clover
Lotus corniculatus	Birdsfoot trefoil
Salix spp.	Willows
Morus rubra	Mulberry
Populus spp.	Poplars
Populus deltoids	Cottonwoods
Phalaris arundinaceae	Reed canary grass
Pinus taeda	Loblolly pines

Accumulation of high concentrations of metals in an environment may result in irreversible damage to plants as the metals can accumulate within the tissues of the plant (Sheoran *et al.,* 2011:168; Klos *et al.,* 2012:1829). However, little information is available on the method of metal uptake by wetland plant species, including *Cyperus*. Uptake by root crops is of particular importance, as many plant species concentrate Al in the root system (Babourina & Rengel, 2009:189). Furthermore, contamination by Fe and Al may not only affect plants and micro-organisms in the environment (Weis *et al.,* 2004:685) but invariably also pose a threat to both animals and humans who can be exposed to these metals through the food web (Sarfraz *et al.,* 2007:130).

Table 1.2: Drivers of metal pollutants and environmental implications

Drivers	Pressures	State	Impacts
Urbanization	Waste generation	Degraded air quality	Coastal erosion
Energy consumption	Gas emissions into atmosphere	Degraded water quality	Decline in biodiversity
Transportation	Alteration of hydrological and sediment flux	Degraded potable water	Altered ecosystem functions
	Pressures on groundwater recharge and supply	Decrease in coastal vegetation	Human health impacts
Agriculture	Habitat loss	Land subsidence	Socioeconomic impacts
Water consumption	Pressures on fish stock	NA	NA
Tourism	NA	NA	NA

Apart from their causing pollution of marine and estuarine water bodies, heavy metals exert negative effects elsewhere (Table 1.2). For example, the hydrosphere is affected by the associated structural changes to estuaries and coastal strips. This can alter regimes of erosion and sedimentation in a manner that disrupts ecosystems by damaging existing structures. Ecosystems can also be destroyed by pollution from extractive industries. In wetlands, the increased nutrient levels associated with pollution can cause major plant community changes (Mitsch & Gosselink, 2007:582; Van der Welle *et al.,* 2007:222; Korejo *et al.,* 2010:1451). This has invariably resulted in alterations in the composition of wild animal species inhabiting these areas. Furthermore, increases in the level of metal pollution in the urban industrial areas lead to major health concerns for future generations (Sinha *et al.,* 2006:651). Therefore, to reduce general environmental pollution, research and development is focusing on sector-specific

methods and technologies to remove heavy metals from different types of waste streams. In view of the toxicological effects of heavy metals on environments and all life forms within these environments, it becomes imperative to treat and remove these toxic compounds in wastewater effluents.

This should be done by determining methods which successfully remove heavy metals from the aquatic ecosystems before they are discharged into freshwater bodies. Metals including Al, Fe, Zn, Cd, Mn and Co originating from pollution of the air, soil, or water have been shown to be deposited on the leaves and soils of wetland plants (Mitsch & Gosselink, 2007:582; Van der Welle *et al.,* 2007:222).

These toxic pollutants can be directly absorbed by the leaves and are then retained by the plants (Deng *et al.,* 2004:29). As one solution, the process of phytoremediation whereby it is possible to utilize the natural ability of plants or plant products to take up, accumulate, store, or degrade organic and inorganic substances offers an innovative and cost-effective option to address recalcitrant environmental contaminants and thereby restore or stabilize contaminated sites (Cunningham *et al.,* 1995:393; Salt *et al.,* 1998:643; Matthews *et al.,* 2004:46; Lesage *et al.,* 2007a:102; Vymazal *et al.,* 2007:162; Maine *et al.,* 2009:363; Scholz & Hedmark, 2010:323).

The roots of wetland plants (*Phragmites australis*, *Arabidopsis thaliana*, *Typha latifolia*, *Iris pseudacorus*, *Scripus lacustris*, *Cyperus alternifolius*, *Cyperus prolifer* and *Cyperus textilis*) tend to accumulate metals and, as a result, these roots release a variety of substances that may include oxygen, enzymes, allelopathic chemicals and antibacterial agents (Yang & Ye, 2009:282). Many of these can affect the rhizosphere directly by altering the pH and oxidative status of the environment. This in turn positively or negatively affects the growth and activity of bacteria, algae and higher organisms hence influencing the chemistry of the environment (Quan *et al.,* 2007:21).

Aluminium and Fe occur naturally in the soil and sediment environments. However, the concentration of these metals in both river water and sediments increases several thousand-fold when present in effluents from industrial and mining wastes (Sharma & Dubey, 2007:2027). Metals, including Fe, are essential to life and play essential roles such as in the functioning of critical enzyme systems. However, when in excess, these metals become toxic. The methods of nutrient cycling in wetlands differ between aquatic and terrestrial ecosystems. Anthropogenic changes have caused considerable changes in chemical cycling in most

wetlands. It is notable that approximately one-third of the water which evaporates from the ocean surface returns back to the land as rain and/or snow. This water fills rivers, lakes, swamps, marshes, and other wetlands. Wetlands have the ability to filter and maintain much of the freshwater that humans and other animals depend on for life (Darwal *et al.,* 2011:474). Hence, it is apparent that the wetlands constitute a critically important environment.

There is extensive published information relating to the danger imposed on the environment as a result of metal pollution (Adekunle *et al.,* 2007:307; Zhang *et al.,* 2007a:435 and 2007b:2269; Sundaramoorthy *et al.,* 2010:597). Metal toxicity severely impacts the plants themselves and therefore also the ecosystems of which the plants are an integral component. Plants growing in a metal polluted environment exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation all of which pose problems for human health (Van der Welle *et al.,* 2007:222; Korejo *et al.,* 2010:1451).

Toxic substances continually produced by industries can contaminate community water supplies. Phytotoxic effects of metals such as Al and Fe include their direct interaction with plant growth and development (Sharma & Dubey, 2007:2027; Sharma & Dietz, 2009:43). Increasing levels of metal contaminants due to anthropogenic processes are causing rapid accumulation of metals in soil and water. Thus, these metals are likely to increase in concentration in plants particularly where plants are located in industrial areas and near large emission sources. In this manner the metals enter the food chain (Munns & Tester, 2008:651; Miller *et al.,* 2010:453).

The role played by some wetland plants in influencing wastewater treatment processes in wetlands is well documented (Mazeij & Germ, 2009:642). Over time, the reclamation of metal-contaminated soils by phytoremediation requires an overall and permanent plant cover. In order to select the most suitable plant species for phytoremediation, the effects of metals on wetland plants need to be assessed. Accumulation of high levels of toxic metals may result in irreversible damage to the plant tissues, and consequently to heterotrophs in the human food-chain (Cui *et al.,* 2004:407; Butt *et al.,* 2005:338; Sinha *et al.,* 2006:651).

Plants experience oxidative stress when exposed to heavy metals and this leads to cellular damage and disturbance of cellular ionic homeostasis. Although plants often survive such conditions, it is unknown what mechanisms within the plant enable this survival. Elsewhere, studies have been done wherein the metal pollution load and bio-monitoring thereof in aquatic

plants were investigated (Baldantoni *et al.,* 2005:48; Sarfraz *et al.,* 2007:130; Yeh, 2008:96; Krems *et al.,* 2013:353).

Global climate change and pollution threaten ecosystems. Wetlands have three main sources of water; precipitation, groundwater and water which moves over surfaces. Some wetland macrophytes are able to rapidly remove pollutants from the environment (Baldantoni *et al.,* 2005:48). Wetlands used for bioremediation of the environment have shown that this is achieved through biological uptake mechanisms in the plants as well as surface adsorption of pollutants to the plants (Table 1.3). Wetland plants offer a means of restoring polluted ecosystems. In addition, these same plants provide biofuel, sequester carbon and improve air quality (Davies et al., 2009:961). Thus, there is an overall beneficiation of the environment.

Shahi *et al.* (2013:379) described the use of constructed wetlands to treat a variety of pollutants present in wastewater. These included organic materials, detergents, nitrogen and phosphorus compounds, heavy metals, suspended solids and trace elements (Cu, Zn and Al). They tested the efficiency of two plants viz. *Cyperus alternifolius* and *Phragmites australis* for phytoremediation of a wetland and found that *C. alternifolius* could be successfully used for treatment of municipal wastewater.

Advances have been made in research to identify and optimize the ability of plants to reduce risk and enhance the environment. The functional role of natural wetlands in water quality improvement has offered a compelling argument for wetland conservation and preservation. The removal of metals by wetland vegetation can be greatly enhanced by the selection of appropriate wetland plant species to be used to remove pollutants from their environment. Selection of a suitable plant species is based on factors such as the type of metal to be removed, geographical location, environmental conditions and the known metal accumulation capacities of the species. Genetic engineering is now recognized as one of the recent technologies which provide a growing number of methods to breed plants which show enhanced heavy metal accumulation or degrade persistent contaminants more effectively in constructed wetlands (Munns & Tester, 2008:651; Miller *et al.,* 2010:453).

The use of wetland plants for water treatment procedures has major implications for the monitoring of metals in aquatic environments (Rucandio *et al.,* 2011:51). However, the rate of metal removal by wetland plants varies widely, depending on plant growth rate, plant species and concentration of the heavy metals (Barley *et al.,* 2005:107). Such plants should have a

competitive edge over existing vegetation such as in their ability to adapt to harsh conditions and high biomass.

Achieving a better understanding of the complex interactions occurring in the cycling of elements by the plant will assist in the development of phytoremediation. In particular, insight into the active reaction zone located in the rhizosphere is critical (Fig.1.1). In the rhizosphere, important physicochemical and biological processes occur, induced by the interaction of plants, microorganisms, soil and pollutants. Such knowledge would enable basic scientific aspects to be combined optimally with the various technical possibilities available. This would make wetland technologies more widely and successfully implemented (Dixit, Wasiullah, Pandiyan, Singh, Sahu, Shukla, Singh, Rai, Sharma, Lade, & Paul, 2015: 2189). Hence, it would be advantageous to develop knowledge regarding the abilities of different wetland plant species to absorb and transport trace elements when exposed to variable environmental conditions (Baldantoni *et al.,* 2005:48; Sarfraz *et al.,* 2007:130; Yeh, 2008:96; Krems *et al.,* 2013:353).

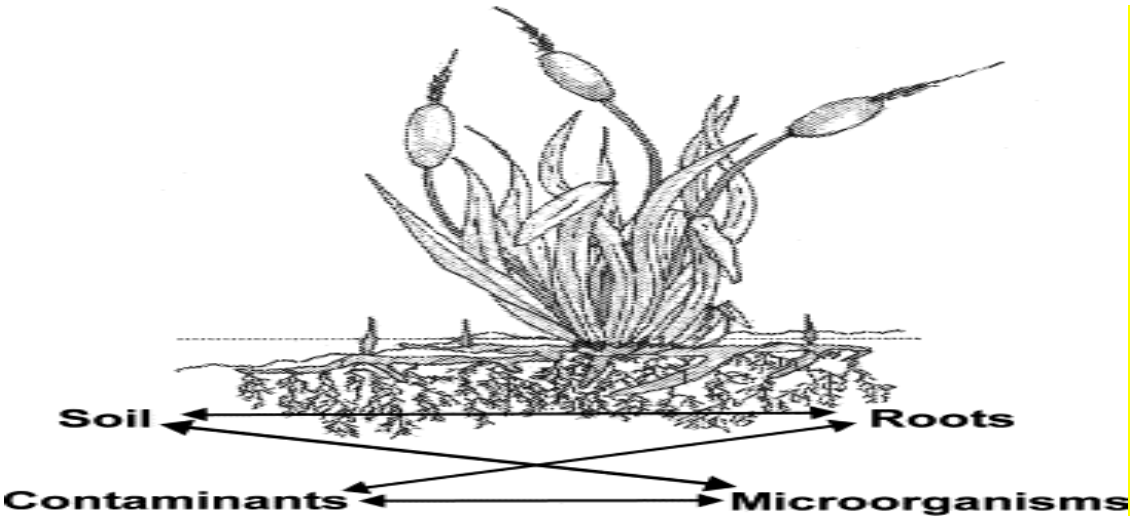

Figure 1.1: The active reaction zone of constructed wetlands is the root zone (or rhizosphere)

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Research assumes that accumulation of a particular metal in wetland plants is affected by immobilization and uptake from the soil, compartmentalization and sequestration within the root, the efficiency of xylem loading and transport, distribution between metal sinks in the aerial parts of the plant, and sequestration and storage in leaf cells (Yamamoto *et al.,* 2002:63). This

in turn can inhibit the photosynthetic activity of aquatic plants. Additionally, there are direct toxic effects on the plants in particular; change in water quality caused by pollution can adversely affect wetlands, which cause considerable loss in biodiversity and resource productivity (Chaudhry, 2010:1; Pilon-Smits & Freeman, 2006:203).

The tolerance of plants to a particular heavy metal is governed by a complex interrelated network of physiological and molecular mechanisms (Dixit, Wasiullah, Pandiyan, Singh, Sahu, Shukla, Singh, Rai, Sharma, Lade, & Paul, 2015:2189). Under stress conditions, the mechanism of defence collapses and production of activated oxygen exceeds the capacity of the plant to detoxify the oxygen. This causes adverse degenerative processes such as the loss of osmotic responsiveness, wilting and necrosis.

Treating wastewater in semi-natural plant systems is a technique which can in principle be applied in natural wetlands such as marshes, moors, wet fields, in artificial ponds and lagoons, and in constructed wetlands. Constructed wetlands offer many basic designs. When designing constructed wetlands to ensure that wastewater is to be treated as efficiently as possible, a detailed understanding of the effectiveness of various plant species and particular contaminants (wastewater components) that interact with the filter bed material is essential (Russi *et al.,* 2013:1).

Examples of some wetland plants employed for wastewater treatment are listed in Table 1.3. Research has shown that at varying concentration levels, dependent on the species, plants can extract or remediate toxins from soil through the root systems (Oancea *et al.,* 2005:561). An understanding of these mechanisms and their genetic basis is important to develop plants as agents of phytoremediation (Jadia & Fulekar, 2009:921). The reuse of wastewater is an important strategy for conserving water resources, particularly in areas affected by water shortage (Fig.1.2).

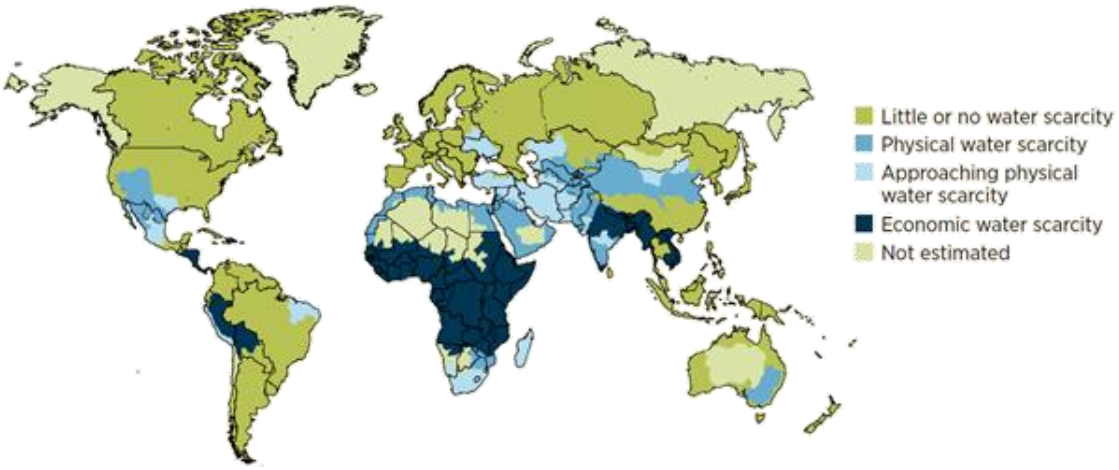

Figure 1.2: Water-scarce countries of the world (Source: 4th Edition of the United Nations Water Development Report 4 Volume 1, 2012).

Water resources are of great importance to both natural ecosystems and human developments. Turton (2000:1) reported that South Africa stands out as one of the most water-scarce countries (Fig. 1.2). However, with increasing environmental pollution from industrial wastewater particularly in developing countries such as South Africa, the tendency for heavy metal contamination existing in aqueous waste streams of many industries, including that from metal plating facilities, mining operations, tanneries should be of major concern (Sekomo *et al.,* 2011:321). Some of the metals associated with these activities are Cd, Cr, Fe, Ni, Pb and Hg. Water is essential for human life, food production, sanitation, energy, production of goods, and transport. The entire biosphere is dependent on the availability of water. Water ensures not only survival of humans, but also social well-being and economic growth. However although water is renewable it is not an inexhaustible resource. It is possible that in the future water could be depleted faster than it is renewed. Therefore, it is a natural resource which should be well conserved for posterity (Saunders, Jones, & Kansiime, 2007: 489).

Table 1.3: Examples of wetland plants employed for wastewater treatment

Plant Scientific names	***Plant Common names***	***Wastewater sources***	***Element removed***	***Process used***	***References***
Zizanopsis bonariensis	Juncus	septic tank	Removal of biological oxygen demand (BOD) and total suspended solids (TSS)	via sequential nitrification-denitrification, plant uptake	Yang & Chang, 1998
Scirpus validus	Bulrush	BOD and TSS from primary municipal wastewater	removal of nitrogen	via sequential nitrification-denitrification	Gersberg *et al.* 1986.
Phragmites communis	Common Reed	BOD and TSS from primary municipal wastewaters	removal of nitrogen	via sequential nitrification-denitrification	Gersberg *et al.* 1986.
Typha latifola	Cattail	BOD and TSS from primary municipal wastewaters	Removal of nitrogen	via sequential nitrification-denitrification	Gersberg *et al.* 1986.
Eichhornia crassipes		Purification from piggery, cattle-pen and poultry wastewater	Removal of nitrogen Removal of ammonia Removal of phosphorus	With harvesting via volatilization /nitrification-denitrification plant uptake	Vymazal, 1998 Roquette *et al.* 1998
Cyperus papyrus	Sedge	municipal wastewater	Removal of nitrogen,phosphorous	via sequential nitrification-denitrification	Brix, 1994
Phragmites australis	Common Reed	municipal wastewater abattoir wastewater	Removal of nitrogen phosphorous	via sequential nitrification-denitrification, plant uptake	Brix, 1994
Eichhornia crassipes	Water Hyacinth	municipal wastewater	Removal of nitrogen,phosphorous	via sequential nitrification-denitrification, plant uptake	Brix, 1994
Typha latifolia	Cattail	municipal wastewater	Removal of nitrogen phosphorous	via sequential nitrification-denitrification, plant uptake	Brix, 1994
Potamogeton pectinatus	Fennel Pondweed or Sago pondweed or Ribbon weed.	municipal wastewater	Removal of nitrogen Phosphorous	via sequential nitrification-denitrification, plant uptake	Brix, 1994
Ceratophylum demersum	Hornwort, or Contrail	municipal wastewater	Removal of nitrogen phosphorous	via sequential nitrification-denitrification, plant uptake	Brix, 1994
Pistoia stratiodes	Water Cabbage, Water Lettuce	municipal wastewater	Removal of nitrogen phosphorous	via sequential nitrification-denitrification, plant uptake	Brix, 1994

The goal of this study was to estimate the capacity of three selected wetland plant species (*C. alternifolius, C. prolifer* and *C. textilis*) to remove metals (Al and Fe) from the aquatic environment as generally indicated in Table 1.3. Many scientists stated that a majority of wetland plants retain higher levels of metals in their roots than in other plant tissues (Liphadzi & Kirkham, 2006:737; Sinha & Saxena, 2006:1340; Munns & Tester, 2008:65; Miller *et al.,* 2010:453). It would therefore be of interest to assess the levels of metals in wetland macrophytes bearing in mind the implications of metals to ecological processes. This can be done through laboratory analysis in combination with greenhouse studies of selected plants. If successful, any standardized procedures which are developed could be implemented to test other plant species for comparative purposes as phytoremediants.

Several studies have shown that certain plants can accumulate high concentrations of various metals. *Amorpha fruitcosa* (Indigo Bush) accumulates lead (Pb), *Azolla pinnata*- (Water Velvet) biosorbs metals, and *Bacopa monnieri* (Water Hyssop) accumulates various metals (McCutcheon & Schnoor, 2003:29).

Molecular understanding of plant metal accumulation has numerous biotechnological implications. However the long term effects of genetically engineered plants in the environment may yet have to be established. For this study *Cyperus* was selected as it is commonly found in wetlands, is fast growing, adapts well to various aquatic conditions, plays an important role in the extraction and accumulation of metals from waters (Kotze *et al.,* 2005:1; van der Welle *et al.,* 2007:222; Korejo *et al.,* 2010:1451; van Dam *et al.,* 2014:469). This study compared the bioaccumulation of the metals Al and Fe in three *Cyperus* spp. This was because of the known toxicities of these two elements to animals and humans and also because they are widespread in the environment (Haines & Lye, 1983:404; van Dam *et al.,* 2014:99).

Aluminium and Fe enter biological systems through different routes and are capable of inducing biological responses at different levels of biological organization. At the molecular level, these metals bind to the DNA, alter its structure, and induce the expression of certain genes (Crichton *et al*., 2002:9). This initiates or reduces synthesis of certain protein products thereby altering normal molecular functions. At the biochemical level, Al and Fe may directly induce or suppress enzyme activities; thus altering essential biochemical pathways leading to impairment of normal metabolism by competing with metabolites for active binding sites (Sood *et al.,* 2008:35). Research has indicated that certain toxic persistent and bio-available contaminants occur throughout the global environment and these originate from a variety of sources (Deng *et al.,* 2004:29).

The choice of wetland vegetation for the removal of trace elements could be greatly enhanced by using appropriate plant species (Adriano *et al*., 2004:121; Sarfraz *et al.,* 2007:130). For this to be achieved, it is important to understand the physiology of different plants (Yamamoto *et al.,* 2005:12). In wetland plants, molecular and biochemical alterations may progress to physiological changes (growth parameters) as a result of physiological disturbances that negate the growth and reproduction. This may directly or indirectly affect the species in their natural environment (Sarfraz *et al.,* 2007:130; Mitsch & Gosselink, 2007:582). Physiological responses such as changes in respiration rate in plants are readily detected through measuring changes in oxygen consumption rate. In certain wetland plants, photosynthesis may be affected by the metal contaminants Al and Fe (Oancea *et al.,* 2005:107; Ashraf & Harris, 2013:163). The inhibition of photosynthesis occurs by preventing electron transport between photosystem 1 and photosystem 11 (Oancea *et al.,* 2005:107). Certain toxic chemicals which bio-degrade slowly can accumulate in body tissues and are harmful to human health. Pesticides are an example as these can contaminate agricultural products, groundwater and surface waters, and in extreme cases can enter the human food chain and are ingested (Fonkou *et al*., 2005:457; Akinola & Ekiyoyo, 2006:597). The toxic effects of Al and Fe should be of primary importance when considering environmental protection and management strategies (Roquette *et al.,* 2009:289)**.**

The time taken for toxicity to manifest in a living organism depends on both the nature and dose of the contaminant (Archer, 1978:533; Arora *et al*., 2006:97). A toxic response can be observed within minutes but may also only appear years after initial exposure (Deng *et al.,* 2004:271). Individual physiological responses may be observed within hours or days; but at population levels, the response/s may only be noted many months or years after initial contamination. An example of a rapid toxic response in plants is Al-induced inhibition of root elongation which occurs only 30 minutes after exposure (Kidd *et al.,* 2001:1339).

From an ecotoxicological point of view, it would be useful to be able to predict or extrapolate the occurrence of the toxic responses initially observed at lower organizational levels (molecular, biochemical, physiological, and individuals) to the response at higher organizational levels (populations). To have advanced knowledge of higher organizational toxic responses in an ecosystem could prevent long term environmental degradation. The use of biological data (biological monitoring and assessment) to evaluate ecosystem health could be a powerful tool to measure and interpret the consequences of human activities on wetland ecosystems (Ho *et al.*, 2012:21).

Oxidation-reduction (redox) and associated pH changes that occur in wetland conditions can affect the retention and release of metals. Plants, particularly those in wetlands, experience oxidative stress upon exposure to heavy metals that leads to cellular damage and disturbance of cellular ionic homeostasis (Rascio & Navari-Izzo, 2011:169). When changes occur in the oxidation status of wetland environments, transformations of metals among different chemical forms may occur, thus affecting the mobility and biological availability of metals. It is well established that oxides of Al and Fe effectively adsorb most trace and toxic metals (Gomes-Junior *et al.,* 2006:420). It has also been reported that heavy metals, for example Al and Pb, can significantly affect the uptake and the translocation of some nutrients in plants (Sinha *et al.,* 2006:651). Thus, nutrient imbalance may be a symptom of heavy metal toxicity in plants (Sinha *et al.,* 2006: 651).

The potential effects of future climate changes on ecosystems particularly wetlands will occur through the interactions of several types of forcing. A noticeable potential effect of climate change would be the increased loss of biodiversity and natural habitats. Healthy, functioning ecosystems form global defences against climate change and storm damage, so it becomes imperative to ensure conservation of wetland ecosystems to maintain their full potential (Mitsch & Gosselink, 2007:582). A number of wetland plants are known to show vigorous growth in different metal-contaminated sites suggesting that plant species or (populations) within these systems have some degree of metal tolerance (Prasad, 2004:345; Keddy, 2010:1). However, the information related to metal tolerance in wetlands remains scarce. It is not even understood whether the tolerance of metals by these wetland species is a result of the plants being exposed to varying concentrations and/or times of the polluting metals.

1.2 The ecological and environmental usefulness of wetlands

Hategekimana and Twarabamenye (2007:12) reported on the impact of wetland degradation on water resources management in Rwanda. The wetlands ecosystem Rugezi Marsh has been shown to play a key role in water quality and quantity management, and conversely, the quantity and quality of water resources provided key services to ecosystem health (Uluocha & Okeke, 2004:151). Local women used wetland plants by picking the vegetation and using the material to make mats and other handcrafts.

The most harvested plant species were the *Miscanthus violaceus, Xrisvalida, Cyperus latifolius, Typha spec, Junctus oxycarpus* and *Papyrus* spp*.* (Nerimaa & Orikirizab, 2016:75).

Degradation of the wetland area, caused by extensive proliferation of both industrial and metallurgical activities in recent years resulted in a decrease of the water table, the drying of the swamp which affected transport in the canoeing channels, losses of both of animal biodiversity and livelihood by weavers, and a catastrophic decline in hydroelectric power production (Hategekimana & Twarabamenye, 2007:12).

Hails (1996:1) reported that wetlands are among the most productive life-support systems in the world and are of immense socio-economic and ecological importance to mankind. Wetland plants form an important component of wetlands, and the plants have several roles linked to wastewater treatment processes. The ability of wetlands to transform and store organic matter and nutrients has resulted in widespread use of wetlands for wastewater treatment worldwide (Brix & Schierup, 1989:100). Wetlands can be used for primary, secondary, and tertiary treatments of domestic wastewater, storm wastewater, combined sewer overflows (CSF), overland runoff, and industrial wastewater such as landfill leachate and that from petrochemical industries (Calheiros *et al.,* 2007:1).

Wetland ecosystems can be natural or man-made habitats that are saturated by water and are often viewed as wasted land. Wetlands, with water being the most striking feature, encompass a wide range of habitats where water inputs exceed rate of water loss, at least seasonally. They consist of flooded environments or water-soaked areas covering, in many cases, plants that subsist in waterlogged areas. The International Ramsar Convention defined wetlands as areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters (Ewart-Smith *et al.,* 2006:174).

Few understand what lies at the heart of the need for wetland conservation - real economic worth. Wetland systems have enormous monetary value and are responsible for direct contributions to national economies and human well-being. According to the journal, Nature - one of the most respected scientific journals in the world - reported recently that worldwide, wetlands are worth some US $4 trillion a year (Russi *et al.,* 2013:1). The value of wetlands is related to their primary task of processing water and regulating runoff. It has been estimated that the demand for water in South Africa is likely to meet the economically exploitable supply for the country as a whole by about the year 2030.

Without sufficient water, sufficient crops cannot be cultivated, the expansion of industry and mining is prevented and the tourism industry remains static. The global economy is therefore

dependent on a continual supply of water of sufficient quality and quantity. Wetlands protect and regulate the water resources. Wetlands may be considered as giant sponges, as they retain water during floods and release water during droughts. During times of floods, wetlands reduce water flow thereby decreasing flood damage and soil erosion (Sánchez-Chardi *et al.,* 2009:387; Siwela *et al.,* 2009:648).

Human activities impact considerably on metal levels in the environment. In particular waste water disposed by chemical and mining activities has a high content of waste metals. Contamination of soils and groundwater with toxic metals is now of global concern (Biswas & Tortajada, 2011:5). Economic development throughout the world has created stress on the pollution-carrying potential of the environment. It has been estimated that more than 60% of contaminated sites are polluted with hazardous metals, which leach into local groundwater, presenting serious health risks (Adal & Weimer, 2013:1). In a dry country such as South Africa, this is crucial.

Wetlands recharge ground water sources, and also remove pollutants from the water. Being natural filters, they enhance water purification by trapping many pollutants, including sediment, heavy metals and pathogens. Some wetlands, such as estuaries, serve as important breeding grounds for oceanic fish. Many wetlands (such as floodplains) can be used as grazing areas, if done on a sustainable basis. Besides performing these vital functions at very little financial cost, wetlands, in association with appropriate buffer strips, are also natural storehouses of biological diversity, providing life support for a wide variety of plant species, some totally reliant on wetlands for their survival. Many of these species are important economically as they are used for food, craft manufacture, medicines, building material and fuel for both subsistence and commercial gain (Kotze *et al.,* 2005:1; van der Welle *et al.,* 2007:222; Korejo *et al.,* 2010:1451; van Dam *et al.,* 2014:469).

Despite this, wetlands are among most threatened habitats in the world today. In some catchment areas in South Africa, studies revealed that over 50% of the wetlands have been destroyed. Responsible for this has been drainage of wetlands for crops and pastures, poorly managed burning and grazing that has resulted in head cut and donga erosion, planting of alien trees in wetlands, mining, pollution and urban development. All of these impact on water flow and quality which destroy or damage the wetland. The pollution of wetlands cannot continue. Neither should they be drained, starved of water or exploited unsustainably for food and short-term economic development, without paying a heavy price in the long-term. Continued wetland destruction will result in less pure water, less reliable water supplies,

increased severe flooding, lower agricultural productivity, and more endangered species (Kotze *et al.,* 2005:1; van der Welle *et al.,* 2007:222; Korejo *et al.,* 2010:1451; van Dam *et al.,* 2014:469).

One known result of climate change will be increased loss of biodiversity and natural habitats. Wetlands may be the key ecosystems for mitigating the effects of fossil fuel emissions on climate (Anderson & Mitsch, 2006:779). There have been numerous studies that indicate that plants have much potential for removing dispersed pollutants from aquatic environments (Kamal *et al.,* 2004:1029). A number of wetland plants have been found growing robustly in different metal-contaminated sites indicating that these species (or populations) have some degree of metal tolerance (Vymazal *et al.,* 2007:154; Jiang & Wang, 2008:697). However, it remains to be established whether the tolerance of metals by the wetland species used during the course of this study (*C. alternifolius, C. prolifer* and *C. textilis*) will enable these plants to be successfully utilised for phytoremediation of wetlands.

1.3 Effects of human activities on wetland ecosystems

The past decade has recorded the remarkable impact of humans on the environment. This is due to soaring increases in population, rapid rates of urbanization and the intensification of the use of fragile and marginal ecosystems (Dietz *et al*., 2007:16). Eutrophication, acidification, river regulation and diversion alter wetland plant communities (Quan *et al.,* 2007:21). Other factors which impact on these plants include industrial pollution, excessive sewage enrichment, and agricultural runoff (Hibbard *et al*., 2007:342). In all, increased nutrient levels have caused substantial plant community changes in some wetlands (Fig. 1.3) (Mitsch & Gosselink, 2007:582). While nutrients are necessary for optimum plant growth, excess nutrient load in the growth media exerts adverse effects on the environment including aquatic life. Furthermore the transfer of toxic elements into the human food chain is of global concern. Wetland ecosystems are under serious threat and understanding the scale of this destruction is essential.

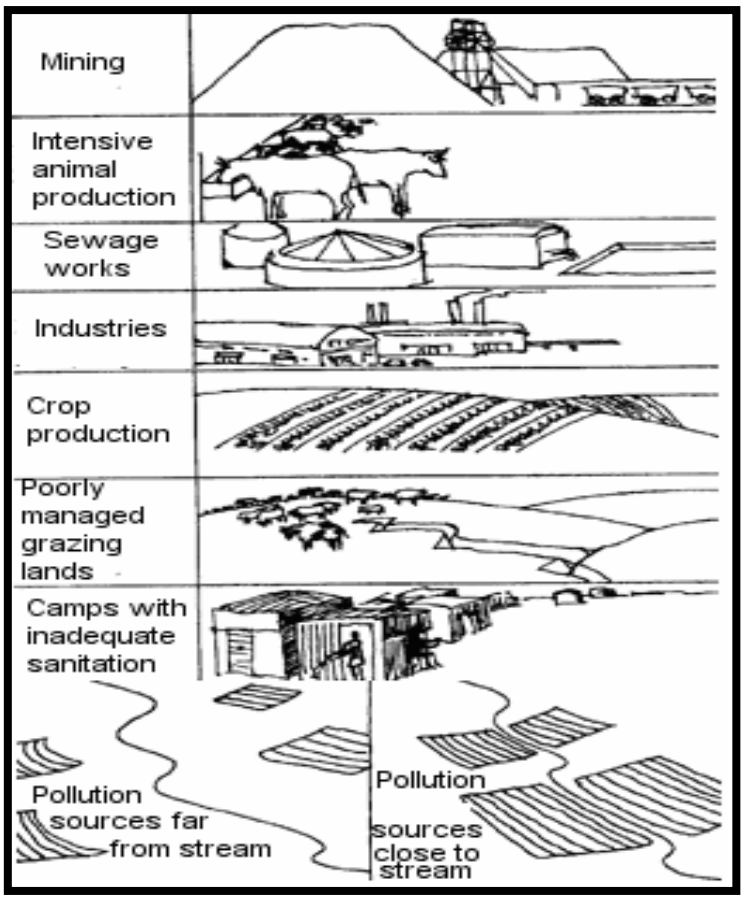

Figure 1.3: A schematic illustration of the most common off-site impacts on wetlands (Adapted from Kotze & Breen, 1994:1)

Among the most important factors influencing wetland ecosystems, are the numbers of *Homo sapiens* particularly in densely populated countries (Fig.1.4). Human activities have critically enhanced widespread biodiversity loss and ecological damage (Kotze & Breen, 1994:1). Some of this damage could be reversed by implementing targeted policies (Roquette *et al.,* 2009:289).

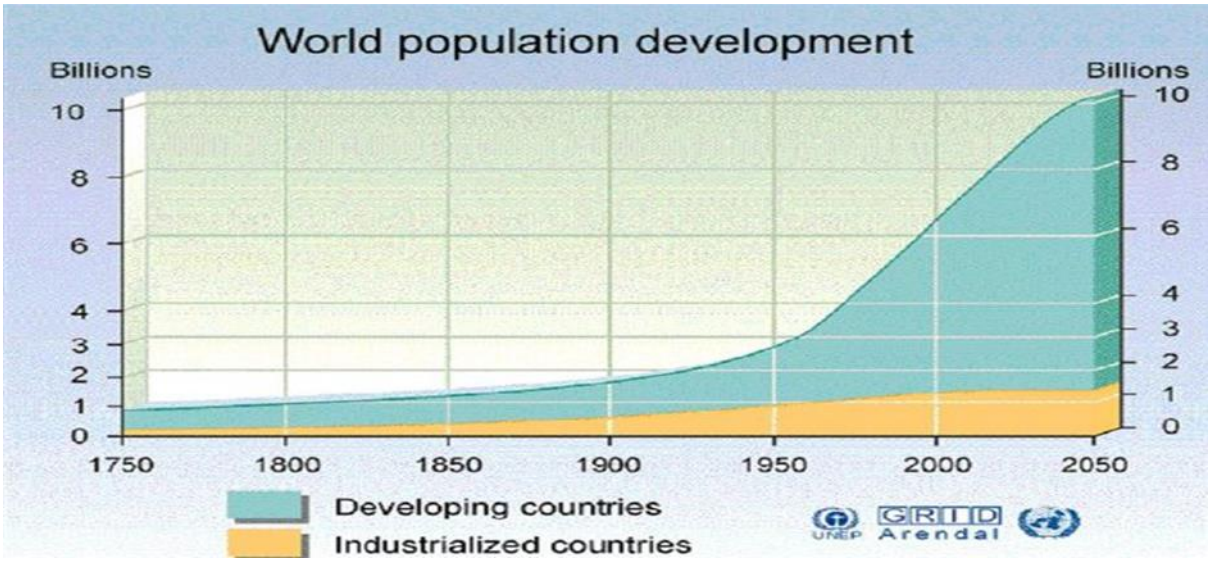

Figure 1.4: The world population progression (Adapted from Daly & Farley, 2004:1)

There is much information available which emphasises how shortages of fresh water supplies affecting more than 1 billion people could be eliminated through the use of phytoremediation (Ebrahimi *et al.,* 2013:5; Muradov *et al.,* 2014:7). Recent studies have shown that functioning wetlands may have many benefits for society (Keddy, 2010:1). Some of these benefits, particularly those termed “indirect” are not obvious and can be easily overlooked. Such as increased storm water and pollutants generated by land development within a wetland’s contributing drainage area (CDA) that stress the plant and animal community by increased lowland flooding, the dying out of species, and pollution of streams. The groundwater will also begin to disappear (Uluocha, 2004:151).

1.3.1 Functions of wetlands

Wetlands are habitats for many different species of organisms and the extensive biological productivity of these wetland environments is an important factor in the food chain for many plants and animals. Natural wetland provides a habitat for a number of biological species including animals, birds, crabs, fishes and shrimps. The wetlands protect these species from extinction by providing breeding, resting, and nesting areas as well as escape cover and travel corridors (Kamal *et al.,* 2004:1029; Wang *et al.,* 2008:55). Wetlands play important roles in filtering and cleansing water and in serving as reservoirs for floodwaters, thereby supporting fisheries, wildlife habitat and nursery areas (Memon & Schroeder, 2009:162).

Wetlands assist water purification by cleansing the water as it travels through the system which provides natural filtration (Dai *et al.,* 2012:169). Often water flow rate is reduced by the wetlands thus enhancing nutrient uptake by soil and plants.

Retarding the movement of flood waters can provide protection against the damaging effects of these waters, particularly in coastal areas. (Brown & Magoda, 2009:1). Wetland vegetation which includes reeds and sedges plays an active role in retarding the rate at which water flows through the system (Ayeni *et al.,* 2010:2045).

Wetlands also aid groundwater recharge by slowing the rate of movement of water and spreading it laterally thus allowing time for vertical infiltration into the substratum and ultimately into the groundwater. The accumulation of organic materials in soils contributes to their high content within the wetland soil. Many wetlands act as sediment traps, protecting marine resources such as coral reefs and sea grass beds from being smothered by silt brought down by rivers and streams (Brown & Magoda, 2009:1). Macrophytes are used in constructed wetlands to support algal growth by shielding the water column from light; absorbing nutrients and by assisting oxygen transfer into the water. Shorelines are protected from erosion by the roots of wetland plants such as *Cyperus* spp, and *Phragmites australis* which bind lakeshores and stream banks (Dai *et al.,* 2012:169).

Wetlands are important to humans because they provide a source of natural raw materials. Some of the latter include medicinal plants and these are used in the manufacture of western pharmaceutical drugs. Some plants in these systems may provide possible cures for life-threatening diseases. This is attributable to the fact that certain plant tissues are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity (Yamamoto *et al.,* 2002:63).

1.3.2 Challenges, barriers and constraints facing wetlands

Wetlands cover only approximately 1% of the earth's surface, yet they are responsible for a much greater magnitude of biogeochemical flux between the land surface, the atmosphere and the hydrological systems (Dork, 2013:1). Challenges facing wetlands are many and include land reclamation, agricultural practices, pollution, river regulation, drainage canals, human population increase, deforestation, overgrazing, water diversion for irrigation, increased salinity, war damage, flood control, diversion of water for domestic and industrial consumption, settlement, land conversion for rice fields, gem and sand mining, construction of roads, dams

and canals, burning and grazing, changes in water regimes, growth of invasive species, economic development and other physical alterations (Richardson *et al.,* 2005:1307).

Many studies have quantified the contribution of biodiversity to human livelihood in terms of income and revenue (Lokhande & Suprasama, 2012:1). The management of wetlands is associated with several common functions and value attributes classified as hydrology, biogeochemistry and habitat, which are linked to the self-maintenance of the wetlands and their surroundings (Mitsch & Gosselink, 2007:582). There is a need to elucidate the methodology required for the conservation of wetlands, their economic utility, and to promote scientific and application-orientated research into their productivity (Kotze *et al.,* 2005:1).

Barrier and constraint facing wetlands such as variations in environmental factors affect the distribution and abundance of wetland plants similar to the manner in which these factors influence other life forms. This is important as it provides useful information for further investigation into the physiological mechanisms of metal tolerance. Plant-metal interactions are complex and depend on many factors. Most important are the plant species affected, their developmental stage and the chemistry and concentration of the particular metal concerned. Plant-metal interactions may exert beneficial, harmful or neutral effects on a plant (Yan *et al.,* 2012:2016). A major wetland management procedure is conservation, which is perceived to be costly both in terms of management and opportunity costs involved. The promotion of conservation strategies such as those that highlight the degree to which ecosystem goods and services contribute to human well-being and economic output will emphasise the value of wetland ecosystems.

1.4 Metal accumulation in wetland soil/sediment

Metal accumulation is of interest, particularly with regard to protection of the environment. Pollution is not a recent phenomenon and living organisms have had to attempt to adapt to environmental contamination since life began. Sources of pollutants are commonly divided in two groups: point and nonpoint (Nouri *et al.,* 2008:1337; Kabata-Pendias, 2011:48). Point sources refer to discrete and localized contamination processes which emanate from human activities (sewage treatment plants, industrial discharges such as mining and smelters). Nonpoint sources of pollution are diverse, and cannot be pinpointed to the initial discharging source/s, as they are related to diffuse human activities that cover large areas. For instance, in the city situation, pollutants may enter rivers and coastal waters through run-off from storm

water. The storm water which enters the sea originates from diverse environments in the city and therefore contains contaminants from roads, (petroleum hydrocarbons), gardens and parks (fertilizers and pesticides), factories and industrial areas.

It should be noted that metals from both natural and polluted sources enter the plant system mainly through uptake by the roots. Inside the plant, metal toxicity influences both physiological and metabolic processes which include altering the activities of several key enzymes, synthesising metal-detoxifying compounds and inducing of oxidative stress (Mittler, 2002:405). Plant tissues have been reported to be capable of accumulating and magnifying pollutants such as heavy metals to concentrations considered highly toxic to life (Arora *et al.,* 2006:97; Cabrera *et al.,* 2006:40; Liu *et al.,* 2006:787).

1.4.1 Role of wetland plants in the removal of heavy metals in wetlands

Wetland plants are essential components in the functioning of the wetland environment. Plant growth may be affected by heavy metal pollution (Deng *et al.,* 2004:29) in ways which would make it difficult to predict environmental impacts of the heavy metals. The information available suggests that many wetland plants, regardless of their origin, are able to grow under conditions of high metal concentrations (Ayeni *et al.,* 2010b:2045; Yusuf *et al.,* 2010:1428). The physiological effect of metal pollution is closely related to their accumulation. Similarly, Cd was found to reduce water content in roots and shoots of terrestrial maize, rye and wheat (Stoltz & Greger, 2002:271).

Wetland plants have shallow root systems when compared to their non-wetland counterparts (Vymazal, 2007:947). Wetland plants adapt to soil saturation and flooding associated with wetland hydrology (Vymazal, 2010:530). As soil becomes saturated, the amount of oxygen available to plant tissues below the surface of the soil decreases rapidly as it is used by plants and microorganisms. The rate of movement of oxygen from air into water or saturated soil is much slower than in a well-aerated soil and creates an oxygen deficit. Plants rely on a range of transitional metals as essential micronutrients for normal growth and development (Agunbiade & Fawale, 2009:267). These elements are essential for most redox reactions which are fundamental to cellular functions. Higher plants produce reactive oxygen species (ROS) during different metabolic processes in cellular organelles; however, during metal stress, the rate of production is dramatically elevated (Jones *et al.,* 2006:1309).

Plant physiological processes, commencing from seed germination through to the production of fruit can be altered in many ways when levels of Al and Fe are elevated (Vansuyt *et al.*,

2007:441; Dhir *et al.,* 2011:1678). These include: inhibition of physiological processes (Clijsters& van Assche, 1985:31; Masarovičová *et al.,* 1999:189; Agunbiade & Fawale, 2009:267) as well as plant water relations and photosynthesis (Ali *et al.,* 2008:177).

Sinha and Saxena (2009:1340) revealed that protein, superoxide dismutase (SOD), ascorbic acid, proline, and Fe uptake are dominant in root tissues, whereas malondialhyde (MDA), guiacol peroxidase/GPX, cysteine and non-protein thiol (Np-SH) occur in the shoots of the stressed plant.

In conclusion, plant tolerance to a particular metal is governed by an inter-related network of physiological and molecular mechanisms and an understanding of these mechanisms and their genetic basis is important for developing plant as agents of phytoremediation (Seregin & Kozhevnikova, 2006:257).

1.5 Accumulation of aluminium in plant components

In acid soil, Al misuse due to indiscriminate use of acid forming nitrogenous fertilizers has resulted in toxic soil conditions. This is the most common cause of reduced plant growth and development. Al exerts a number of adverse effects on both physiology and biochemical processes in wetland plants (Becker, 2005:1; Dhir *et al.,* 2011:1678). Plants can withstand relatively high concentrations of chemicals without exhibiting toxic effects and are able to convert the absorbed chemicals to less toxic metabolites (Seregin & Kozhevnikova, 2006:257). It is important to use native plants for phytoremediation as these plants which mediate the clean-up *in situ*, are adapted to the soil properties, metal toxicity levels and the climate of the contaminated site (Chaney *et al.,* 2005: 190; Küpper & Kroneck, 2005:97; Chaney *et al.,* 2007:1429).

Plant species and cultivars of the same species differ considerably in their ability to take up and translocate Al to above-ground tissues of the plant (Ayeni *et al.,* 2010b:2045). Plants vary in their responses to metals, in mechanisms of uptake, avoidance of damage and in the type of damage caused. With regard to Al accumulation, there appear to be two groups of plants: Al excluders and Al accumulators. Plants known to contain high levels of Al internally include *Lycopodium* (Lycopodiaceae), ferns such as *Symplocos* (Symplocaceae), and *Orites* (Proteaceae) (Milner & Kochian, 2008).

1.5.1 Toxicity of Aluminium

The causes of Al toxicity are still misunderstood (Prasad, 2004:345). It is known however that various physiological processes that occur throughout the life of the plant commencing at seed germination and terminating with the production of fruit can be altered when levels of Al are high (Meda & Furlani, 2005:309). In Al-stressed plants, root elongation is reduced (Prasad, 2004:345). This is caused by an inhibition of root cell division and a decrease in cell expansion in the elongation zone (Prasad, 2004:345).

According to Meda and Furlani (2005:309) in *Limnobium stolonifrum*, an aquatic pondweed, the root hairs respond rapidly to Al toxicity. These authors detected a decrease in root hair growth within 30 minutes.

An understanding of the controlling mechanisms of cell elongation is key to elucidating the mechanism of plant growth and development and may provide important information on Al-induced root growth inhibition. It is generally understood that the inhibition of growth of root apices is the initial effect caused by Al toxicity. This is a result of Al^{3+} inhibiting root growth by binding to sensitive binding sites in the apoplast of the epidermis and the outer cortex (Meda & Furlani, (2005:309). The cellular components and processes which have been proposed to be affected by Al are wide ranging and some of the most important ones are presented in Table 1.4.

Table 1.4: Effects of Al on wetland plant growth and development

Wetland plants growth and development	Wetland Plant	Effects	References
Germination	*Spartina alternifolius*	Reduction	Becker, (2005:1)
Root growth	*Spartina alternifolius*	Decreased Decreased	Becker, (2005:1) Meda & Furlani, (2005:309)
	Limnobium stolonifrum	Inhibition	Barcelo & Poschenrieder, (2002:75)
	Thhlaspica erulescens	Inhibition	Magalhaes *et al.*(2007:1156)
	Phaseolus vulgaris	Inhibition	Milner & Kochian, (2008:1)
Shoot growth manifesting as cellular and ultrastructural modifications in leaves, reduced stomatal opening, decreased photosynthetic activity, chlorosis and foliar necrosis.	*Spartina alternifolius* *Thlaspi arvense*	Reduction Reduction	Becker, (2005:1); Ciamporova, (2002:161)
Leaf growth	*Spartina alternifolius*	Reduction	Becker, (2005:1); Matsumoto, (2000); Silva, (2012:8)
Yield and dry matter production	*Lemna gibba* *Triticum aestivum*	Reduction	Obek & Sasmaz, (2011:217); Boscolo *et al.* (2003:181); Yamamoto *et al.* (2002:63); Rengel & Zhang, (2003:295)

1.6 Accumulation of iron in plant components

Iron is one of the essential mineral elements for plant growth and plays an extremely important role in physiological processes such as plant photosynthesis, respiration, nitrogen fixation, protein and nucleic acid synthesis (Table 1.5). Iron is a constituent of cytochromes (electron transfer proteins) and metalloenzymes, and it is essential for many biochemical and physiological processes in plants. These include: photosynthesis, utilization of N and S, production of plant hormones, ethylene and biosynthesis of chlorophyll (Deng *et al*., 2009:353). Iron is generally incorporated into heme and non-heme proteins, such as the cytochromes. Heme proteins are involved in the formation of lignin, suberin, and catalase enzymes which degrade hydrogen peroxide in cells (Deng *et al*., 2009:353). Heavy metals are absorbed by the roots and the leaves (Agunbiade & Fawale, 2009:267; Ayeni *et al.,* 2010a: 2045). The pathway of transport is from root to shoot through the stem in the xylem and the exit is through

the transpiration stream However, Fe may be stored in certain tissues in plants, particularly in older leaves (Agunbiade & Fawale, 2009:267; Ayeni *et al.,* 2010a: 2045).

Alloway (2013:195) reported that Fe deficiency and toxicitiy adversely affect the plant leading to reduction in growth rate, overt symptoms of physiological stress and in extreme cases death. The most common symptoms of Fe deficiency in plants is interveinal chlorosis of young leaves.

Genotypic variations in efficiency of Fe uptake have been reported in plants (Rengel *et al.,* 1998:433). Differences in Fe uptake efficiency are probably due to genotypic variation in the following: volume and length of roots, root-induced changes in rhizosphere, increased absorption through vesicular mycorrhizae, release of root exudates to facilitate uptake, efficiency of utilization of the Fe once absorbed into plants, recycling of elements within the tissues of the growing plant, or tolerance of factors which inhibit uptake e.g. HCO_3^- and Zn in rice (Gao *et al.,* 2007:283).

Table 1.5: Effects of Fe on wetland plant growth and development

Process	Wetland Plant	Effects	References
Germination	*Oenanthe javanica, Leersia hexandra, Juncus effusus*	Decreased	Deng et *al.* (2009:353)
Root growth	*Cyperus malaccensis, Juncus effuses, Cyperus flabelliformis*	Inhibition of root elongation	Deng *et al.,* (2009:353)
Shoot growth	*Lactuca sativa* leaves	Decreased	Tyksinski & Komosa, (2008:3)
Leaf growth			Badr *et al.,*(2012:1292)
Yield and dry matter production	*Lactuca sativa* leaves	Yields were higher in other concentrations than in the control	Tyksinski & Komosa, (2008:3)

Rapid industrialization and urbanization have resulted in extensive environmental pollution and thus enrichment of metals in soils and the aquatic environments (Agunbiade & Fawale, 2009:267; Ayeni *et al.* 2010a: 2045). Global contamination of soil with metals such as Al and Fe has been a focus of the international concern in recent years, especially in areas where anthropogenic pressures are high (Hibbard *et al*., 2007:341). The vulnerability of the wetland environment to chemical damage depends on factors such as the physical and chemical properties of the chemical substances entering the wetland ecosystem and their transformation products, the duration of the exposure and properties of the ecosystem which enable the

system to resist changes caused by the presence of the chemicals (Bornette & Puijalon, 2011:1).

The implementation of environmentally friendly protocols that utilize biological sources such as aquatic plants for wastewater treatment have been accepted worldwide (Table 1.9). *Eichhornia crassipes, Elodea canadensis, Heteranthera dubia, Myriophyllum spicatum, Potamogeton pectinatus, Potamogeton richardsonii, Vallisneria americana, Vallisneria spiralis, Wolffia globosa, Lemnatris ulca, Hydrilla verticillata* and *Typha latifolia* have been the most extensively studied plants both in the laboratory and field (Table 1.9). Furthermore *Cyperus* spp. has been reported to tolerate high concentrations of heavy metals such as Al and Fe (Al-Hamdani & Sirna, 2008:71). *Phragmites australis* (Bragato *et al.* 2006:967); *Bolboschoenus maritimus* (Almeida *et al.,* 2006:424; Bragato *et al.,* 2006:967), and *Spartina alterniflora* (Weis & Weis, 2004:685; Weis *et al.,* 2004:409) have been reported to accumulate and store metals in their roots.

Table 1.6: Plants screened for the possibility of using in biomonitoring of water ecosystems and water sewage phytoremediation

Tested plants	**References**	
Bacopa monnieri (Water hyssop)	McCutcheon & Schnoor, 2003:29	
Potamogeton pectinatus (Fennel-Leaved Pondweed)	Peng *et al.* 2008:1	
Potamogeton malaianus (Bamboo-leaved Pondweed)	Peng *et al.* 2008:1	
Ceratophyllum demersum (Coontail)	Fawzy *et al.* 2011:980	
Azolla filiculoides (Water Fern)	Schor-Fumbarov *et al.* 2005:69	
Nelumbo nucifera (Sacred Water Lotus)	Kumar *et al.* 2008:193; Ramadan, 2003:1108	1.7
Phragmites australis (Common Reed)	Ramadan 2003:1108	
Lemna gibba (Duckweed)	Bennicelli *et al.* 2004:141	
Typha domingensis (Cattail).	Sasmaz *et al*. 2008:278	
Eicchornia crassipes(Water Hyacinth)	Krems *et al.* 2013:353	

Wetland protection, sustainability and amelioration

There is concern regarding environmental protection, conservation and policies for sustainable development. This is due to the extent of the pollution of the biosphere caused by an industrial revolution which has accelerated to such an extent that metal levels in surface waters pose health risks to humans and to the general environment (Fonkuo *et al.,* 2005:457). The earth is under threat from global warming, associated climate change and ecological degradation due to unchecked human consumption of natural resources. Extensive damage to the growth and development of plants, thereby causing a marked decline in the biota, is one consequence of the abundance of Al and Fe in the environment (Krems *et al.* 2013:353). Climate change will

decrease biodiversity and natural habitats. Wetlands impacted by anthropogenic activity will require remediation. Enhancement, restoration, and creation of wetlands offer means of remediating these negative trends. The use of phytoremediation to remove excessive levels of chemicals in the soil has gained importance in research and development (Pilon-Smits & Freeman, 2006:203). Over time, the reclamation of metal-contaminated soils through the use of phytoremediation requires an overall and permanent plant cover. The selection of the most suitable plants as candidates for phytoremediation makes it essential to assess the effect of polluting metals on wetland plants. Vigorously functioning ecosystems offer a global defence against climate change and storm damage. The preservation and remediation to ensure the conservation of wetland ecosystem is essential (Comin, 2010:175).

Although there is much information available relating to heavy-metal tolerance in plants, information concerning this tolerance in the wetland plant *Cyperus* is scarce (Memon & Schrooder, 2009:162; Maestri *et al.,* 2010:1). Generally, physiological acclimatization is a form of phenotypic plasticity by which an organism can adjust its metabolism as an acute response in order to cope with altered environmental conditions such as the challenges caused by excessive heavy metal presence. Tolerance to metals in some plants is based on multiple mechanisms such as the metal binding to the cell wall, active transport of ions into vacuoles, and the formation of complexes with organic acids or peptides (Liu *et al*., 2007:947).

The primary question is why are these studied plants tolerant of Al and Fe? In which features do they differ from their less tolerant relatives especially from within the same genus? According to the Industrial Toxicology Research Centre (ITRC) (2003:1), contaminant accumulation must be monitored to maintain ecological health of the environment, particularly in wetland ecosystems. Kabata-Pendias, (2011:1) also reiterated that an improved understanding of the biogeochemical processes that control trace element cycling and the availability of a comprehensive dataset on the abundance of trace elements in abiotic and biotic environmental compartments may be key to better management of trace elements. Such management is a prerequisite for sustainable land use and presumably to diminish health risks due to trace inorganic pollutants.

1.8 Statement of research problem

Water emanating from mine drainage, household wastewater discharges or industrial effluents contains elevated concentrations of metals such as Al and Fe (Englar, 2007:1). This water may

enter and pollute wetlands. Wetland macrophytes readily take up metals as reduced forms from sediments which may be anaerobic and oxidize these in the plant tissues. This immobilises and bio-concentrates the metals (Deng *et al.* 2004:29). Metals may also become available for epiphytic phytoplankton and herbivorous invertebrates. These represent potential major routes for the incorporation of polluting metals into the aquatic food chain. It would be of value to assess the levels of heavy metals in macrophytes due to their importance in many ecological processes.

Wetland plants are today used in artificial ecosystems for their proven abilities to decontaminate waters polluted by heavy metals (Ye *et al.,* 2004: 413; Davies *et al.,* 2009: 961). The methods by which these plants survive after receiving high concentrations of metals remain unclear. Therefore this study aims to elucidate some of the physiological mechanisms which have been altered in wetland plants subjected to stressful conditions induced by the presence of Fe and Al.

1.8.1 Research questions applied during this study

Scientists have conducted numerous investigations which revealed that plants are able to concentrate and detoxify pollutants (Ye *et al.,* 2004: 413; Davies *et al.,* 2009: 961). Careful selection of the appropriate plant family and genotype to match the particular pollutant and environment under study is crucial for successful phytoremediation (Audebert & Sahrawat, 2000:1877).

- Which of the studied plants *C. alternifolius, C. prolifer* or *C. textilis* accumulate more Al and Fe in their tissues?
- How does each wetland plantselected (*C. alternifolius, C. prolifer* and *C. textilis*) offer a positive physiological and biochemical response after exposure to Al & Fe exposure?
- Is there variation in (*C. alternifolius, C. prolifer* and *C. textilis*) ability to bioaccumulate Al and Fe?
- What physiological features are exhibited by the *Cyperus* spp to enable tolerance of Al and Fe at various concentrations?

1.8.2 Aims of the study

The overall aims were to:

- Investigate the effects of Al and Fe exposure on selected physiological mechanisms and adaptation to environmental stress, in order to evaluate factors that might explain differences in the growth responses in the three selected wetland plants viz. *C. alternifolius, C. prolifer*and and *C. textilis* grown under hydroponic conditions, and to
- Assess the effect/s of varying levels of Al and Fe on growth and chemical composition of *C. alternifolius, C. prolifer* and *C. textilis*.

1.8.3 Specific objectives

These were to:

- Compare Al accumulation and tolerance in *C. alternifolius*, *C. prolifer* and *C. textilis* grown under hydroponic conditions in Al or Fe-amended nutrient solutions in a greenhouse,
- Evaluate Fe uptake, accumulation and tolerance in *C. alternifolius, C. prolifer* and *C. textilis* grown hydroponically in Al- or Fe-amended nutrient solutions in a greenhouse,
- Correlate the effects of Al concentrations on growth responses of *C. alternifolius, C. prolifer* and *C. textilis* grown hydroponically in Al or Fe-amended nutrient solutions in a greenhouse,
- Establish a comparison of metal uptake among the *C. alternifolius, C. prolifer* and *C. textilis* grown hydroponically in nutrient (Al/Fe)-amended solutions in a greenhouse.

1.8.4. Research significance

A broader understanding of the manner in which wetland plants respond to and are affected by their exposure to metals may be used to develop environmental strategies to curb environmental degradation. A secondary benefit would be that a greater knowledge of these plant responses may be used to determine the pollution state of a site. An understanding of how the presence of Al and Fe can affect the growth, physiological and biochemical processes within the selected wetland plants (*C. alternifolius, C. prolifer* and *C. textilis*) could create novel solutions to the problems caused by heavy metal contamination in natural wetland habitats. Furthermore, this research could contribute to the development and maintenance of viable optimally functioning wetland environments which could reduce the deleterious effects of global climate change. The research presents an opportunity to promote the need for the conservation of wetlands, to maintain their economic utility and to stimulate scientific and application-oriented research into the productivity of wetlands.

This study is important as much ecotoxicological research has focused on a single species, which is inadequate when predicting the effects and hazards of a chemical on natural ecosystems.

It is well known that natural ecosystems are more complex than artificial laboratory environments. By using three different species of *Cyperus* the biodiversity of the study was enhanced. Conservation of biodiversity is imperative not only for its own sake and for future generations, but also for the preservation of intact natural habitats such as wetlands and the associated benefits of these environments.

1.8.5 Justification of the study

The Sedges are a focus of this study due to their widespread presence in South African Rivers and their potential for use in phytoremediation. They display relative structural simplicity, presented by their common occurrence in monospecific stands and the existing evidence of their important influence on morphological change and ecological functioning (Brown & Magoda, 2009:1; Saltonstall, 2008:1).

Govindasamy *et al.* (2011:145) indicated that the concentration of heavy metals is increasing in the environment. Much research work on the effect of pollution on plants has been carried out on cultivated plants particularly agricultural crop plants. Over the past 30 years there has been a growing interest in the use of metal-accumulating roots and rhizomes of aquatic or semi-aquatic vascular plants for the removal of heavy metals from contaminated aqueous streams (Pilon-Smits & Freeman, 2006). Some examples include the Water Hyacinth (*Eichornia crassipes*) (Kay *et al.,* 1984:117), Pennywort (*Hydrocotyl eumbellata* L.) (Dierberg *et al.,* 1987:1), Duckweed (*Lemna minor*) and Water Velvet (*Azolla pinnata*) (Jain *et al.,* 1989:115). The ability of wetlands to transform and store organic matter and nutrients has resulted in the widespread use of wetland for wastewater treatment worldwide. Thus wetland plants which are an important component of wetlands have possible roles as agents of phytoremediation in wastewater treatment processes. Successful phytoremediation will be dependent on a variety of factors such as the type of metals to be removed, the geographical location of the area, environmental conditions and the known metal accumulation capacities of the plant species. It is therefore important to select appropriate wetland plants to remove the metal of interest (Calheiros *et al.,* 2007:1790). Thus knowledge of metal uptake by candidate phytoremediation plants including *Cyperus,* as undertaken during the current study, is essential prior to use of these plants for water purification.

1.9 Limitations of the study

During the past, numerous studies have made it apparent that heavy metals, often required in trace amounts by plants for normal growth, when in excess can exert adverse effects on plants (Gomes-Junior *et al.,* 2006:420). Linking research to environmental policy decisions creates a challenge. An ecotoxicological study of plant responses is usually short-term, extending to perhaps a month or a season. In addition since an individual plant within an ecosystem makes a small contribution to that environment, it is difficult to extrapolate results obtained from a single plant studies such that they apply to an entire complex ecosystem and global climate change. Although any notable responses from the study of single plants exposed to high levels of Al and Fe in an artificial environment may enhance knowledge and understanding of phytoremediation of an environment, the contribution and complex interactions of entire plant communities in a given ecosystem cannot be underestimated.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of wetland ecosystems

This chapter presents an overview of general published information relevant to the Interrelation of Biodiversity Dynamics, Ecosystem Processes and Abiotic Factors, Conservation and Restoration of Wetlands: a Strategic Approach for the Management of Aquatic Ecosystems, Al and Fe, Key Elements in Wetland Sustenance and Risk Assessment of Wetland Exposed to Toxic Levels of Al and Fe.

Phytotechnology is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants in soil and water. In phytoremediation, vegetation plays

an important role in decontamination. Macrophytes (floating, emergent and submerged) readily take up reduced forms of metals from the sediments which are anaerobic. Subsequent oxidation of the metals in plant tissues makes them immobile and as a result bio-concentration of the toxic metal occurs in plant tissues (Kamal *et al.,* 2004:1029). In this manner the toxic trace metal bioavailability is reduced (Adriano *et al.,* 2004:121). Aquatic macrophytes are of paramount importance in ecosystems as they provide carbon substrates for microbes (Adekunle *et al.,* 2007:307; Tack & Vandecasteele, 2008:283).The latter are important in processing wastewater contaminants. The accumulation potential of both Al and Fe in *Cyperus* has not as yet been extensively researched and is therefore unknown.

Rooted and emergent macrophytes are particularly satisfactory and effective as bio-indicators of metal pollution, as these plants represent the real levels present at the contaminated sites (Kamal *et al.* 2004:1029). The chemical composition of the habitats of the macrophytes, the associated environmental conditions and monitoring of trends of metal concentrations could reflect changes in species composition over time. Metabolism in plants requires micronutrients including Al and Fe (Vansuyt *et al*., 2007:441). Aluminium is toxic to many plants at concentrations in excess of 2-3 ppm where soil pH measures < 5.5 (Magalhaes *et al.,* 2007:1156). The metabolism of Al interferes with cell division in root tips and lateral roots, increases cell wall rigidity or maintains an acceptable cellular redox state (Darko *et al.,* 2004:583). Various other biochemical, physiological and growth responses are also influenced by Al. Excess concentration of reducible Fe in acidic soils creates constraints for wetland plants (Comin, 2010:175).

The impact of metal toxicity (Al and Fe) requires an understanding of aspects related to Al and Fe uptake, and their transport and distribution in wetland ecosystems. This chapter provides an overview of the environmental risk associated with remobilization of metal contaminants and recycling into the food chain, particularly by infiltration into ground water. It has been reported that groundwater contamination poses ever increasing health and environmental risks throughout the world (Adekunle *et al.,* 2007:307; Tack & Vandecasteele, 2008:283).

The importance of wetlands to optimum functioning of ecological systems surrounding wetlands is being increasingly recognized. This is due to the fact that biological resources (which include organisms or parts thereof of genetic material, population or any other biotic components of ecosystems) are of actual or potential use or value to humans. (Kotze *et al.,* 2005:174; Ewart-Smith *et al.,* 2006:174). Hence biotic functions and ecosystems have become exceedingly important, not only for understanding the global environment but also because of

the commercial significance of biodiversity particularly in wetland ecosystems (Persley & Macintyre, 2002:25; Baldantoni *et al.,* 2005:48; van Dam *et al.,* 2014:469). Many studies have estimated the contribution of biodiversity to human livelihood in terms of income and revenue. There is a need to elucidate methodologies required for the conservation of wetlands, their economic utility, as well as for promoting scientific and application-oriented research into productivity of these environments.

2.2 Wetland contamination and pollution

The management of wetlands is associated with several common functions and value attributes which are classified as hydrology, biogeochemistry and habitat, all of which are linked to the self-maintenance of the wetlands and their surroundings (Mitsch & Gosselink, 2000:1). A major wetland management procedure is conservation, which is perceived to be costly both in terms of management and opportunity costs involved. The promotion of conservation actions such as those that highlight the degree to which ecosystem goods and services contribute to human well-being and economic output, projects the value of ecosystems.

Wetlands contamination often occurs as a result of various anthropogenic activities and rapid developments of technology. However if unlimited the generation of waste could become excessive and disposed of with little regard to impacts on the environment into which it is discharged. Wetlands are subject to pollution and degradation because they receive extensive volumes of water and wastewater from inland sources, including domestic and industrial waste waters, storm water off the land and other dispersed sources. These waters could contain nutrients, dissolved and suspended metals and organics (pesticides, phthalates), which are capable of causing disruption to wetland functionality and/or severe damage to wetland diversity.

Wetland plants are essential for the permanent functioning of wetland environments. Studies suggest that wetland plants, regardless of their origin, are able to grow in high metal concentrations (Ayeni *et al.,* 2010b:2045). Yang and Ye (2009:282) reported the vigorous growth of wetland plants in different metal-contaminated sites. This showed that such plant species (or populations) have some degree of metal tolerance. As plants can accumulate

metals without showing any obvious effect, the time required to detect that wetlands have become polluted could be considerable. Such delays would necessitate expensive restorative measures. Very little attention has been accorded to the monitoring of wetlands. Thus, unless correctly and efficiently managed, wetland ecosystems are at risk of degradation. Poor understanding of the value of wetlands will continue to encourage resource overuse and environmental degradation.

2.2.1 Wetland protection, sustainability and amelioration

There is much concern regarding environmental protection, conservation and policies for sustainable development. This is a consequence of the pollution of the biosphere which has rapidly accelerated due to the industrial revolution. The latter is also responsible for the presence of excess metal levels in surface waters which constitute a risk to human health and the environment (Atafar *et al.,* 2010:83). Wetlands impacted by anthropogenic activity frequently require remediation. Enhancement, restoration, and creation of wetlands present ways of remediating these adverse impacts (Kent, 2000:38). Wetlands are altered by pollutants from upstream or local runoff and these inflowing waters therefore alter the quality of the water flowing out of the wetlands (Milovanonic, 2007:159). The use of phytoremediation to remove excessive levels of chemicals in the soil has become important in the research and development of wetlands.

Over the years, the reclamation of metal-contaminated soils by phytoremediation requires a complete and permanent plant cover. The selection of the most suitable plants for phytoremediation purposes has necessitated that the effects of various metals on particular wetland plants need to be assessed.

2.2.2 Metals in wetlands: sources, accumulation and distribution

Heavy metals or metallic chemical elements in the environment cannot be degraded or destroyed even at fairly low concentrations (Yadav *et al.,* 2009: 4616). These are deemed toxic or poisonous to both animal and plant life (Landner & Reuther, 2004:1; Govindasamy *et al.,* 2011:145). Heavy metals, which are hazardous to humans, include Pb, Hg, Cd, As, Cu, Zn, and Cr (Lokeshwari & Chandrappa, 2007:121). Such metals are found naturally in the soil in trace amounts, and these pose few problems. When concentrated in particular areas, however, they present a serious danger. For examples, As and Cd are carcinogenic; mercury can cause mutations associated with genetic damage, and Cu, Pb and Hg can cause brain and bone damage according to the Toxicological profile for mercury (update) reported in Vol. 199.

Atlanta: (Agency for toxic substances and disease registry; Agency for toxic substances and disease registry; p. 485).

The increased levels of toxic metal emissions due to anthropogenic activities and processes are causing a rapid accumulation of metals in soil and water. The release and deposition of metals such as Al, Fe, Zn, Cd, Mn and Co result in pollution of the atmosphere, water and soil. These are likely to aggregate in plants particularly in industrial areas and in the vicinity of large emission sources. Human and animal activities also enhance the accumulation of metals in plant tissues (Deng *et al.,* 2004:29*).*

Heavy metals are of major concern because of their persistent and bio-accumulative nature (Chang *et al.,* 2009:1275; Yadav *et al.,* 2009:4616). Contamination of the environment with metals in the urban industrial areas is a major health concern (Lokeshwari & Chandrappa, 2007:121).

Substantial quantities of toxic metals deposited on the leaves of wetland plants were reported to be directly absorbed and retained through leaf uptake (Deng *et al.,* 2004:29*).* Wetland plant roots accumulate metals and this causes the roots to release a variety of substances that include oxygen, enzymes, allelopathic chemicals and antibacterial agents. Many of these affect the rhizophere directly by altering the pH and oxidative status of the environment (Foyer & Noctor, 2000:359). In addition these metals in the rhizosphere can positively or negatively influence growth of bacteria, algae and higher organisms (Lokeshwari & Chandrappa, 2007:121). All of these collectively through their activities thus alter the chemistry of the environment (Chang *et al.,* 2009:1275; Yadav *et al.,* 2009:4616).

Oxidation-reduction (redox) and associated pH changes that occur in wetland conditions can affect the retention and release of metals. When changes occur in the oxidation status of wetland environments, transformation of metals among the various different chemical forms may occur affecting the mobility and biological availability of metals (Chang *et al.,* 2009:1275; Yadav *et al.,* 2009:4616). It is well established that oxides of Al and Fe effectively adsorb most trace and toxic metals (Foyer & Noctor, 2000:359). It was also reported that heavy metals such as Al and Pb can significantly affect the uptake and the translocation of certain nutrients in plants (Sinha *et al.,* 2006:651). Thus, nutrient imbalance may be a symptom of heavy metal toxicity in plants. However, information relevant to metal tolerance in wetlands is limited. It is not known whether the tolerance of metals by these wetland species is related to oxidative stress.

2.3 Effects of metals on plants

Metals are studied worldwide as many are non-degradable, stable in the environment and are associated with toxic effects on many life forms. Accumulation of excessive toxic metals often causes irreversible damage to various plant tissues. As these plants eventually enter the food chain the metals contaminate heterotrophic species in the human food-chain (Adekunle *et al.,* 2007:307; Tack & Vandecasteele, 2008:283). Eventually, this results in ecological risks and human health problems (Cui *et al.,* 2004:785; Butt *et al.,* 2005:338; Sinha *et al.,* 2006:65). The accumulation of metals has been reported to be responsible for various phytotoxic side effects (Sinha *et al.,* 2006:651). These include stunted growth, chlorosis and necrosis (Fodor, 2002:149).

The phytotoxicity of metals could arise partly from the generation of Reactive Oxygen Species (ROS), which cause direct damage to lipids, proteins and DNA. In plants, ROS are continuously generated as by-products of photosynthesis and other cellular metabolic processes (Foyer & Noctor, 2000:359). The ROS originate principally from the dissipation of electrons across chloroplastic and mitochondrial membranes; concentrations are normally controlled by complex mechanism (Apel & Hirt, 2004:373). At the cellular level enzymatic activity is altered resulting in enzyme activation or inhibition (Darko *et al.,* 2004:583; Dazy *et al.,* 2009:297).

2.3.1 Aluminium and iron: functionality of wetland plants and their effects

Aluminium and Fe occur naturally in the soil and sediment environments. They are also released into the environment in significant amounts by anthropogenic activities (Sharma & Dubey, 2007:2027). Aluminium and Fe do not catalyse redox reactions although Fe is a transition metal and therefore has catalytic ability. Both Al and Fe can cause oxidative damage to major biomolecules (DNA, lipids, and proteins) and can induce anti-oxidative defence mechanisms (Boscolo *et al.,* 2003:181; Choudhary *et al.,* 2007:204). Several studies have proposed that the pro-oxidant activity of Al could be explained by the formation of an Al superoxide semi-reduced radical cation (Khan *et al.,* 2006:223; Choudhary *et al.,* 2007:204). Involvement of the mitochondrial electron transport chain was inferred in both Fe and Al-induced ROS generation (Yamamoto *et al.,* 2002:63). Antioxidant enzyme induction was proposed to play an important metabolic role under conditions of metal stress (Matysik *et al.,* 2002:525).

The physiological effects of metal pollution are closely related to their accumulation. For example, Al and Fe were indicated to inhibit root growth and decrease fresh weight (Landner & Reuther, 2004:1; Govindasamy *et al.,* 2011:145). Similarly Cd was found to reduce water content in roots and shoots of maize, rye and wheat (Stoltz & Greger, 2002:271). Plant growth may be affected by heavy metal pollution (Deng *et al.,* 2004:29). This in turn inhibits the photosynthetic activity of aquatic biota. Direct toxic effects are also known to occur. Thus there is a need for fundamental research to yield an understanding of the biology of wetland ecosystems, including the role the system plays in determining the specific character of plants.

2.3.2 Mechanisms of Al and Fe toxicity

Anthropogenic activities are responsible for on-going and excessive accumulation of Al and Fe in the environment (Moustakas *et al.,* 1995:669; Jones & Ryan, 2003:656). The sources of these metals in wetlands are various industrial processes and household-related activities (Englar, 2007:1).

The bioavailability of Al and Fe to plants is controlled by many factors such as soil and climatic conditions, plant genotype, and plant processes (active/passive transfer, redox states of metals) (Landner & Reuther, 2004:1; Govindasamy *et al.,* 2011:145). Also important are the responses of plants to elements in relation to seasonal cycles, the type of plant root system, sequestration and speciation of metals which influence bioavailability (Baldantoni *et al.,* 2005:48). Research into elucidating the mechanisms of metal ion transfer from soil to plant would assist in ecological risk assessment.

2.3.3 Plant varietal selection and screening for resistance to Al and Fe toxicity

Varietal differences in tolerance to metals are often observed among species and cultivars (Baldantoni *et al.,* 2005:48). Moreover differences are observed even within tissues of the same plant grown under identical conditions. These differences are related to organic acid biosynthesis and accumulation which show a marked increase in response to environmental stress (Balazsy, 2000:1). Generally, intra-specific variations (between varieties or cultivars) can often be greater than differences in susceptibility between species. However, all plants are affected by a severe deficiency or toxic overload of any of the micronutrients.

Plant species in tropical areas are notably resistant to Al stress (Jones & Ryan, 2003:656). Some of these species can accumulate high concentrations of Al in the leaves to levels comprising 1% of their dry weight (Jones & Ryan, 2003:656). In contrast, cereals such as *Secale cereal, Zea mays, Hordeum vulgare, Triticum aestivum, Sorghum bicolor* and *Avena*

sativa do not accumulate high concentrations of Al internally but rather use an Al exclusion mechanism through organic acid exudation (Caniato *et al.,* 2007:863).

The principal difference noted for various genotypes is the critical concentration at which the supply of particular micronutrients becomes inadequate. These levels are significantly lower for more metal tolerant genotypes (cultivars). Absorbability of metals by plants varies among different species (An, 2004:21; Rai, 2009:697; Cao *et al.,* 2010:2777).

2.3.4 Iron content as physiological indication of toxicity in wetland plants

Iron is a trace element necessary for photosynthesis in all plants. Green plants utilize Fe for energy transformation processes (Alcantara *et al.,* 1994:1983). The effects of Fe on other physiological indicators have also been noted (Sinha *et al*., 1997:286; Sinha & Saxena, 2006:1340).

Free Fe within the cellular system can catalyse the conversion of hydrogen peroxide to free radicals, which can cause damage to a wide variety of cellular structures, and ultimately kill the cell (Crichton *et al.,* 2002:9). Commonly observed symptoms are rusty leaf spots (bronzing), stained leaf edges, and a dark brown and poorly developed root system (Dobermann & Fairhurst, 2000:191).

In wetland plants, iron uptake and transport occurs primarily through the root system by passive diffusion and active transport (Seregin & Kozhevnikova, 2006:257). The Fe is taken up together with other nutrients such as Ca, Zn and Cd through the root system via the apoplast, including the cell wall continuum and intercellular space. The iron concentrates in the rhizosphere surrounding the root cell membranes and enters metabolic pathways (Nakanishi *et al.,* 2006:464). When soils are depleted of Fe, or water soluble Fe, plants usually experience growth problems (Alcantara *et al.,* 1994:1983). Shanker *et al.,* (2004:1035) reported that when Fe is deficient in dicotyledonous plants enhanced root Fe^{3+}-reductase activity occurs. This increases the capacity to reduce Fe^{3+} to Fe^{2+}. Ferrous iron is the form in which roots absorb Fe (Alcantara *et al.,* 1994:1983).

Plant iron-uptake capacity varies widely, and does not only depend on soil Fe concentrations, but also upon pH values (Hell & Stephan, 2003:541), phosphate concentration (Richardson *et al*., 2004:267) and competition between Fe and other heavy metals (Sood *et al.,* 2008:35). Lime soils are often Fe deficient even when sufficient amounts of iron are present. This is a result of the alkaline pH value, which leads to iron precipitation (Shanker *et al.,* 2004:1035).

Iron usually occurs in soils in tertiary forms, but in water-saturated soils it is converted to binary iron, thereby enabling plant iron uptake (Batty & Younger, 2003:801; Shanker *et al.,* 2004:1035). Plants may absorb water insoluble iron compounds by releasing H^+ ions, causing Fe to dissolve (Sood *et al.,* 2008:35).

2.3.5 Accumulation, toxicity symptoms and conditions enhancing iron content in plant components

Studies done elsewhere have indicated that several factors influence the uptake of metals by plants (Sheoran *et al.,* 2011:168). These factors include the type of plant, its size, the root system, the growth environment and soil pH (Matthews *et al.,* 2004:39; Yamamoto *et al.,* 2005:12*).* Heavy metals are absorbed by the roots and leaves. The pathway of transport is from root to shoot through the stem, and xylem, and Fe exits by means of the transpiration stream. Iron may be stored in certain plant organs, particularly in older leaves.

High concentration levels of heavy metals may lead to metabolic imbalances which are detrimental to plant growth and development (Cortes-Esquivel, *et al*., 2012:871). This is as a result of the effect of the metal on the robust inerratic fibrillar networked cell wall, consisting of cellulose, hemicelluloses, glycoproteins and pectin and other compounds (Vert *et al.,* 2002:1223).

Several studies have reported that Fe toxicity is a problem in wetland plants, particularly in rice (Batty & Younger, 2003:801). Fe deficiency is first evident in young leaves (Sood *et al.,* 2008:35). Symptoms of Fe toxicity often occur adjacent to unaffected plants. Young plants may overcome symptoms as the plant matures and the root system develops (Batty & Younger, 2003:801). Symptoms include: damage to the root system, chlorosis, dark green foliage, stunted growth, thickening of roots, brown spots on leaves which commence at the tips of lower leaves, and dark brown and purple leaves (Prasad, 1999:1).

Fe is an important element for all living organisms; notably in biogeochemical processes because of its unique ability to serve as both an electron donor and acceptor (Lalonde *et al*., 2012:198). Free iron within the cell can catalyse the conversion of hydrogen peroxide to free radicals. When in high concentration hydrogen peroxide can damage cellular structures; and even cause death of cells (Crichton *et al.,* 2002:9).

2.3.6 Conditions which reduce iron toxicity

Many life forms have evolved biochemical protection mechanisms by binding iron atoms to proteins, thereby limiting the ability of Fe to induce damage and simultaneously permitting the cells to take advantage of Fe presence (Andrews, 1999:1986).

The most important group of iron-binding proteins is the heme molecule, all of which contain Fe at the centre. Organisms use variants of heme to perform redox reactions and electron transport processes (Batty & Younger, 2003:801). In higher organisms, however, Fe is an essential component of myoglobin, which stores oxygen in muscle cells (Abbaspour *et al*., 2014:164). At a redox potential of approximately +120 mV, the insoluble oxidized form of Iron (Fe^{3+}) is reduced to (Fe^{2+}). In the reduced form Fe^{2+}, iron becomes soluble and is more readily bio-available (Alcantara *et al.,* 1994:1983). The Fe concentration in wetland plant tissues is often greater than that of terrestrial plants (Mittler, 2002:405). The tolerance of wetland plants to Fe^{2+} is related to root porosity, root oxidizing ability and flood tolerance (Matthews *et al.,* 2005:1). The flood resistant species *Eriophorum angustofolium* and *Juncus effuses* tolerate Fe in a similar manner (Matthews *et al.,* 2005:1).

2.3.7 Human exposure to iron

Iron is an absolute requirement for all forms of life. Iron compounds may often exert more of an effect on human health than does the relatively harmless element itself. Extensive use of Fe by industries may lead to its accumulation in ecosystems, thus inducing the toxicity to crops and vegetables (Yazgan & Tanik, 2005:687). Iron can occur in meats, whole meal products, potatoes and other vegetables (Yazgan & Tanik, 2005:687). The human body absorbs Fe present in animal products more rapidly than Fe which occurs in plant products. Iron is an essential part of haemoglobin; which transports oxygen in blood (Abbaspour *et al*., 2014:164).

In humans, iron may cause conjunctivitis, choroiditis, and retinitis if it contacts and remains in the tissues (Bartzokis *et al.,* 2004:1012224). Chronic inhalation of excessive concentrations of iron oxide fumes or dusts may cause the development of a benign pneumoconiosis, called sclerosis, observable as an X-ray change (Abbaspour *et al*., 2014:164). No physical impairment of lung function has been associated with sclerosis (Abbaspour *et al*., 2014:164). Inhalation of excessive concentrations of iron oxide may enhance the risk of lung cancer in workers exposed to pulmonary carcinogens (Bartzokis *et al.,* 2004:1012224).

2.4 Aluminium in the environment

Aluminium is an abundant element in the earth thought to constitute 7.5% - 8.1% of the earth's crust material (Pereira *et al.,* 2010:1496). Aluminium is a reactive metal making it difficult to extract from aluminum oxide (Al_2O_3) ore (Grjotheim & Welch, 1988:1). Hence Al is very rare in its free form. Aluminium is one of the most difficult earth metals to refine as it is rapidly oxidised to form an extremely stable compound (Andrews, 1999:1986). Aluminium contributes much to the properties of soil, where it is present mainly as insoluble aluminium hydroxide (Andrews, 1999:1986; Prasad, 2004: 345). Aluminium is one of the most widely used metals in industry (Pereira *et al.,* 2010:1496).

2.4.1 Toxicity of aluminium

In Al-stressed plants, it has been shown that root elongation is retarded (Prasad, 2004: 345). This is due to the inhibition of root cell division and decreased cell expansion in the root elongation zone (Barceló & Poschenrieder, 2002:75; Prasad, 2004: 345). According to Meda and Furlani (2005:309), root hairs in the aquatic pondweed *Limnobium stoloni*- ferum. respond rapidly to Al toxicity and decreased root hair growth is detected within 30 minutes of exposure to Al. Understanding the control of cell elongation would be a key step towards elucidating the mechanisms of plant growth and development and may provide important information regarding the method/s of Al-induced root growth inhibition. It is generally understood that the inhibition of growth of root apices is the first response to Al toxicity by plants as Al^{3+} inhibits root growth through binding to sensitive sites in the apoplast of the epidermis and the outer cortex (Barceló & Poschenrieder, 2002:75; Prasad, 2004:345; Bhalerae & Prabhu, 2013:447).

Figure 2.1: Effect of Al on plant roots (Inostroz-Blancheteau *et al.*, 2005).

2.4.2 Uptake, transport and distribution, and environmental influences of aluminium

Attention has been drawn to the effects of Al in the environment caused principally by acidification (Watanabe & Osaki, 2002:1247). Aluminium may accumulate in plants and subsequently cause health problems for animals that ingest these plants (Watanabe *et al*., 2006:1243). The environmental concentrations of Al appear to be greatest in acidified lakes (Watanabe & Osaki, 2002:1247). In the latter, the number of fish and amphibians is declining due to the reactions of Al ions with proteins in the gills of fish and in the embryos of frogs (Watanabe *et al*., 2006:1243; Fonkuo *et al*., 2005:457). High concentrations of Al also occur in acidified lakes, the atmosphere, and in the groundwater of acidified soils (Watanabe & Osaki, 2002:1247). High Al concentrations not only affect fish, but also birds and other animals that consume contaminated fish and insects (Fonkuo *et al*., 2005:457).

The consequences for birds that consume contaminated fish include eggshell thinning and chicks hatching with low birth weights (Fonkuo *et al*., 2005:457). On the inhalation of Al present in the atmosphere, animals may be adversely affected through weight loss, damage to lungs and the appearance of malaise (Sharma, 2009:1).

Aluminium enters the environment naturally through the weathering of rocks and minerals (Sharma, 2009:1). Anthropogenic releases are in the form of air emissions, waste water effluents, and solid waste primarily associated with industrial processes, such as Al production (Watanabe & Osaki, 2002:1247; Prasad, 2004:1). As Al is the most abundant metal and the third most abundant chemical element on the earth's crust, it is absorbed by the roots and via leaves (Watanabe *et al*., 2006:1243). The pathway of transport is from root to shoot through the xylem, and the element exits by means of the plant transpiration stream (phytovolatization). It may also be stored in selected organs in plants, particularly in older leaves (Fig. 2.2). Additionally, Al is often taken up and concentrated in root tissue (Prasad, 2004:1; Inostroz-Blancheteau *et al.,* 2005).

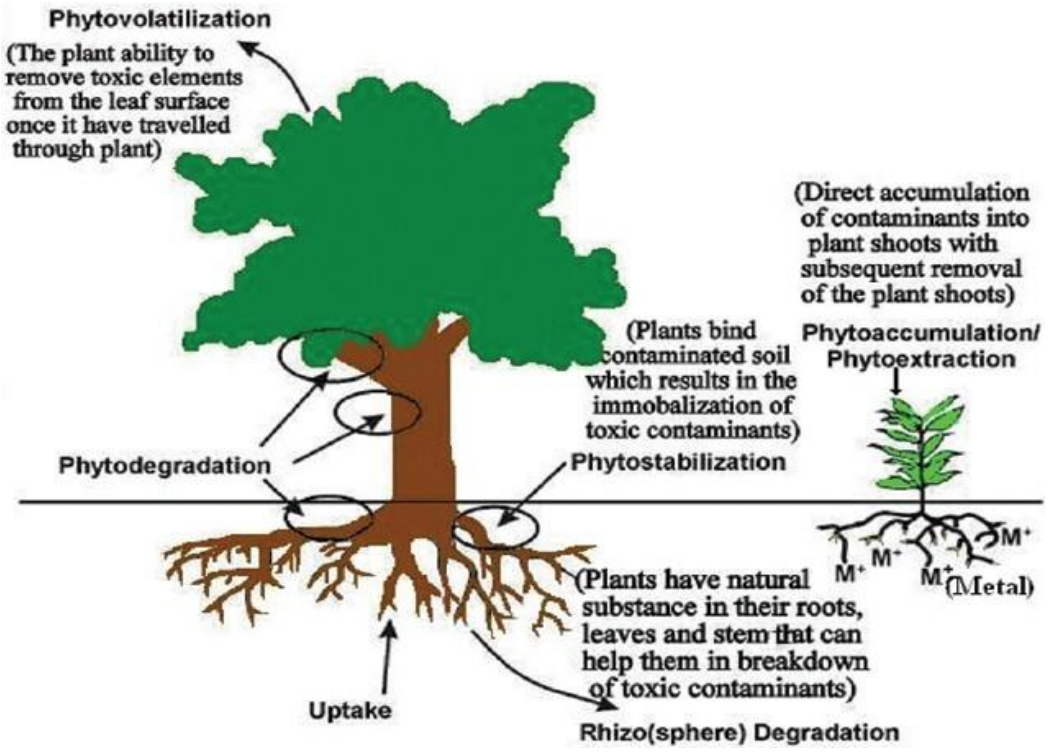

Figure 2.2: Transition mechanisms in plants for metal accumulation (Adapted from Singh *et al*. 2011:246)

2.4.3 Accumulation of aluminium in plant components and aluminium toxicity

The toxicity of Al is the primary factor limiting crop production on acidic soils (pH values of 5 or below), and because 50% of the world's potentially arable lands are acidic, Al toxicity is a very important limitation to worldwide crop production (Bot *et al.,* 2000:114). Aluminium tolerance is undoubtedly an ecological and agronomical advantage to plants and crops. Aluminium toxicity causes significant changes in the biochemical and structural patterns of plant cells, and impacts on cell multiplication and cell growth. There are strong indications that Al can damage the roots of trees, (specifically the root tip) when the metal is located in groundwater. As a result, root elongation, rather than shoot and dry plant matter would provide the most sensitive monitor over a short period of time to use for comparative analyses. Silva *et al.* (2000:123) found that Al entered into the cell symplast within 30 minutes, accumulating in the nucleus of meristematic cells of soybean roots, and causing a decrease in root growth. Another negative environmental effect of Al is that where water is present in the soil, Al ions can react with phosphates, thereby making phosphates less available to organisms.

2.4.4 Symptoms of, and conditions enhancing or reducing aluminium toxicity

Symptoms of Al toxicity include a delay in the vegetative growth of the plants, with fewer leaves forming and there is a decreased development of shoots, directly proportional to Al levels in solution (Meda & Furlani, 2005:309). Prasad (1999:1) listed Al toxic symptoms as stunting, dark green leaves, purpling of stems, leaves and leaf vein yellowing and death of leaf tips, curling of young leaves and collapse of growing points or petioles, thickening of root tips and later roots, and inhibition of root elongation. In the shoot, purple colouration and interveinal leaf chlorosis were observed in shoots of Al-stressed plants. These may translate to a reduction in crops in both vigour and yields (Kochian *et al.,* 2005). Many symptoms become evident after a few minutes exposure to micromolar concentrations of Al in hydroponic solutions (Rengel & Zhang, 2003:295). Furthermore, Al also triggers membrane lipid peroxidation and apoptosis or programmed cell death (PCD) (Barceló & Poschenrieder, 2002:75). Prolonged exposure to Al can induce and produce responses of rapid change in other biochemical and physiological processes (Rengel & Zhang, 2003:295). Excesses of Al also induce symptoms of Fe deficiency, observed in *Sorghum bicolor* (Caniato *et al.,* 2007:863; Magalhaes *et al*., 2007). Furthermore, up to 60% of the acid soils in the world occur in developing countries, where food production is critical (Bot *et al*., 2000:114).

Aluminium phytotoxicity is one of the major agronomic problems in acid soils. On acid soils, land forming operations or erosion can expose acid subsoil. Aluminium toxicity can occur where soil pH is less than 5.2 and can be alleviated by the use of ammonium fertilizers and

acid rain which increase soil pH. Toxic forms of Al are solubilized into the soil, inhibiting root growth and function, and thus reducing crop yields (Kochian *et al.,* 2004; Watanabe *et al.,* 2006).

2.5 Health effects of human exposure to aluminium

Aluminium, termed an “innocent” compound, is one of the most widely used metals and is one of the most frequently occurring compounds in the earth's crust. Hence humans are exposed to high concentrations which can cause health problems. People working in certain environments, such as Al mines are at risk of Al exposure, especially from water bodies into which the element leaches. People working in factories where Al is applied during production processes may develop lung problems when they inhale Al dust. The route of Al into humans occurs through food consumption, inhalation and also through contact with the skin. Lengthy exposure to and uptake of significant concentrations of Al can lead to serious health problems (Adekunle *et al.,* 2007:307).

The water-soluble form of ionic Al also exerts harmful effects. They usually occur in a solution of Al in combination with other ions. An example is Al chlorine. Aluminium can cause problems for patients with kidney disease when it enters the body during dialysis. Inhalation of finely divided Al and Al-oxide powder has been reported as a cause of pulmonary fibrosis and lung damage. This effect, known as Shaver’s Disease, is complicated by the presence in the inhaled air of silica and oxides of iron. Other effects include damage to the central nervous system, and various dementias; including Alzheimer’s disease (Bot *et al.,* 2000:114).

2.6 Wetland monitoring using plants: threat and conservation

Wetland plants are particularly useful as biological indicators because they comprise a universal component of wetland ecosystems. These plants are common, and are present in sufficient diversity to provide clear and robust signals of human disturbances in the environment. The use of biological data to evaluate ecosystem health (biological monitoring and assessment) is a powerful tool to measure and interpret the consequences of human activities on wetland ecosystems. Wilcox (1995:240) reported on the extensive use of wetland plants to distinguish among environmental stresses including hydrologic alterations, excessive siltation, nutrient enrichments, and other types of disturbances.

Disturbance is a natural element of all ecological systems and many biotas have adapted to these natural disturbance regimes. However, in recent times, technology has advanced at a

rapid rate causing the generation of greater concentrations of contaminants. Some of the contaminants accumulate in wetland biota and wetland sediments. As human activities increase with the passing of time, the ecological integrity of the wetlands will simultaneously diminish. This would be caused by associated changes in processes such as photosynthesis, hydrology and nutrient cycles (Karr, 1993:83). The rapidity and magnitude of disturbances associated with human activity has led to a reduction of numerous wetland plant species.

Vegetation plays an important role in waste treatment wetlands (Table 2.1 and Table 2.2). Plants provide a substrate for microbes, which are the most important processors of wastewater contaminants. Plants provide microbes with sources of carbon. The composition of plant species reflects both current and historical environmental conditions and for this reason changes in species composition over time may indicate environmental change (Aksoy *et al.,* 2005:241). The degradation and loss of wetland makes the elucidation of the need for the conservation of wetlands and their economic utility, as well as the promotion of scientific and application-oriented research into their productivity, expedient. Healthy functioning ecosystems form global defenses against climate change and storm damage. Overall the conservation of wetland ecosystems will be essential in order to reap their full potential (Mitsch & Gosselink, 2007:582).

2.6.1 Wetland plants as bioindicators

Wetland plants are particularly useful as biological indicators because they are a universal component of the wetland ecosystem. The continued exploitation and degradation of wetlands has caused concern among scientists and stakeholders, with attention turning to understanding the dynamics of the responses of plant communities. The response of a plant species to disturbances is a function of its auto-ecological tolerance to different environmental factors. The utilization of biological data to evaluate ecosystem health (biological monitoring and assessment) is a useful tool for the measurement and interpretation of the consequences of human activities on wetland ecosystems (Wilcox, 1995:240).

2.6.1.1 Removal of heavy metals from wetlands by wetland plants

Anthropogenic changes have led to considerable changes in chemical cycling in many wetlands. Nutrient cycling in wetlands differs from that of both aquatic and terrestrial ecosystems. Nutrient cycling has both temporal and spatial dimensions.

The phytoremediation potential of plant species has been considered by many (Singh *et al.,* 2007:223; Zhang *et al.,* 2010:1315). Wetlands and wetland plants retain nutrients and heavy

metals by accumulating these in the sub-soil or by storage within the vegetation (Tables 2.1). Many wetland plants have developed specialized morphological adaptations that enable them to survive and proliferate with their roots in an anoxic environment. These adaptations have developed in response to root zone saturation, enabling the plants to capture molecular oxygen and transport it to the stems and roots (Kent, 2000:38).

Table 2.1: Overview of pollutant removal mechanisms in wetland ecosystem (Adapted from Kent, 2000:38)

Pollutant removal mechanism	Removal Processes
Organic material (measured as BOD)	Biological degradation, sedimentation, microbial uptake
Organic contaminants (e.g., pesticides)	Adsorption, volatilization, photolysis, and biotic/abiotic degradation
Suspended solids	Sedimentation, filtration
Nitrogen	Sedimentation, nitrification/DE nitrification, microbial uptake, volatilization
Phosphorous	Sedimentation, filtration, adsorption, plant and microbial uptake
Pathogens	Natural die-off, sedimentation, filtration, predation, UV degradation, adsorption
Heavy metals	Sedimentation, adsorption, plant uptake

Table 2.2: Contaminant removal mechanisms in constructed wetlands (Adapted from: Choudhary *et al.* (2011:1)

Parameters / contaminants	Physical	Chemical	Biological
Suspended solids	Sedimentation Filtration		Biodegradation
Biochemical oxygen demand	Sedimentation	Oxidation Reduction	Biodegradation
Chemical oxygen demand	Sedimentation	Oxidation Reduction	Biodegradation Phytodegradation Phytovolatilization Plant uptake
Nitrogenous Compounds	Sedimentation Volatilization	Adsorption	Bio-denitrification-nitrification Plant uptake
Phosphoric Compounds	Sedimentation	Adsorption Precipitation	Microbial uptake Plant uptake
Metals	Sedimentation Filtration	Adsorption Precipitation	Plant uptake
Pathogens	Filtration	Adsorption	Natural death

UV ray action	Oxidation	Exposure to natural toxins Bacteriophage attack

2.6.1.2 Wetlands as water purifiers

A wetland plant exerts an important influence on water quality. This influence can be positive, as plant root systems assist in stabilising sediments, and their tissues may accumulate nutrients or metals, thus eliminating them from the water column (Potter *et al.,* 2010:2104). Wetlands are sensitive to seasonal changes and rainfall. Wetland plants have a cosmopolitan distribution and display high levels of phenotypic polymorphism. There are many plant species which have adapted specifically to life in wetland environments, and in particular to water soaked soils/lands. Many of these plants have adapted to taking up nutrients from the water, which enables them to survive. This removal of substances from the water enhances the water quality. Wetland soils serve as medium in which many of the wetland chemical transformations occur and they act as a primary storage facility for available chemicals for most wetland plants. The distribution of wetland plants depends on the distribution of wetland ecosystems. The factors determining the distribution and types of wetland globally are climate, topography, and geology (Mitsch & Gosselink, 2000:1).

2.6.2 Degradation and wetland destruction

The problems of wetlands can be perceived as the result of a mismatch between extrinsic resources and natural resources. This often arises from humans deliberately or inadvertently misusing or abusing the natural environment. The rapid increase in numbers of the global population of human beings is the principal cause of increased human impacts on the environment. Other key concerns are the modification of river flows by damming, irrigation, and pollution emanating from land, marine and atmospheric sources. Wetland degradation has increased during the past 50 years, creating further losses of biological resources. The principal reasons for this degradation, apart from natural disasters, are poverty among human populations and the pressures of economic development occurring both locally and globally. Economic gains, many with short-term benefits, are being made at the expense of the integrity of ecosystems and the vulnerable communities that they support. The over-exploitation of resources impacts on the livelihood, survival and food security of the human population (Lokhande & Suprasama, 2012:2). Wetland destruction has the potential to exacerbate the effect of both climate change and associated increasing loss of natural habitats and biodiversity. The protection and restoration of aquatic ecosystems and their vital functions are

subjected too often to adverse pressures from land-use changes, urbanization, global warming/climate change, rising sea levels, coastal erosion and lowland flooding.

2.6.3 Wetland management

The management of wetlands has been associated with several functions and values commonly attributed to wetlands. The functions, classified into three main groups (hydrology, biogeochemistry and habitat) are linked to the self-maintenance of the wetlands and their surroundings (Mitsch & Gosselink, 2000:1).

Conservation is perceived by many to be costly both in terms of management, and the opportunity costs involved. However, the values of ecosystems could be realised through the promotion of conservation actions that highlight the degree to which ecosystem goods and services contribute to human well-being and economic output. Thus, it is understandable why conservation of wetlands should be taken seriously. Many studies have estimated the contribution of biodiversity to the livelihoods of humans in terms of income. There is a great need for the conservation of wetlands themselves, and their economic utility. It is also important that scientific and application-oriented researches are promoted to enhance the productivity of wetlands (Uluocha, 2004:151).

2.6.4 Adaptations of wetland plants to metal toxicity

Adaptation of wetland plants may aptly be referred to as mechanism of tolerance leading to either passive or active interaction with the environment/metal. Plant tolerance to heavy metals may refer to the ability of plants to survive in a soil that is toxic to other plants (intolerant), and is manifested by an interaction between a genotype and it environment (Macnair *et al.,* 2000:235). The mechanisms of response to metals in some wetland plants are based on one or more of the following:

- aerenchyma. This term refers to air spaces or pore in the roots and stems through which oxygen can enter the plant and be transported to its roots.
- hollow stems for transporting oxygen to the roots.
- woody plants actively pump oxygen from the stems to the roots.
- many wetland trees have very shallow root systems, swollen trunks, or roots that grow above the ground. Examples of this are the cypress knees observed in swamps.
- some plants develop adaptations that allow them to tolerate salt water. These plants are called halophytes. Adaptations of these plants include:

- reduction of salt intake by the roots, by a process known as salt water exclusion.
- specialised glands which excrete excess salt.
- glands often found in fleshy leaves which collect concentrations of salt followed by shedding of the leaf.
- succulent leaves, which accumulate and store water which is used to dilute salt concentrations.
- development of a waxy outer protective covering thus preventing salt uptake.
- reduced leaf surface area to minimize exposure to salt and .
- sequestration of salt into specialised internal organs (Macnair *et al.,* 2000:235).

Many physiological processes in plants, commencing with those associated with seed germination through to the production of fruit can be variously altered when levels of metals (Al and Fe) are elevated. These processes include induction of oxidative stress (Chen *et al.,* 2009:2350), inhibition of enzymes (Gianfreda & Rao, 2004:339) and plant water relations and photosynthesis (Ashraf & Harris, 2013:163). Studies on the formation of ROS and the consequences within cells when subjected to particular chemical substances are of great importance in the elucidation of answers to essential questions in stress physiology. Induction of the activity of anti-oxidative enzymes has been suggested as a convenient model for the investigation of stress in plants (Nimptsch *et al.,* 2005:147). Thus, plant tolerance to a particular metal is governed by an inter-related network of physiological and molecular mechanisms and an understanding of these mechanisms and their genetic basis is important for the development of plants as agents of phytoremediation (Seregin & Kozhevnikova, 2006:257).

2.6.5 Metal uptake and tolerance mechanisms in wetland plants

Wetland plants have shallow root systems when compared with their non-wetland counterparts. Wetland plants have adapted to the soil saturation and flooding associated with wetland hydrology. Roots are the primary site of metal (and all elements) uptake and therefore, the concentrations of element are usually much higher in roots when compared with leaves (Mittler, 2002:405). As soil becomes saturated with water, the amount of oxygen available to plant tissues below the surface of the soil decreases rapidly because it is used by plants and microorganisms. The movement of oxygen from air into water or saturated soil is much slower

than in a well-aerated soil and creates an oxygen-deficit. Plants rely on a range of transition metals as essential micronutrients for normal growth and development. These elements are essential for most redox reactions which are fundamental to cellular functions.

Higher plants produce ROS during different metabolic processes in cellular organelles; however, during metal stress, their rate of production is dramatically elevated (Sytar *et al.,* 2013:985).

2.6.5.1 The application of wetland remediation systems

One of the key indicators of the quality of life is a healthy environment, which can be further disaggregated into atmosphere, water, plants, micro-organisms, soil, animals, and humans. Over the centuries, humans have altered the composition of the atmosphere through pollution. All pollutants discharged into the atmosphere in excess of critical concentrations are harmful to plants, animals and humans. Furthermore as discussed in the foregoing, many pollutants enter water bodies. As one of the ultimate goals of preserving an environment is to preserve biological diversity, the most direct means of achieving this could be by measuring the quality of a wetland by assessing its biota (Cunningham *et al.,* 1995:393).

The interactions of physical, biological and chemical components of a wetland, such as soils, water, plants and animals enable the wetland to perform many vital functions. The latter include: water storage; storm protection and flood mitigation; shoreline stabilization and erosion control; groundwater recharge (the movement of water from the wetland down into the underground aquifer); groundwater discharge (the movement of water upward to become surface water in a wetland); water purification through retention of nutrients, sediments, and pollutants; and stabilization of local climate conditions, particularly rainfall and temperature (Obek & Sasmaz, 2011:217). Wetland ecosystems support high numbers of birds, mammals, reptiles, amphibians, fish and invertebrate species. Of the 20,000 species of fish in the world, more than 40% live in fresh water.

2.6.5.2 Amelioration and phytoremediation of metal contamination using wetland plants

Phytoremediation is a biotechnology that utilises plants and their associated rhizosphere micro-organisms to remove, degrade, metabolize or detoxify contaminants. These include pesticides, metals radionuclides explosives located in the soil, sediments, groundwater, surface water and even the atmosphere (Marmiroli *et al.,* 1999:169). Phytoremediation offers several advantages; it is inexpensive and associated with minimal environmental disturbance. It constitutes a group of strategies meant not only to reduce metal loading at the contaminated

site but also to stabilize the site (Almeida *et al.,* 2006:424; Bashmakov *et al.,* 2006:2210). Ma *et al.* (2000:273) indicated that suitable plants for phytoremediation purposes include herbs, shrubs, or trees which could accumulate organics and heavy metals in excess of the levels found in nature. This is possible through mechanisms such as: phytoaccumulation, phytoextraction, phytostabilization, phytotransformation, phytovolatilization and rhizodegradation. According to Aken (2008:225), phytoremediation using a variety of plants acts as natural solar–powered pump-and-treat systems for cleaning up contaminated environments.

2.7 Wetland dynamics and associated problems

Wetlands are constantly under threat due to a mix of social, economic and political factors. A detailed study of the functions, uses and issues affecting wetlands is necessary in order to ensure the sustainable management of these resources. Conservation of wetland ecosystems is essential not only for sustainable freshwater supply but also for preserving biodiversity and ensuring other services necessary to the health and well-being of people around the world. In contrast, wetlands degraded through human activities especially those that reduce water quality and availability often have reduced capacity to deliver ecosystem services, which can directly or indirectly affect human health and further impacts such as the loss of food production and local livelihoods.

Degraded wetlands can cause the emergence of infectious diseases and the resurgence of water related diseases. Human health is compromised. Furthermore, degradation of wetlands will reduce the availability of wetland plants and animals that have medicinal value of particular importance to indigenous people and local communities.

2.7.1 Metal accumulation in wetland soil/sediment

The vulnerability of the wetland environment to chemical damage depends on factors such as the physical and chemical properties of the chemical substances entering the wetland ecosystem and their transformation products. The duration and properties of the ecosystem that enable it to resist changes may even be altered by the presence of these chemicals (Schiff *et al.,* 2002:115). Rapid industrialization and urbanization have resulted in environmental pollution and thus enrichment of metals in the soils and aquatic environment (Agunbiade & Fawale, 2009:267; Ayeni *et al.,* 2010b:2045). Contamination of soil with metals such as Al and Fe has created a concern for the global environment in recent years, particularly in areas with high anthropogenic pressures. The reactions of heavy metals with soil are important when

determining the fates of metals in the environment. This is due to the fact that soil has a high metal retention capacity (Verschueren, 1983:578).

2.7.2 Metal uptake, translocation and distribution by wetland vegetation

The ability of living tissues to accumulate, magnify and transform pollutants in the environment has made them of great importance in environmental studies. Plant and animal tissues have been reported to be capable of accumulating and concentrating pollutants such as heavy metals to levels that are toxic to life (Cabrera *et al.,* 2006:40, Liu *et al.,* 2006:787, Madejon *et al.,* 2006:1). This issue is emphasized by Kamran (2013:1029) in that author's review of heavy metal contamination and the impacts for living organisms.

2.7.3 Metal accumulation by wetland vegetation

Kumar *et al.* (2008:193) used energy dispersive analysis of X-Rays (EDAX) to investigate elemental composition of the aquatic plants *Vallisneri aspiralis*, *Hydrilla verticillata* and *Azolla pinnata* and found high levels of heavy metals such as Al, Mn and Fe.

2.7.4 Problems affecting wetlands plants

Variations in environmental factors affect the distribution and abundance of wetland plants. These variations are important as they should provide useful information for further investigations into the molecular mechanisms of metal tolerance. According to Richardson *et al.* (2005:1307), wetland plants are affected by anthropogenic disturbances and pollution of their habitat, including changes in hydrology, associated with reservoir development and canalization as well as pollution by nutrients and toxic chemicals.

Approximately one-third of the water which evaporates from the ocean surface returns back to land in the form of rain and snow. This water fills rivers, lakes, swamps, marshes, and other wetlands. Wetlands filter and maintain much of the freshwater on which humans and other animals depend. This makes wetlands one of the most important environments.

2.8 Climate change and wetland ecosystems

The global environment is regulated by climate changes and biosphere dynamics. In recent times there has also been a proliferation of large numbers of various organic compounds such as pesticides and insecticides which have exacerbated the pollution problems experienced by certain societies. Some of these compounds have potentially serious side-effects for non-target organisms and ecosystem viability as a whole.

Uncertainty about the density and composition of air pollution often intensifies the perception of risk to natural ecosystems and can lead to inappropriate management decisions. Such a situation demands effective ecosystem management; that is the fostering of beneficial livelihood or community practices that promote sustainabilty and overall ecosystem health. The solution to environmental problems typically involves the cooperation of multidisciplinary teams: scientists, engineers, sociologists, and lawyers frequently work together to design and implement processes or procedures to solve or prevent real or perceived environmental problems (Roquette *et al.,* 2009:289; Lokhande & Suprasanna, 2012:2; Alloway, 2013:195). Hence management requires an integrated approach between industrial chemists and environmental scientists towards sustaining wetlands. New technologies and stricter environmental laws are expected to improve enforcement and compliance regarding climate change.

2.8.1 Effect of iron on climate change

Climate change is one of the greatest environmental issues of the present. The burning and clearing of tropical forests is a major threat to stable ecosystems. It is now generally recognized that it will be impossible to achieve any of the required targets for mitigating climate change without significantly curbing the clearing and burning of tropical forests. Reducing greenhouse gas (GHG) emissions and stabilizing atmospheric concentrations at 350-450 parts per million CO_2 equivalent (ppm CO_2e) is essential. The current GHG level is approximately 390 ppm CO_2e.

Intact forests and other natural ecosystems – including wetlands, peat lands, coral reefs and mangroves – are required to reduce the risk of catastrophic impacts such as floods and droughts. This will allow for species migration and ecological adaptation, and support the livelihoods of indigenous and local communities. Maintaining these ecosystems will ensure that humans and other species can remain as resilient as possible to the impacts of climate change. Protecting the Earth's ecosystems can yield immediate, cost-effective climate change solutions that will be forever lost if immediate action is not taken. Iron could play a role in alleviating climate change. Iron fertilization is the intentional introduction of iron into the upper ocean to stimulate a phytoplankton bloom. This is intended to enhance biological productivity, which can benefit the marine food chain and remove carbon dioxide from the atmosphere. However, this attempt to remedy global warming is not yet finalized.

2.8.2 Effect of aluminum on climate change

As referred to in the foregoing, the Earth is threatened by global warming, climate change and ecological degradation due to unchecked human activities. Natural plant growth and development has been damaged by an abundance of Al in the environment thus causing a decline in biota, biodiversity and natural habitats. In this manner Al contributes toward climate change.

The study of a wetland plant species could elucidate on the importance of wetland ecology. For example, one of the reasons that ecosystems such as wetlands and estuaries are becoming increasingly threatened globally is that the benefits of damaging activities are usually perceived to be greater than the benefits of conservation and sustainable use. Conservation is perceived by many to be costly both in terms of management and the opportunity costs involved. However, when the values of ecosystems are seen through the promotion of conservation actions, such as those that highlight the degree to which ecosystem goods and services contribute to human wellbeing and economic output, conservation efforts would be more appreciated and enhanced.

The wetland ecosystem is very complex, as these seldom if ever are isolated systems; rather they interact strongly with adjacent terrestrial and aquatic ecosystems. Our understanding of the fundamental functioning of ecosystems, without the interaction of chemical stressors is usually limited. Wetland ecosystems are threatened by metal pollution throughout the world. There are more than two million types of commercially produced chemicals, with about 2000 added annually (Connell, 1999:219). Metals are introduced into wetland naturally through volcanic eruptions and rock weathering (Thawley *et al.,* 2004:180; Van Aardt & Booysen, 2004:57). Anthropogenically, metals can be released directly into rivers mainly through effluent from wastewater treatment plants, industries and mining, or indirectly through surface runoff from roads, farming lands and metal-contaminated groundwater, among others (Dalvie *et al.,* 2004:43).

To prevent cross-contamination or direct threats of heavy metals to humans, studies on the transfer and sub-chronic toxicity of soil, water, plant, animals are common. These could provide managers with a means of determining, implementing and monitoring the ecological reserve of wetlands.

To date, variability has been commonly reported in all morphological, biochemical and physiological parameters of plants investigated as potential phytoremediants. Thus variability will play a significant role in the selection of plant species suitable for phytoremediation. It is

possible that the search for suitable indigenous ecotypes may be enhanced by studying these plants whilst undergoing induced heavy metal stress when grown under standardized greenhouse conditions. Such studies would offer a useful approach as they would provide information on survival, growth biomass and reproduction of selected plant species which show tolerance to heavy metals and may even be able to accumulate high concentrations of these metals within their tissues. For the purposes of the current study, *Cyperus* spp. indigenous to the Western Cape were selected and investigated under controlled conditions to ascertain their suitability for the removal of Al and Fe from an aquatic environment. Table 2.3 lists *Cyperus* spp. investigated to date as candidate phytoremediants.

Table 2.3: Examples of *Cyperus* spp used for wastewater treatment

Cyperus species	**References**
Cyperus exaltatus	Ojo & Mashauri, 1996:1
Cyperus papyrus	Okurut *et al*. 1998:265
Cyperus papyrus	Erina & Wiyono, 2012:110
Cyperus alternifolius	Ebrahimi *et al.*2013:5
Cyperus papyrus	Foukuo *et al.* 2011:160

2.8.3 Toxicity thresholds of plants

Critical concentration thresholds vary considerably across metals and plant species (Tables 2.4-2.6). Most research has been done on crop plants. There has been little research into the effects of metal toxicity on wetland plants. In particular, there is little reported on metal toxicity within Cyperaceae (*Cyperus* spp). It is important that an understanding of the toxicity responses of wetland plants to metals is developed in order to utilize appropriate species for the rehabilitation of contaminated wetlands areas, the identification of metal toxicity levels, and the efficient regulation of metal emissions (Reichman, 2000:14).

Table 2.4: Aluminium concentrations in selected plants species

Al conc	**Species**	**Common name**	**References**	**Exposure time**
3 and 9 mg l^{-1}	*Ceratophyllum demersum*	Hornwort	Umebese & Motajo, 2008:197	15 d
50, 100, 200, 300, 400 and 500 µM	*Eleusine coracana*	Finger millet	Hemalatha *et al.* 2005:501	4 d

100 µM	*Triticum aestivum*	Wheat	Delhaize *et al.* 1993:685	4 h
200 µM 20 µM	*Phaselous vulgaris*	Common bean	Rangel *et al.* 2007:1	24 h.
50 pM	*Triticum aestivum*	Wheat roots	Jones & Kochian, 1995:1913	<2 h
0.21 ± 0.01 mg L^{-1}, 0.11 ± 0.03 mg. L^{-1}, and 0.18± 0.04 mg. L^{-1},	*Cabomba piauhyensis, Egeri adensa*, and *Hydrilla verticillata*	*Cabomba haynesii* Wiersema, Brazillian waterweed, and Water Thyme	Bakar *et al.* 2013:7	14 d

Plant roots directly remove metals from the soil solution and responses to metals are dose dependent (Silva, 2012:8). For essential metals, these responses cover the phases from deficiency through to sufficiency/tolerance to toxicity. For non-essential metals, only the tolerance and toxicity phases occur. The idea of critical or threshold toxicity is often used to establish the point at which metals cause significant growth decreases. These are often defined as the metal concentrations corresponding to a yield decrease of 10%.

Chenery (1955:174) first reported on the accumulation of unusually large quantities of Al (5000 to 16,000 mg.kg^{-1}) by tea plants. It was observed that the older tea leaves contained more aluminium than did young leaves (Chenery, 1955:174). From the foregoing it is apparent that a variety of plants is capable of removing heavy metals from the environment and accumulating these within various tissues and could function as phytoremediants. Therefore an aim of current study was to investigate the possible phytoremediation potential of native *Cyperus* species by investigating their ability to remove Fe and Al from the environment and to accumulate these metals in the roots and shoots.

Table 2.5: Iron concentrations in some plants species

Fe Conc	Plant scientific name	Plant common name	References	Exposure
0mM Fe; 20 mM Fe EDDHA; 3 mM Fe EDDHA + 10 mM NaHCO3	*Spinacia oleracea*	Spinach	Assimakopoulou, 2006:21	20 d

3.3 to 403 nM	*Crocosphaera watsonii*	filamentous cyanobacteria	Jacq *et al.* 2014:86749	Not specified
0 to 400 nmol.L^{-1}	*Oryza sativa*	Rice	Silveira *et al.* 2007:127	Not specified
Not specified	*Bacopa monnieri, Eichhornia crassipes, Hydrilla verticillata, Ipomoea aquatica* and *Marsilea minuta*	Water Hyssop, Water Hyacinth, Water Thyme, Water Spinach and Water Clover	Mishra & Tripathi 2008:7091	Not specified
	Arabidopsis spp.		Sun *et al.* 2010:347	

Table 2.6: Aluminium concentrations in tea plants in China

Part of plant	Concentration range (mg kg-1)	Reference
Fresh tea leaves	300-1600	Chen, 1984:1
Tea leaves	1510-3364	Fung & Wong, 2004:1469
Young leaves	370-1526	Xie *et al.* 2007:376
Tea leaves	2034-3322	Chen *et al.* 2009: 2350
Fresh tea leaves	1080-2020	Cao *et al.* 2010:2777

Despite physico-chemical conditions that favour limited metal mobility, some plants (Table 2.6) can exhibit elevated metal concentrations in the above-ground parts (Vandercasteele *et al.,* 2002:191). It should be borne in mind that the litter fall from these plants could recycle the stored metals into the food web (Mertens *et al.,* 2004:209) thus contributing to the quantity of such metals in the food chain. Therefore the consequences of high metal concentrations in vegetation in any ecosystem development should be carefully considered.

CHAPTER THREE

DESCRIPTION OF CYPERACEAE

3.1 Cyperaceae

Kingdom Plantae; Family: Cyperaceae; Order: Poales.

Table 3.1: General description of the family (Cyperaceae) and genus (*Cyperus*)

General description of the family: Cyperaceae

- Comprises approximately104 genera and more than 5 000 species world-wide.
- Approximately 90 *Cyperus* species occur in North America and in arid Southern Africa; there are estimated to be 40 genera and 80 species
- Cyperaceae (Sedges) resemble grasses, but most sedges lack stem nodes. All sedges have a closed sheath, if present. Most of the species in the family are dependent on wetlands.
- They are aquatic macrophytes, which have a cosmopolitan distribution.
- They are endemic to Africa, and display high levels of polymorphism and phenotypic plasticity in relation to variation of environmental factors (Bernez *et al.* 2004:43; Yin & Yin, 2010:429).

General description of the genus: *Cyperus*

- Large genus comprised of 600 species of sedges, distributed throughout all continents in both tropical and temperate regions except in Antarctica. There are approximately 100 species in South Africa.
- They are either annual or perennial plants. Most are aquatic and grow in still or slow-moving water which has a maximum depth of 0.5 m deep.
- The species vary markedly in size. Heights vary between 5 cm and 5 m.
- Common names include Basket Grass, Papyrus Sedges, Flat Sedges, Nut Sedges, Umbrella-Sedges and Galingales.

3.1.1 Cyperus: economic and environmental impacts

Cyperus spp are widely used in the reconstruction, creation and rehabilitation of wetlands, mainly to assist with water purification (Foukuo *et al.,* 2011:160; Erina & Wiyono, 2012:110; Ebrahimi *et al.,* 2013:5). They are used as test species in ecotoxicological studies in various countries around the world (Liao *et al.,* 2005:156; Almeida *et al.,* 2006:424; Madejón *et al.,* 2006a:1). Other uses for *Cyperus* include as a food source for humans, e.g. the starchy, protein-rich corms of tiger-nut or chufa (*Cyperus esculentus* var. *sativus*), for construction of boats and houses on Lake Titicaca, Peru (*Schoenoplectus californicus*), thatching, paper-making (*Cyperus papyrus*) and for weaving household items which include mats, baskets, beer-strainers and other utensils (Almeida *et al.,* 2006:424; Madejón *et al.,* 2006a:1; Liao *et al.,* 2005).

Shahi *et al.* (2013:379) cited the use of constructed wetlands to treat a variety of pollutants available in wastewaters including organic materials, detergents, nitrogen and phosphorus compounds, heavy metals, suspended solids and trace elements (copper, zinc, aluminium. They tested the efficiency of two plants viz. *Cyperus alternifolius* and *Phragmites australis* for their potential to decontaminate municipal wastewater. Results showed that *C. alternifolius* was suitable for this purpose. Wetlands represent one of the most efficient ways of reducing

the volume and high nutrient loads of piggery effluent (Liao, 2000:1). Liao (2000) conducted a study to screen for the most suitable plants for purifying this effluent. Vetiver grass and 11 other species, including *Cyperus* were selected. Of these only Vetiver and *C. alternifolius* were suitable. To date no similar studies have been done in South Africa. However, *Cyperus* occurs commonly along riverbanks in the Cape Peninsula and further north (Trinder-Smith, 2003:21). Thus it would be possible to conduct research into local species to determine their suitability for phytoremediation in polluted South African wetlands.

3.1.2 Cyperus

Cyperus alternifolius (Fig.1), *C. prolifer* (Fig. 2), and *C. textilis* (Fig. 3), belong to the family Cyperaceae and are important in the wetland environment. A general description of the differences among these three species is given in Table 3.2. These plants are endemic, have high nutrient assimilative capacity, grow rapidly, are desirable in contained systems and have a high pollutant removal capacity. A recent use for sedges is their cultivation in artificially constructed water purification beds. This is related to the ability of the rhizomes of several species to grow anaerobically, at least for a period of time. Thus, *Cyperus* has the potential to play an important part in waste treatment of wetlands where conditions may be anaerobic. To date Sedge species have been used in studies to determine the stress effects in wetlands caused by heavy metals including Cd, Cr, Cu, Pb, and Zn (Sekomo *et al.,* 2011), Cd, Zn, Pb, and Cu (Fonkou *et al.,* 2005:457); Al (Zheng *et al.,* 1999:1537; Rai, 2009:697).

Cyperus spp. are suitable for use as a vegetative buffer or wetland plant species due to the following morphological and physiological features (Cull *et al.,* 2000:407; Misra *et al.,* 2012:658):

- An ability to tolerate flooded soil conditions making them ideal for use in ephemeral or permanent wetlands.
- The dense stand of stiff, erect stems can reduce flow velocity, increase detention time and enhance deposition of sediment and sediment-bound contaminants (e.g. heavy metals and some pesticide residues).
- The dense, finely structured root system can improve bed stability and nutrient uptake, and provide an environment that stimulates microbiological processes in the rhizosphere.

Figure 3.1: ***Cyperus alternifolius***

Figure 3.2: ***Cyperus prolifer***

Table 3.2: Differences among *C. alternifolius*, *C. prolifer*, and *C. textilis*

Preferred Scientific Name	***C. alternifolius***	***C. prolifer***	***C. textilis***
Preferred Common Name	Umbrella Flat Sedge	Miniature Papyrus	Mat sedge, Umbrella sedge, Basket Grass.
Description	Stem is circular with slender grass-like leaves at the base of the plant. The flowers are greenish and wind pollinated. They are produced in clusters among the apical leaves. Usually makes a clump 2 m tall	Stem is circular with slender grass-like leaves at the base of the plant. The flowers are greenish and wind pollinated. They are produced in clusters among the apical leaves.	a more refined, non-invasive, darker green, more columnar harder and better behaved species.
Origin	Madagascar but cultivated worldwide, Native to East Africa.	Along east coast of Africa, from Kenya, Tanzania and Mozambique, through KwaZulu-Natal (KZN) and into the Eastern Cape at Mkambati Nature Reserve. It can also be found in Madagascar and in the Mascarene Islands. It grows in full sun, in freshwater swamps and along water courses, in wet mud or shallow water. Native to South Africa	Found in the southern part of South Africa, from Piketberg in Western Cape to southern KwaZulu-Natal, where it grows along river banks and streams, in pools, dams or marshes, in wet ravines and even in coastal wetlands and brackish estuaries
Biology	In some the stems are circular in cross-section and triangular in others; usually leafless for most of their length, with the slender grass-like leaves at the base of the plant, and in a whorl at the apex of the flowering stems. The flowers are greenish, and wind pollinated; they are produced in clusters among the apical leaves. The seed is a small nutlet.	Pollination is by wind, during which the mature fruits are released. Grows in water and or moist soil as well as in sun or shade as long as shade is adequate	The stems are circular in cross-section in some, and triangular in others. They are usually leafless for most of their length, with the slender grass-like leaves at the base of the plant, and in a whorl at the apex of the flowering stems. The flowers are greenish, and wind pollinated; they are produced in clusters among the apical leaves. The seed is a small nutlet.

Table 3.2 continued

Significance and environmental relevance	Can be invasive in moist soils. Several species eg. *Cyperus papyrus* is used in horticulture for waterside planting (Erina & Wiyono, 2012:110).	Water feature planting and basket making in Madagascar. *Cyperus papyrus* has been demonstrated as highly effective for treating domestic waste water in a constructed wetland (Erina & Wiyono, 2012:110).	Reed beds consisting of reeds, bulrushes and sedges like *C. textilis* are used all over the world to clean polluted water and factory effluents.
Likely Environmental impacts	Environmental impacts are likely to be small, but this will depend on growth rate and seeding habit if it establishes. The impact of *C. alternifolius* could occur in wetland marshes and other freshwater riparian habitats. It may jeopardize the natural state of the environments, and could displace native species, or even threaten local populations of rare or endangered plants	*Cyperus papyrus* has been used for domestic wastewater treatment and accumulates more nutrients from wastewaters than from their natural habitats (Agendia, 1995:1). It is a typical shallow water plant with thick rhizomes and well-developed roots. It may perform long-term absorption and accumulation of nutrients. The plant is suitable for growing indoors.	The plants absorb the excess nitrogen and phosphates from treated sewage, and have also proved to be effective in removing heavy metals and phenolic compounds from waste water (Kivaisi, 2001:545; Goetghebeur, 1998:141; Haines & Lye, 1983:404). Robust growth occurs on the water's edge and in boggy sites.

Figure 3.3: *Cyperus textilis*

3.2 Ecological and hydrological significance of the studied sedges

Cyperaceae are an important component present in many ecosystems. Cyperaceae constitutes a cosmopolitan family consisting of approximately 100 genera and 5 000 species which vary widely in appearance and natural history (Haines & Lye, 1983:404; Goetghebeur, 1998:141). *Cyperus* spp are generally tropical (Thompson, 1985:45). A modern usage for sedges is in artificially constructed water purification beds, because the rhizomes of several species are able to grow anaerobically, at least for a period of time. Several species are used in horticulture for waterside planting. They include *Cyperus papyrus,* various umbrella sedges (*C. involucratus, C. textilis*), and smaller species (e.g. *Carex*).

The importance of sedges in many ecosystems, the large number of species, their diversity in lifecycles, forms, and habitats, makes them of ecological importance as notable wetland species. Following recent surges in urbanization and burgeoning developments in developing countries, vast tracts of various habitats have been adversely affected (Ladwani *et al.,* 2012:73). Noticeable impacts of the increasing eutrophication and ecological degradation in urban water bodies are the widespread progressive decline of aquatic vegetation cover and associated changes in plant communities (Peng *et al.,* 2008:22). Recent research emphasises the rehabilitation of degraded urban waters in which the recovery of aquatic vegetation is an important component (Dai *et al.,* 2012:169). The aim of ecological rehabilitation is to restore the original components and function of the ecosystems. This would include macrophytes, which are characteristic features of shallow aquatic systems (Janauer, 2006:19).

Hydrological change and exploitation of wetlands by humans are widespread in the papyrus-dominated wetlands. Studies show that *C. papyrus* often dominates the inner and wetter zones of the wetland while other *Cyperus* spp and *Cynodon dactylon* commonly occur on the periphery often in disturbed areas (Kipkemboi *et al.,* 2006:75). There is little published information concerning the response of Papyrus wetland plant communities to changes in water depth and human exploitation of this environment. A better understanding of the natural and human influences on plant communities is important for the formulation and operation of sustainable management strategies for wetlands.

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Experimental design

Experimental design was artificial, but attempted to mimic conditions similar to those associated with metal toxicity in a wetland. Therefore the pH was maintained at ≤ 4 to ensure that Al was mobilised (Sun & Wu, 1998:255; Schier & McQuattie, 2000:637; U.S. EPA. 2008). The pH was adjusted by using 20 ml (55%) HCl. All experiments were carried out at the Cape Peninsula University of Technology Horticultural Unit Research glasshouse complex, Cape Town campus, South Africa. The experimental set up is shown in Fig. 4.1.

Figure 4.1: Experimental set up, Cape Peninsula University of Technology Horticultural Unit Research glasshouse complex, Cape Town, South Africa

4.1.1 Species selection for experimental studies

It was important to select the appropriate plant species and varieties. Selected plants were to be capable of achieving the desired treatment objective, and to be adapted under greenhouse conditions to the irrigation water and the nutrients used. Any factor that compromises plant vigour will reduce performance. Three *Cyperus* spp were selected as they comprise specific assemblages of organisms that should be protected as they provide important ecological functions. They are characterised by a rapid growth rate, resistance to local pests and diseases and their ability to act as indicators of induced metal stresses. Plants were obtained from commercial horticultural enterprises located close to the experimental site (Cape Peninsula University of Technology, Cape Town Horticulture glasshouse) as endemic species should display resistance to local pests and diseases (Jabeen *et al.,* 2009:339). Plants investigated were *Cyperus alternifolius, Cyperus prolifer* and *Cyperus textilis.* These three species are versatile and hardy, produce a large biomass, grow rapidly have an extensive root system and occur in diverse ecological niches.

4.1.2 Plantation

Sand suitable for hydroponic culture viz. Consol filter sand, Grade 14/30 was used. The sand was purchased from Consol Sand located at the Athlone Industrial Estate 2, Cape Town. The sand was prepared for the experiments by thoroughly rinsing with water and leaching with a mixture of hot 17 % hydrochloric acid and 1 % oxalic acid for 12 h (Hewitt, 1952). The sand was then drained. Seedlings of *C. alternifolius, C. prolifer* and *C. textilis* were obtained from stock plants from a commercial producer. The specimens were removed from the containers in which they were supplied and the root systems were meticulously washed with deionised water. The wet weight (g) of each plant at the commencement of the experiment was recorded. The specimens were then placed into the prepared sand media and cultivated using defined hydroponic solutions and conditions.

4.1.3 Glasshouse set up

Tests to evaluate the effect of metal toxicity on *Cyperus* growth were done under glasshouse conditions at the Cape Peninsula University of Technology, Cape Town. All treatments were randomly placed on a designated bench area in the glasshouse and the experimental plants were moved once a week during the growth period of five months (May to September). *Cyperus* was cultivated under ambient light (750 J.lmol m^{-2} S^{-l}) and temperature conditions of 22-25°C to minimize bench effects (Fig.4.1).

Pot culture experiments were performed on ten healthy seedlings of *C. alternifolius, C. prolifer* and *C. textilis.* A randomized sampling system was used throughout this study. All the observations regarding growth (height, weight) and physiological parameters (photosynthesis and chlorophyll) of plants were taken weekly.

4.1.4 Experimental set up

Ideally, at the commencement of the experiments all plant species should be at the same size and growth stage in order to make valid comparisons. Wetland plant species, however, differ substantially in their growth rate, morphology, physiology, and size. Therefore prior to the tests conducted to determine the effects of and tolerance to Fe and Al; C*. alternifolius, C. prolifer* and *C. textilis* were cultivated for four weeks in nutrient solutions for acclimatization purposes. During this period, $\{Al_2(SO_4)_3\}$ and Fe(Na)EDTA were applied at a concentration of 0.1 mmol.L^{-1} and the pH of the solution was adjusted to pH≤4 using 10 ml of NaOH and/or 20 ml (55%) HCl. The study was conducted under hydroponic conditions using black polyethylene pots (20-cm diameter, 1L). After first establishing the plants for two weeks, they were placed in pots with appropriate nutrient solutions. All plants were hand-cropped and rinsed carefully with deionized water to eliminate adhering soil, mud and other debris, and to discard decay and dead parts.

The Al ($AlSO_4.7H_2O$) in four varied concentrations (0.001, 0.01, 0.1, and 1 µM) or Fe ($FeSO_4.7H_2O$) in four different concentrations (5, 10, 15 and 20 µM)-amended nutrient solutions were then added to each pot in the hydroponic set up. There were twelve replicates for each Al or Fe treatment for three different plant populations and appropriate controls. The total number of treatments was 120, and these were organised in a randomized design/manner. During the course of the study, the temperature in the glasshouse was maintained at 22-25°C to minimize bench effects. The nutrient solutions in the growth containers were continuously aerated with pumps. Control samples were not treated with any metals.

4.1.5 Decontamination of plant species

Generally, Al and Fe are among the few elements most affected by soil and dust particles. During the experiments all plant materials had to be free of extraneous contamination by

removing soil and dust particles as well as any foliar spray residues that would influence analytical results (Plank, 1992:1).

This was done by washing fresh, fully-turgid plants samples with deionised water. The decontamination was thorough but at the same time preserved the integrity of sample.

4.2 Data collection and analysis

Once a week, following daily application of metal-enriched nutrient to the plants in the hydroponic system where they were dosed intermittently by a drip system data were collected and recorded. However, data collection commenced only from the second week of dosage application.

These data were used to analyse morphological indicators of metal toxicity viz. stem elongation and stem height (cm) by using a meter ruler. A digital measuring balance, XB 220A Precisa was used to record sample mass (g) (Fig. 4.2).

4.3 Plant species and sampling

Plantlets of *Cyperus* were regenerated from stem nodal embryogenic calli using the methods of Máthè *et al.* (2000:81). For experimental studies, healthy seedlings of *C. alternifolius, C. prolifer and C. textilis* were randomly selected from the stock plants. These were to be passive biomonitors for estimating the toxicity status induced by Fe and Al. Care was taken to remove each plant from the existing containers. This was done by hand, using gloves, and thereafter seedlings were meticulously washed in deionised water to remove periphyton and sediment particles. These were maintained for four weeks in the glass house to acclimatize. The wet weights of each plant were recorded weekly. Seedling samples were transplanted into prepared sand media and nutrient solution.

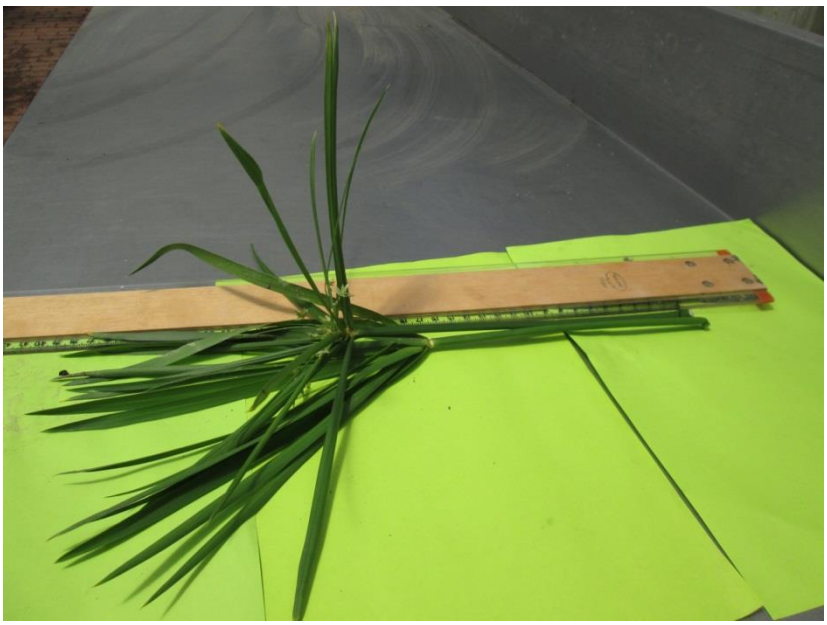

Figure 4.2: Data collection – measuring the stem length

4.3.1 Plant material and growth conditions

To quantify the Al and Fe concentration that induced toxic effects on *C. alternifolius, C. prolifer* and *C. textilis*, the plant material was grown in a controlled hydroponic environment. The application of the metals was graded, gradually increasing in concentration. The experiment was designed such that *Cyperus* plants were exposed to concentrations of Al or Fe in quantities sufficient to cause a toxic response in the plant. The media used was sand culture (coarse sand size particles).

4.4 Hydroponic growth experiments designed to detect metal tolerance

4.4.1 Preparation of hydroponic solutions

A hydroponic nutrient solution was constituted by adding 50% dilute Hoagland's stock solution (Hoagland & Arnon, 1950:1) to 100 L tap water. The pH was adjusted to 4 using 10 ml of NaOH and/or 20 ml (55%) HCl.

4.4.2 Hydroponic experiments to detect metal uptake, accumulation and tolerance

Uniform seedlings were selected and transplanted into 15 cm (length x width x height) lightproof plastic containers. *C. alternifolius*, *C. prolifer* and *C. textilis* were placed into a prepared sand culture and hydroponic solution, carefully labelled and placed on the hydroponic bench set up for a continuous flow drip system (Fig. 4.3.). Polythene tools were used to collect

and store the matrices to avoid any metal contamination. Plant species were identified according to the methods of Simpson and Ingles (2000:257).

Plantlets with two or three primary roots were exposed to Al supplied as ($AlSO_4.7H_2O$) in four varied concentrations of (0.001, 0.01, 0.1, and 1 µM). All plants were cultured in individual containers (replicated x12) for each Al treatment (60 in total). Plants in 10% Hoagland's solution without Al served as controls. The plants were placed in randomised arrangement. The experiments were performed under standard physiological controlled temperature and light conditions in a greenhouse. The day and night regime used was 16h/28 °C and 8h/22 °C. One set of the plants was harvested after 24 h treatment and the remaining four sets were harvested after 48 h., 72 h., 5 d and 7 d. After harvesting, the plants were used for the determination of various bio-chemical parameters as described below.

Plantlets were subjected to Fe supplied as $FeSO_4$ in different concentrations (5, 10, 15 and 20 µM). It was ensured that plant samples of relative uniform biomass (fresh weight) were used for consistency for both the control sets and treated pots. All plants were cultured in individual containers (replicated x12) for each treatment of Fe (60 in total). Plants in 10% Hoagland's solution without Fe served as controls. The plants were placed in a randomised arrangement. The experiments were performed under standard physiological controlled temperature and light conditions in a greenhouse. The day and night regime used was 16h/28 °C and 8h/22 °C. One set of the plants was harvested after 24 h treatment and the remaining four sets were harvested after 48 h., 72 h., 5 d and 7 d. After harvesting, the plants were used for the determination of various bio-chemical parameters as described below.

4.4.3 Chemical analysis

At the completion of each experimental cycle, all the *Cyperus* were harvested, and separated into above- and below-.ground material. These samples were then dried for 24 h at 60 °C. The total dried weight was subsequently determined.

The total Fe content in the different plants samples was determined using methods described by Odendaal and Reinecke (1999:64). For each plant, separated shoot and roots were washed with deionised water. They were dried in an oven as described above, homogenised and then stored for further analysis. Approximately 1 g of each sample was weighed out, labelled and recorded.

To determine the Al or Fe content, the samples were digested by adding 10 ml 55% HNO_3 to each sample (1 g) in a test tube. This was then stirred using a glass rod. The mixtures were heated on a universal block dryer (UBD) heater in a fume cupboard at 40 °C for 1 h followed by 120 °C for 3 h. The samples were removed and cooled to room temperature. Each of the cooled solutions was made up to 20 ml with distilled water and filtered using 0.45 µm cellulose nitrate filter paper. The filtrates obtained were further diluted to a final volume of 100 ml with distilled water. The Al or Fe content was estimated by using a Perkin-Elmer (Analyst Model 300) atomic absorption spectrophotometer equipped with an air-acetylene burner. The Al or Fe content was expressed as mg g^{-1} (DW) of the sample.

4.5 Growth observations

Several parameters were measured to assess Al and Fe tolerance in the different species. Measurements of leaf and root lengths were carried out weekly. Dry weight measurements of shoots and roots were taken when plants were harvested by using an analytical balance. A weekly count of the number of new leaves emerging from the plants was done. Lengths of all leaves were measured from the initial point of growth of the leaf to the tip. The lengths of all leaves were expressed as a total summation of individual leaf lengths following the recommendations of McCabe & Otte, (2000:548) and McCabe *et al.* (2001:141).

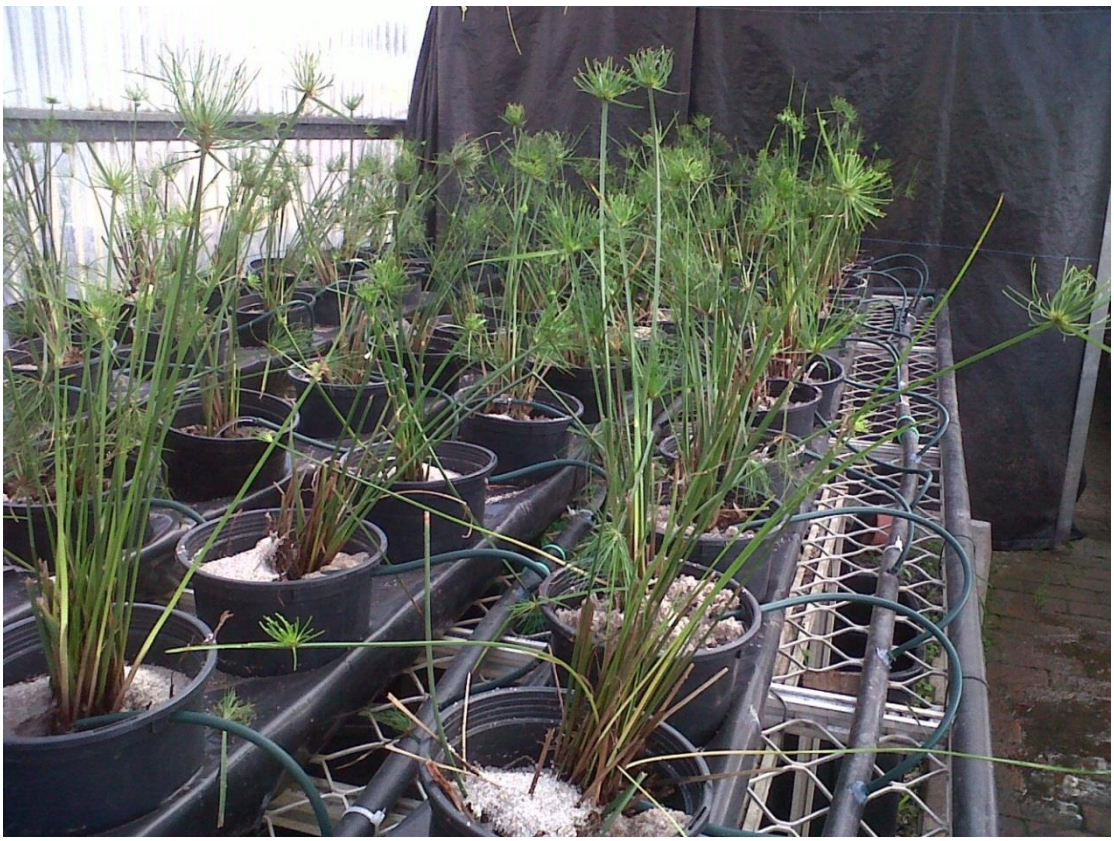

Figure 4.3: Experimental set up in the glasshouse at CPUT, Cape Town

4.6 Photosynthesis

Concerns are being expressed about the long term effects of the increasing concentration of CO_2 in the global atmosphere. This has necessitated questions about CO_2 effects on wetland plants. Global atmospheric enrichment will affect wetland plants both directly and indirectly through global warming. The direct physiological effects of elevated CO_2 on photosynthesis, growth are well documented (Mihailovic *et al.,* 2012:1). Like any other physiological processes, photosynthesis differs greatly among various wetland plant species, particularly between C_3 and C_4 plants, whether growing under normal or under stressed conditions (Pessarakli, 2005:152). Plants with the C_3 photosynthetic pathways are expected to benefit more than C_4 photosynthetic pathways (Edwards *et al.,* 2010:587). Developing wetland plants with enhanced photosynthesis will improve yield and make efficient use of resources in a sustainable manner (Karki *et al.,* 2013:28).

Photosynthesis is the bonding together of CO_2 with H_2O to make CH_2O and O_2 using the sun's energy. The carbohydrate contains stored energy and serves as the raw material from which other compounds are made. Measurements of photosynthesis are important for comparing

and understanding productivity (biomass accumulation) of vegetal systems at the leaf, plant or community level as well as their response to environmental stresses such as metals. Gaseous exchange (CO_2 and H_2O as vapour) by leaves constitutes the basis for the design of most photosynthesis measurements. Since CO_2 intake and H_2O release share the same biochemical pathway, photosynthesis measurements commonly include the estimation of photosynthesis itself (assimilation or CO_2 uptake), stomatal conductance and transpiration (Field *et al.,* 1989:1)

4.6.1 Description of the infra-red gas analysis sensors

Infrared sensors for gas analysis (IRGA) are commonly used for CO_2 measurement and are based on an infrared emitter-photo-detector whose light beam is used to measure the concentration of gas molecules in the atmosphere. This is based on the absorption phenomenon of the light beam by molecules in a gaseous state (Hunt, 2003:314). This phenomenon of absorption occurs because the hetero-atomic gas molecules with odd number of atoms such as CO_2, CH_4, NH_3, to name a few, absorb a portion of the infrared light while the homo-atomic gas molecules such as N_2 and O_2 do not. The CO_2 has a maximum detection at a wavelength of 4.25 $^{-m}$, with peaks of 2.66, 2.77 and 14.99 $^{-m}$ (Hill & Powell, 1968:1).

The calibration of these sensors to zero requires an atmosphere free of CO_2 and other hetero-atomic gases; therefore N_2 is most often used. Also, the adjustment requires a range of known concentrations of CO_2 to be carried out with precision pumps (Hunt, 2003:314).

4.6.2 Determination of photosynthetic pigments

Heavy metals can affect each photosynthetic component at different levels thereby creating changes in certain physiological processes and not in others (Vassilev & Yordanov, 1997:114; Li *et al.,* 2009:1). Krupa and Baszynki, (1995:177) stated that most of the observed physiological disturbances in metal-exposed plants may be focussed on photosynthesic performance. Samples (leaves and/or stems) removed after 24, 48 and 72 h and 3 d and 5 d after metal dose treatment were examined for photosynthetic pigment content.

An infrared gas analyser (IRGA), type G, Harmann and Braun, Siemens, Munich, Germany), accurate to within 1± 0% of full scale (5000 mol $^{mol^{-}}$) was used to measure CO_2 concentration. Measurements were taken using the infra-red gas analyser to read parameters *A*, (photosynthesis); *G*s, (stomata conductance); *C*i, (intercellular carbon dioxide concentration and and *E* (rate of evapo-transipiration) in randomly selected four young leaves (flag leaves) removed from all three *Cyperus* spp from all treatments as well as an untreated control set.

This was done by using a portable infra-red red gas analyser (LCpro+ 1.0 ADC, Bioscientific Ltd., Hoddesdon, and Hertfordshire, UK).

Photosynthetic parameters in the intact plant leaves were assessed in the morning between (08h00 am and 11h00 am) as well as 14h00 pm and 16h00, according to the manufacturer's instructions. Leaves were allowed 4-5 min to acclimatise to the light environment in the leaf chamber. Without troubleshooting, the photosynthetic leaf recording only occurs after about 2 minutes of being kept in the leaf chamber, which was minimum time allowed for the readings to stabilize (Hamid *et al.,* 1990). The carbon dioxide assimilation (C_1), stomata conductance (Gs). Intercellular carbon dioxide concentration (*C*i) and rate of evapo-transipiration *(E)* measurements were taken using the following conditions in the leaf chamber: photosynthetic photon flux density (PPFD) = 1100 µmol (quantum) m–2 $^{s–1}$, relative humidity = 44%, leaf vapour pressure deficit = 1.83 kPa, flow rate = 400 µmol $^{s–1}$, reference CO_2 = 400 ppm, and leaf temperature = 25^{o}C (Hamid *et al.,* 1990).

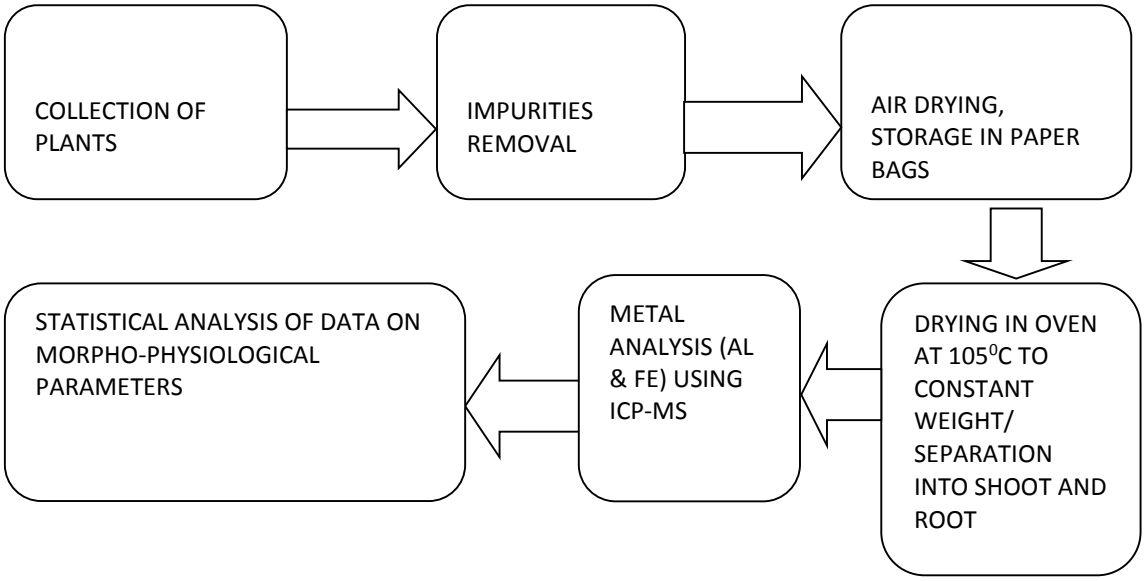

Figure 4.4: Summary of consecutive steps for measurement and analysis used in the study

4.6.3 Determination of pigment content

Chlorophyll content was analysed using a portable version of an imaging-PAM chlorophyll fluorometer (CCM-200) OPTI-SCIENCES, Hudson, connected to a computer with data acquisition software. Chlorophyll has several distinct optical absorbance characteristics that the CCM-200 exploits in order to determine relative chlorophyll concentration.

4.7 Statistical analysis

The parameters were evaluated from a minimum of three replicates. Statistical analyses were carried out using SPSS (SPSS Chicago IL, USA). Differences among treatments for chemical variables, plant biomass and plant nutrient composition were tested with a univariate ANOVA using Turkey's post–hoc test. Mean values for treatments and respective standard deviations were calculated where a value of $P<0.05$ was considered significant. Statistically, significance differences were determined with an appropriate t-test.

CHAPTER FIVE

RESULTS AND DISCUSSION (PART 1)

5.1. Macrophyte biomass response to metal exposure

Plants rely on a range of transition metals as essential micronutrients for normal growth and development (Becker, 2005:1; Umebese & Motajo, 2008:197; Brankovic *et al.,* 2011:11956). Plants require adequate amounts of a micronutrient such as Fe. However, Al has no known biological function and the absorbance and/or accumulation of an excess of non-essential metals can be harmful.

Arthur and Coat (1998:1) reported phytoremediation to be a strategy worth considering for the treatment of contaminated environments. For the phytoremediation of aquatic environments, suitable indigenous wetland plants would include those that are capable of growth and reproduction under conditions of environmental stresses such as excessive metal concentrations (Yoon *et al.,* 2006:456). According to Foukuo *et al.* (2011:160), the selected plants should exhibit a rapid rate of increase in biomass thus enhancing remediation of large amounts of metals in the site. The three *Cyperus* spp studied are commonly found in wetlands and along roadside and agricultural ditches (Polprassert, 2007:1).

The response of individual plant species to chemical stresses such as in phenological behaviour, phytomass production and physiologically is ecologically significant. For this study parameters studied include the actual measurement of the lengths of roots and shoots, the weight of roots and shoots recorded before seedlings were put into treatment of varied concentrations of metals and after treatment respectively which were found to increase over the treatment periods.

The evaluation of heavy metals by their total concentrations in the plants has shown that the amount of metals within the plant is seldom related to their bioavailability (Ren, 2000:68). Variability has been reported in every morphological, biochemical and physiological parameters study and suggest that variability would play a significant role in proper selection of plant species for phytoremediation. During the current study, the total biomass of the three *Cyperus* spp (sum of roots plus shoots) yield per experimental pot increased significantly ($P \leq 0.05$) over the course of the experimental period. Studies by Long *et al.* (2006:315) and Zhu *et al.* (2010:235) showed that the maximum potential biomass produced by a plant is determined by the following five variables: The:

(a) Amount of incident solar radiation available over the growing season of a plant;

(b) Light interception efficiency, that is, the efficiency of the photosynthetic pigments to intercept photosynthetic active radiation;

(c) Energy conversion efficiency, that is, the ratio of the biomass energy produced over a given period relative to the radioactive energy intercepted by the canopy of the plant over the same period;

(d) Translocation of photosynthates to sinks as determined by sink strength; and

(e) Partitioning efficiency that is, the amount of total biomass energy partitioned into seed production per unit ground area, also known as harvest index (HI).

The biomass recorded for the three pre-conditioned hydroponically-grown *Cyperus* spp studied, in response to treatment by either Al or Fe applied in four different concentrations are shown in Tables and Figures 5.1-5.10. Mean values were calculated by standard deviations. In all the data presented, the control water-sprayed samples (0) were plants which had not been exposed to either Fe or Al. Results showed that all three *Cyperus* species demonstrated rapid growth rates. This satisfied one of the requirements of a satisfactory phytoremediation candidate. Further studies are necessary to evaluate the long term changes in response to Al and Fe resistance mechanisms - these were beyond the scope of the present investigation.

Table 5.1: Mean weight and lengths of roots and shoots before and after Al treatment recorded for *Cyperus* spp after day 1 (24 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	after	before	after	before	After	Before	after
	0	4.7	4.7	9.4	9.4	60	60	13.3	13.3
	0.001	6.8	6.8	10.0	10.1	72	74	12.6	12.8
	0.01	6.4	6.7	7.2	7.3	65	68	11.4	11.6
Cyperus	0.1	5.7	6.0	7.0	7.9	68	69	12.7	12.9
alternifolius	1	5.5	5.7	7.1	7.4	69	70	14.5	14.8
	0	1.7	1.7	4.4	4.4	8.0	8.0	8.7	8.7
	0.001	3.1	3.2	4.3	4.4	10.2	10.3	9.0	9.1
	0.01	2.5	2.7	5.4	5.5	14.0	14.1	5.4	5.5
Cyperus	0.1	1.3	1.7	4.5	4.7	17.1	17.2	14.6	14.7
prolifer	1	0.5	0.7	5.7	5.8	18.5	18.6	15.2	15.3
	0	1.7	1.7	2.0	2.0	22	22	4.9	4.9
	0.001	3.1	3.2	2.5	2.7	30	32	6.1	6.3
Cyperus	0.01	2.4	2.5	0.5	0.8	24	25	5.3	5.5
textilis	0.1	1.3	1.3	1.5	1.2	35	36	5.4	5.7
	1	0.7	0.7	1.3	1.4	28	29	6.1	6.3

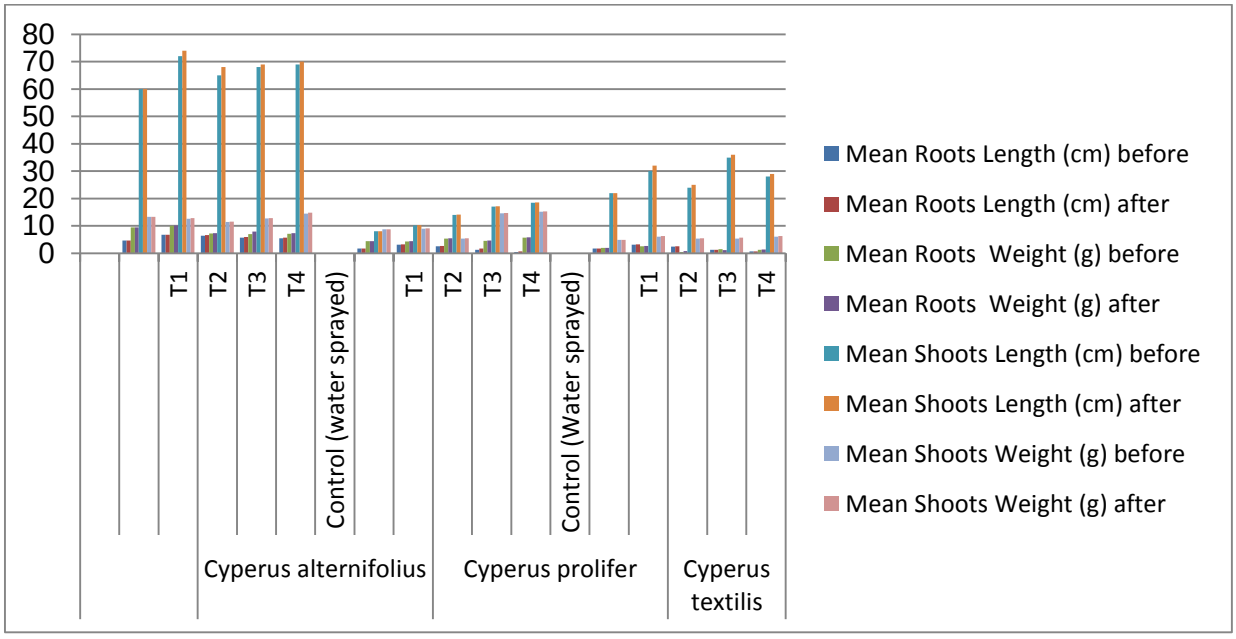

Figure 5.1: Mean weight and lengths of roots and shoots before and after Al treatment in *Cyperus* after day 1 (24 h)

NOTE

T1= first metal dosage treatment
T2= second metal dosage treatment
T3= third metal dosage treatment
T4= fourth metal dosage treatment

Results showed that after 24 h exposure to Al there was no significant difference in the total fresh weight of either roots or shoots. Although there was a general small increase in the fresh weight of roots and shoots after 24 h exposure to Al, this was not significant (P=0.05). No significant changes in fresh weight partitioning from shoot to root, or root to shoot in response to Al were observed.

Table 5.2: Mean weights and lengths of *Cyperus* roots and shoots before and after exposure to Al treatment on day 2 (48 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	after	before	after	before	after	before	after
	0	4.7	4.7	9.4	9.4	67.3	67.3	13.4	13.4
	0.001	6.8	6.9	9.1	9.2	70.2	72.2	22.6	22.7
Cyperus alternifolius	0.01	6.4	6.7	7.2	7.2	66.6	67.2	21.8	21.8
	0.1	5.7	6.0	7.0	7.1	65.3	66.1	17.7	17.8
	1	5.5	5.7	7.1	7.2	65.1	66.3	12.5	12.6
Cyperus prolifer	0	1.7	1.7	4.4	4.4	8.0	8.0	8.7	8.7
	0.001	3.1	3.2	4.3	4.3	10.2	10.3	9.3	9.3
	0.01	2.3	2.5	5.4	5.5	14.0	14.1	5.4	5.5
	0.1	1.3	1.4	4.6	4.7	17.1	17.2	14.6	14.7
	1.0	0.5	0.6	5.2	5.3	18.5	18.6	15.2	15.3
Cyperus textilis	0	1.7	1.7	2.1	2.1	22	22	4.9	4.9
	0.001	3.1	3.2	2.5	2.7	20	20.4	3.1	3.3
	0.01	2.3	2.4	0.8	0.9	24	24.8	4.3	4.5
	0.1	1.5	1.7	1.2	1.5	25	25.6	5.1	5.1
	1	0.5	0.7	1.3	1.4	28	28.7	3.7	3.7

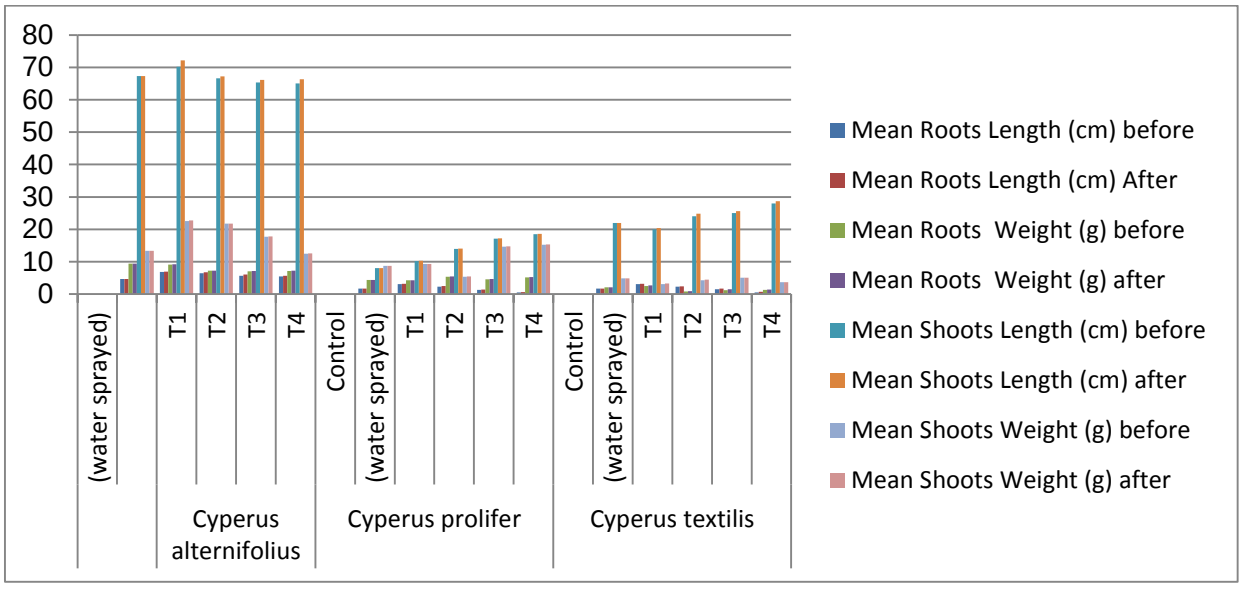

Figure 5.2: Mean weight and mean lengths of roots and shoots recorded for *Cyperus* before and after Al treatment day 2 (48 h)

Table 5.3: Mean weight and mean lengths of *Cyperus* roots and shoots before and after Al at day 3 (72 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	after	before	after	before	after	before	after
Cyperus alternifolius	0	4.7	4.7	9.4	9.4	60	60	13.3	13.3
	0.001	6.8	6.8	10.0	10.1	62	63	12.2	12.4
	0.01	6.4	6.7	7.2	7.3	65	67	11.4	11.8
	0.1	5.7	6.0	7.0	7.2	68	69	11.7	11.9
	1	5.5	5.7	7.1	7.4	69	71	12.5	12.6
	0	1.0	1.7	4.4	4.4	8.0	8.0	8.7	8.7
Cyperus prolifer	0.001	3.1	3.2	4.3	4.3	10.2	10.3	9.3	9.7
	0.01	2.5	2.9	5.4	5.5	14.0	14.1	5.4	5.5
	0.1	1.3	1.7	4.6	4.7	17.1	17.2	14.6	14.7
	1	0.6	0.7	5.2	5.3	18.5	18.6	15.7	15.9
	0	1.7	1.7	2.8	2.8	22	22	4.9	4.9
	0.001	3.0	3.2	2.5	2.7	30	30.4	6.1	6.3
Cyperus textilis	0.01	2.3	2.1	0.8	0.9	24	24.7	5.2	5.5
	0.1	1.3	1.7	1.2	1.5	35	35.6	5.4	5.7
	1	0.5	0.7	1.3	1.4	28	28.5	6.7	7.0

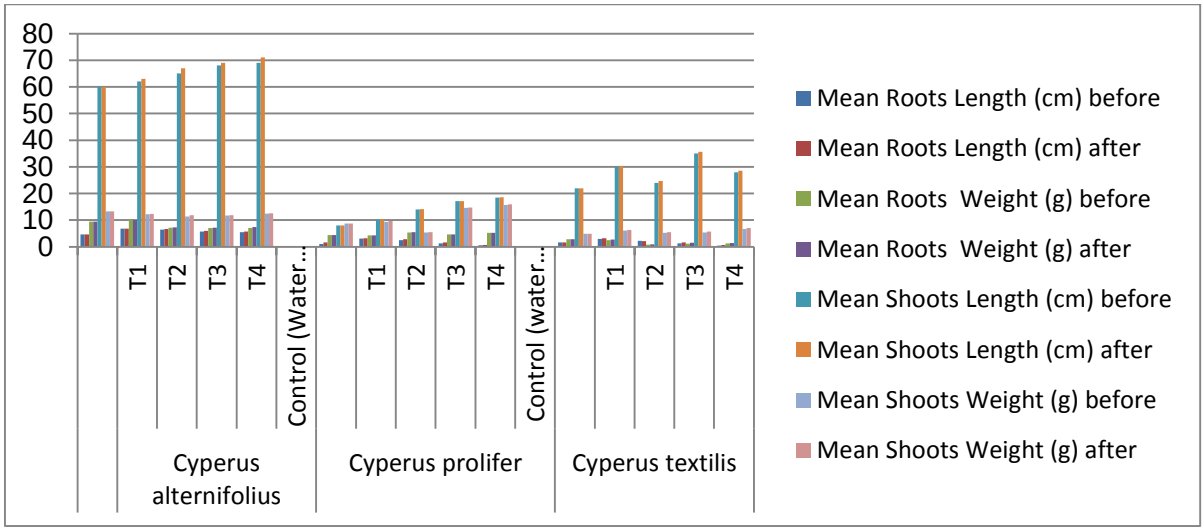

Figure 5.3: Mean weights and lengths of *Cyperus* roots and shoots before and after Al treatment at day 3 (72 h)

After 72 h (Fig. 5.3) increasing elongation in both roots and shoots was observed in *C. alternifolius* and *C. textilis* when compared with *C. prolifer.* The difference noted for *C. alternifolius* was significant ($P<0.05$).

Table 5.4: Mean weight and lengths of roots and shoots in *Cyperus* before and after Al treatment by day 5 (120 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	after	before	after	before	after	before	after
	0	4.7	4.7	9.4	9.4	60	60	13.3	13.3
	0.001	6.8	6.8	10.0	10.1	62	63	12.2	12.6
Cyperus alternifolius	0.01	6.4	6.7	7.2	7.23	65	66	11.4	11.6
	0.1	5.7	6.0	7.0	7.09	68	69	11.7	11.9
	1	5.5	5.7	7.1	7.14	70	72	12.0	12.6
Cyperus prolifer	0	1.7	1.7	4.4	4.4	80	82	8.7	8.7
	0.001	3.1	3.2	4.3	4.7	10.2	10.3	9.3	9.3
	0.01	2.5	2.9	5.8	6.0	14.0	14.5	5.4	5.5
	0.1	1.3	1.7	4.5	4.7	17.1	17.7	14.6	14.7
	1	1.5	1.7	5.7	5.8	18.5	18.9	15.7	16.0
	0	1.6	1.6	2.8	2.8	22	22	4.5	4.5
	0.001	3.0	3.2	5.5	5.7	30	30.4	6.1	6.3
	0.01	2.5	2.9	4.0	4.9	24	24.8	5.2	5.5
Cyperus textilis	0.1	1.5	1.7	2.8	3.0	35	35.4	5.4	5.7
	1	0.5	0.7	1.3	1.4	28	28.7	6.7	6.7

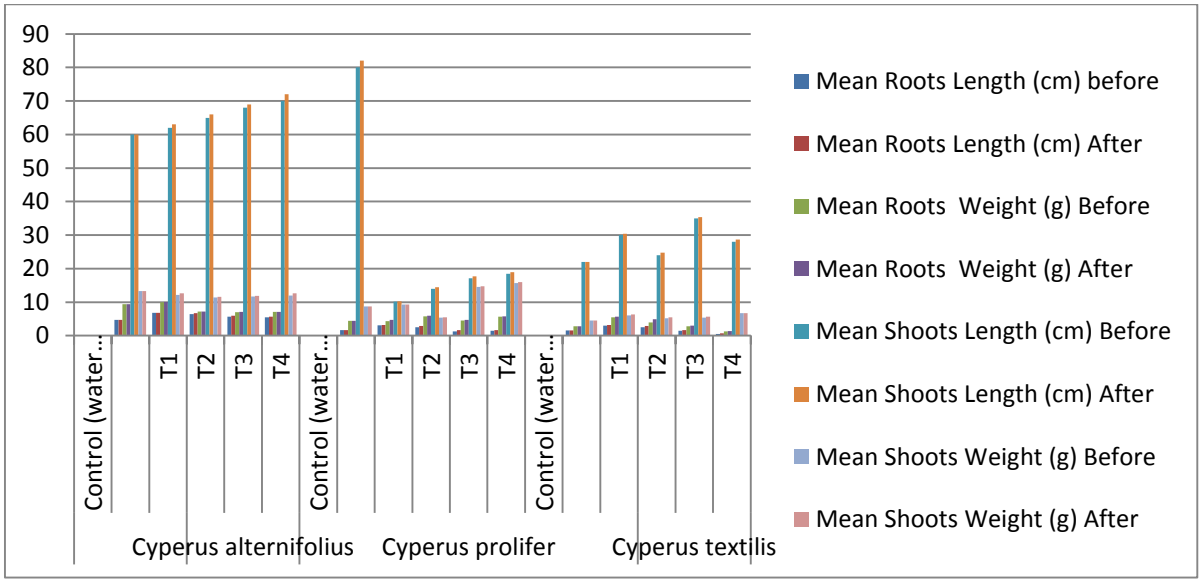

Figure 5.4: Mean weight and lengths of roots and shoots in *Cyperus* before and after Al treatment by day 5 (120 h)

The same trend noted after 72 h for *C. alternifolius* and *C. textilis* was also observed after 120 h exposure to Al. However, the only significant difference was recorded for *C. alternifolius* ($P<0.05$).

Table 5.5: Mean weight and lengths of roots and shoots of *Cyperus* before and after Al treatment at day 7 (168 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
Cyperus		**before**	**after**	**before**	**after**	**before**	**after**	**before**	**after**
alternifolius		4.7	4.7	9.4	9.4	60	61	13.3	13.3
	0								
	0.001	6.8	6.8	10.0	10.2	72	73	12.2	12.4
	0.01	6.4	6.7	7.2	7.3	65	65	11.4	11.6
	0.1	5.7	6.0	7.0	7.2	78	79	11.7	11.9
	1	5.5	5.7	7.1	7.4	69	70	12.3	12.6
Cyperus	0	2.7	2.7	4.4	4.4	8.0	8.0	8.7	8.7
prolifer	0.001	3.1	3.2	4.0	4.3	10.2	10.3	9.0	9.4
	0.01	2.5	2.7	5.8	6.0	14.0	14.5	5.4	5.5
	0.1	2.3	2.5	4.6	4.7	17.1	17.4	14.6	14.9
	1	0.5	0.6	5.7	5.8	18.5	18.8	15.2	15.7
Cyperus	0	1.7	1.7	2.1	2.1	2.2	22	4.9	4.9
textilis	0.001	2.1	2.2	2.9	3.1	3.0	30.4	6.1	6.3
	0.01	2.3	2.6	0.8	0.9	24	24.9	5.3	5.5
	0.1	1.3	1.6	1.3	1.5	35	35.5	5.4	5.7
	1	0.5	0.7	1.3	1.4	28	28.4	6.1	6.3

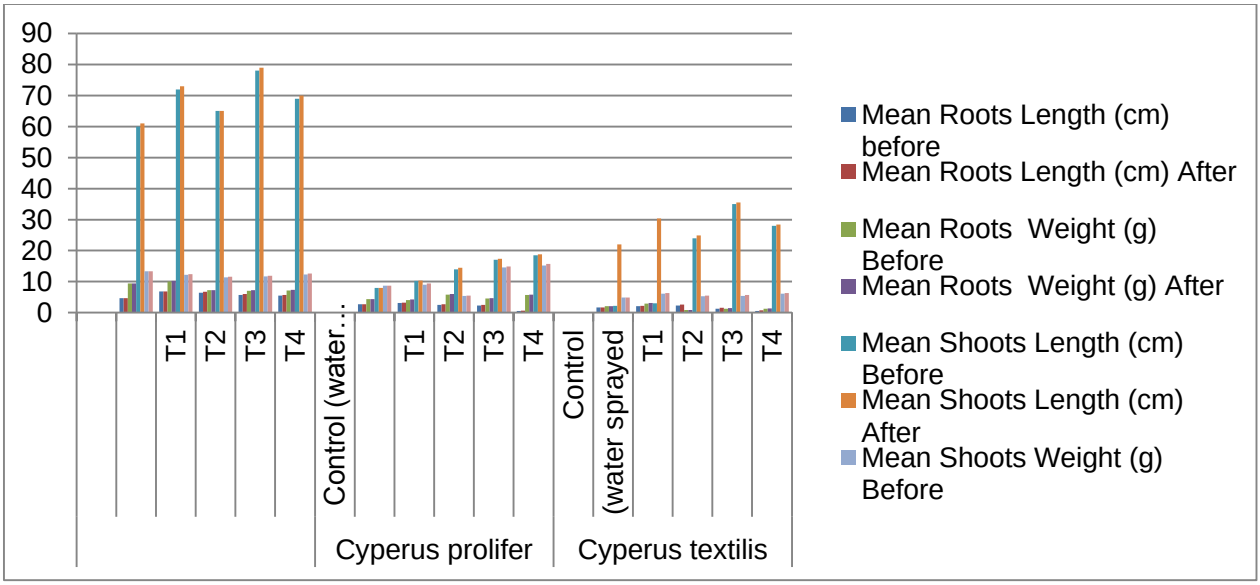

Figure 5.5: Mean weight and lengths of *Cyperus* roots and shoots before and after Al treatment by day 7 (168 h)

Results showed that the root length of *Cyperus* was influenced more by Al concentration than by exposure time. The two species that showed obvious increasing elongation in both their roots and shoots were *C. alternifolius* and *C. textilis* but only differences for *C. alternifolius* were significant ($P<0.05$).

Table 5.6: Mean weight and lengths of roots and shoots in *Cyperus* before and after Fe treatment after day 1 (24 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Roots Weight (g)		Shoots Length (cm)		Shoots Weight (g)	
		before	after	before	after	before	after	before	after
	0	4.7	4.7	9.4	9.4	60	60	13.3	13.3
	5	6.8	6.8	10.0	10.1	62	63	12.2	12.6
	10	6.4	6.7	7.2	7.3	65	66	11.8	11.9
Cyperus	15	5.7	6.0	7.0	7.3	68	69	11.7	11.7
alternifolius	20	5.5	5.7	7.1	7.4	69	70	12.5	12.6
	0	1.7	1.7	4.4	4.4	8.0	8.0	8.7	8.7
	5	3.0	3.2	4.3	4.3	10.2	10.3	9.3	9.3
	10	2.9	3.3.	5.4	5.5	14.0	14.1	5.4	5.5
Cyperus	15	1.5	1.7	4.6	4.7	17.1	17.2	14.6	14.7
prolifer	20	0.5	0.7	5.2	5.3	18.5	18.6	15.7	15.8
	0	1.7	1.7	2.0	2.0	22	22	4.5	4.5
	5	3.1	3.2	2.1	2.7	3.0	3.4	6.1	6.3
	10	2.3	2.4	0.8	0.9	2.4	2.5	5.3	5.5
Cyperus	15	1.5	1.7	1.2	1.5	3.5	3.9	5.4	5.7
textilis	20	0.5	0.7	1.3	1.4	2.6	2.6	6.7	7.1

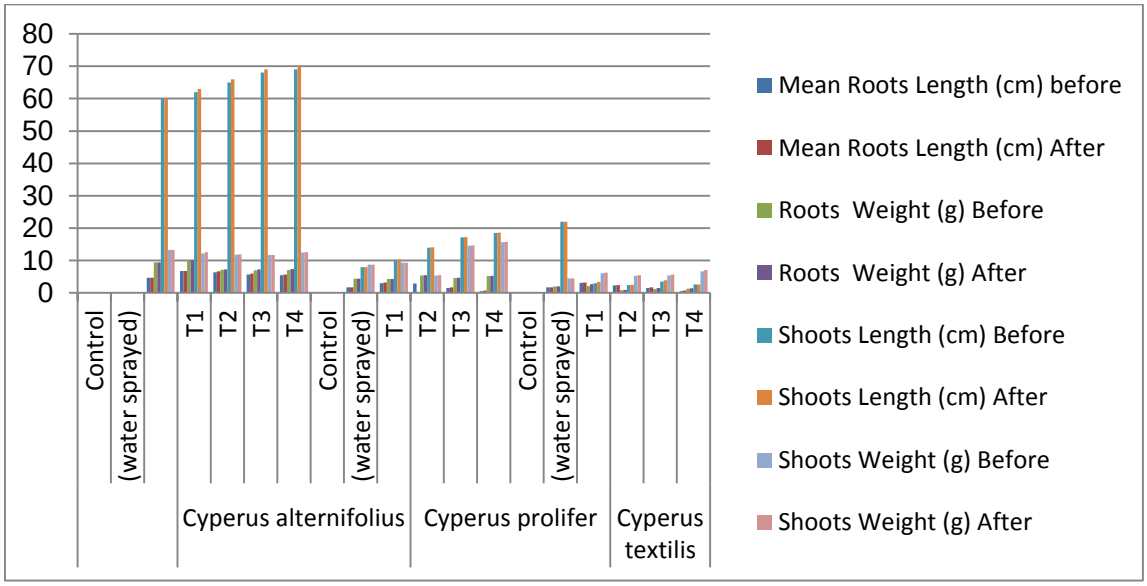

Figure 5.6: Mean weight and lengths of roots and shoots in *Cyperus* before and after Fe treatment after day 1 (24 h)

Table 5.7: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 2 (48 h)

Species	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	After	Before	After	Before	After	Before	After
Cyperus alternifolius	0	4.7	4.7	9.4	9.4	60	60	13.3	13.4
	5	6.8	6.8	10.0	10.1	62	63	12.2	12.4
	10	6.4	6.7	7.2	7.3	65	67	11.4	11.8
	15	5.7	6.0	7.0	7.1	68	69	11.7	11.9
	20	5.5	5.7	7.1	7.4	69	71	12.5	12.6
Cyperus prolifer	0	1.7	1.7	4.4	4.4	8.0	8.0	8.7	8.7
	5	3.1	3.3	4.3	4.3	10.2	10.3	9.0	9.1
	10	2.5	2.8	5.4	5.5	14.0	14.1	5.4	5.5
	15	1.5	1.7	4.6	4.7	17.1	17.2	14.5	14.7
	20	0.4	0.5	5.2	5.3	17.5	17.6	15.2	15.3
Cyperus textilis	0	1.0	1.0	2.8	2.8	2.2	2.2	4.5	4.5
	5	3.1	3.2	2.5	2.7	3.0	3.2	6.1	6.3
	10	2.5	2.7	0.8	0.9	2.4	2.6	5.2	5.5
	15	1.3	1.6	1.5	1.5	3.5	3.6	5.4	5.7
	20	0.5	0.7	1.3	1.4	2.8	2.9	6.1	6.3

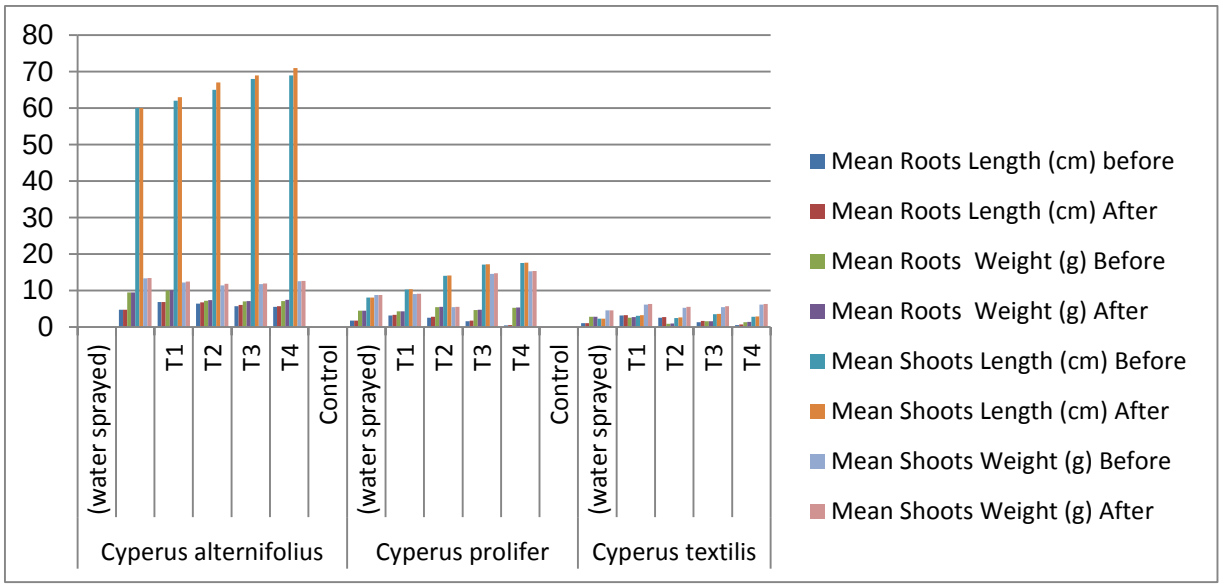

Figure 5.7: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 2 (48 h)

Table 5.8: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 3 (72 h)

S/N	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	after	before	after	before	after	before	after
Cyperus alternifolius	0	4.7	4.7	9.4	9.4	60	60	13.3	13.4
	5	6.8	6.8	10.0	10.1	62	62	13.2	13.6
	10	6.4	6.7	7.2	7.3	65	67	11.4	11.6
	15	5.7	6.0	7.0	7.5	68	68	11.7	11.9
	20	5.5	5.7	7.1	7.4	69	70	12.5	12.6
Cyperus prolifer	0	1.7	1.7	4.4	4.4	8.0	8.0	8.9	8.9
	5	3.1	3.2	4.0	4.1	10.2	10.3	9.3	9.6
	10	2.3	2.3	5.4	5.5	14.0	14.1	5.8	5.5
	15	1.5	1.7	4.6	4.7	17.1	17.2	14.6	14.2
	20	0.5	0.7	5.2	5.3	16.5	16.6	17.2	17.0
Cyperus textilis	0	1.7	1.7	2.0	2.0	2.2	2.2	4.5	4.5
	5	3.1	3.2	2.5	2.17	3.0	3.3	6.1	6.3
	10	2.5	2.6	0.85	0.89	2.4	2.6	5.3	5.5
	15	1.3	1.7	1.25	1.25	3.5	3.7	5.4	5.7
	20	0.7	0.7	1.35	1.40	2.8	2.9	6.7	6.9

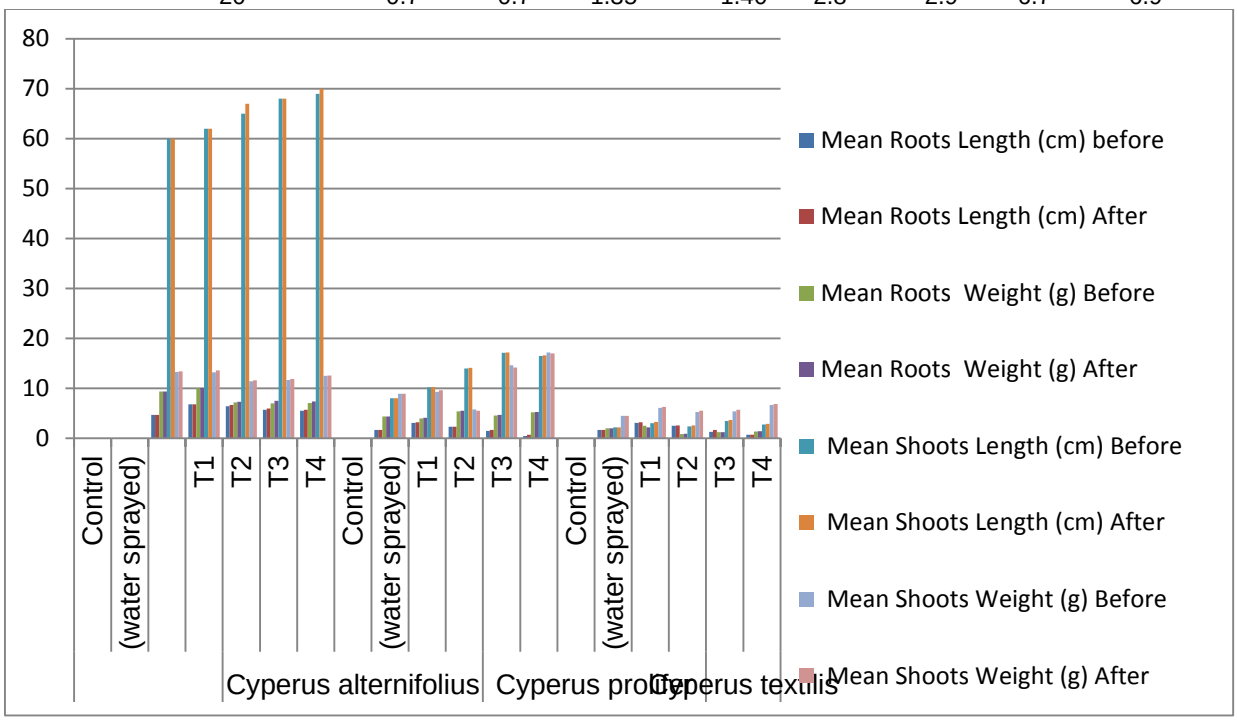

Figure 5.8: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 3 (72 h)

Table 5.9: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 5 (120 h)

S/N	Treatment mg/litre	Roots Length (cm)		Roots Weight (g)		Shoots Length (cm)		Shoots Weight (g)	
		before	after	before	after	before	after	before	after
	0	4.7	4.7	9.4	9.4	60.4	60.4	23.3	23.3
	5	6.8	6.8	10.0	10.1	62.3	63.3	22.2	22.4
Cyperus	10	6.4	6.7	7.2	7.3	65.3	65.6	21.4	21.6
alternifolius	15	5.7	6.0	7.7	7.9	68.2	68.5	16.7	16.9
	20	5.5	5.7	7.1	7.4	68.1	69.2	12.5	12.6
	0	1.7	1.7	4.4	4.4	8.0	8.0	8.7	8.7
	5	3.1	3.2	4.0	4.1	10.2	10.3	9.3	9.4
	10	2.5	2.7	5.4	5.5	14.0	14.1	5.4	5.5
Cyperus	15	1.5	1.7	4.5	4.7	17.1	17.2	14.5	14.7
prolifer	20	0.6	0.7	5.7	5.9	14.5	14.6	15.0	15.3
	0	1.5	1.5	2.1	2.1	22	22	4.5	4.5
	5	3.0	3.2	2.5	2.7	30	30.4	6.1	6.3
	10	2.5	2.8	0.5	0.9	24	24.1	5.3	5.5
Cyperus	15	1.5	1.7	1.5	1.6	35	35.1	5.4	5.7
textilis	20	0.5	0.7	1.3	1.4	28	28.1	6.1	6.3

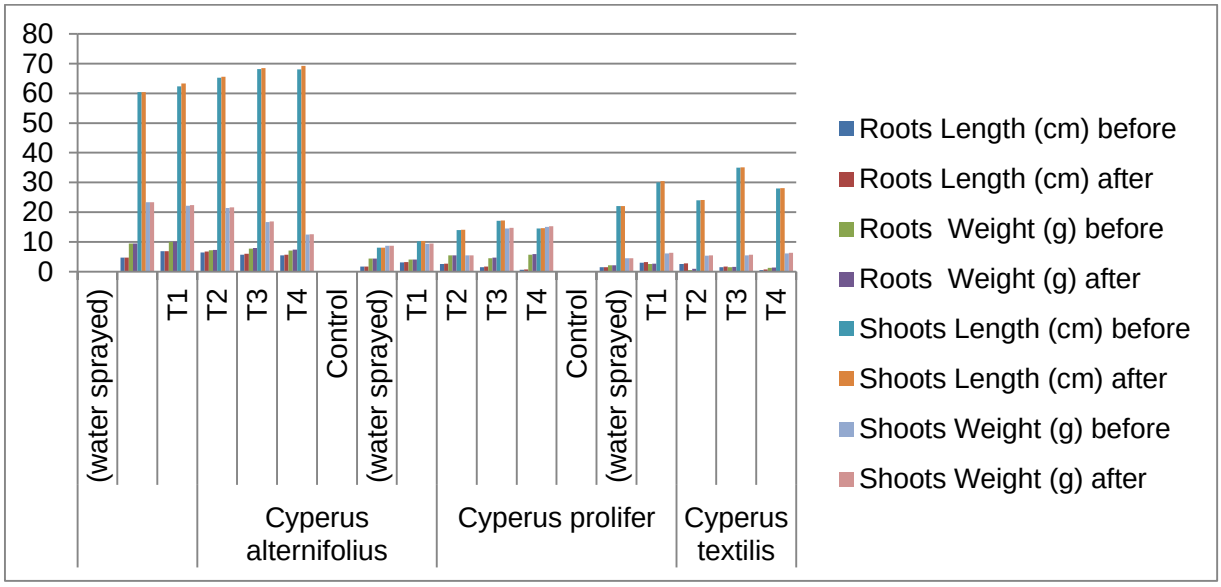

Figure 5.9: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 5 (120 h)

Table 5.10: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 7 (168 h)

Scientific name	Treatment mg/litre	Mean Roots Length (cm)		Mean Roots Weight (g)		Mean Shoots Length (cm)		Mean Shoots Weight (g)	
		before	after	before	after	before	after	before	after
	0	4.7	4.7	9.4	9.4	60.5	60.5	15.3	15.4
	5	6.8	6.8	10.0	10.1	62.4	62.7	12.62	12.6
Cyperus	10	6.4	6.7	7.2	7.23	65.4	65.3	11.84	11.9
alternifolius	15	5.7	6.0	7.0	7.09	68.2	68.7	11.77	11.8
	20	5.5	5.7	7.1	7.14	69.1	69.2	12.50	12.5
	0	1.7	1.7	4.1	4.1	8.0	8.0	8.1	8.1
	5	3.01	3.02	4.30	4.31	10.2	10.3	9.3	9.6
	10	2.35	2.10	5.48	5.50	14.0	14.1	5.4	5.5
Cyperus	15	1.35	1.37	4.65	4.72	17.1	17.2	14.6	14.7
prolifer	20	0.55	0.57	5.27	5.30	18.5	18.6	15.2	15.3
	0	1.0	1.0	2.8	2.8	22.8	22.8	4.9	4.9
	5	3.1	3.2	2.5	2.7	30.2	30.4	6.1	6.3
	10	2.5	2.8	0.5	0.9	24.0	24.4	5.2	5.5
Cyperus	15	1.5	1.7	1.2	1.5	35.1	35.4	5.4	5.7
textilis	20	0.5	0.7	1.5	1.8	28.3	28.8	6.1	6.3

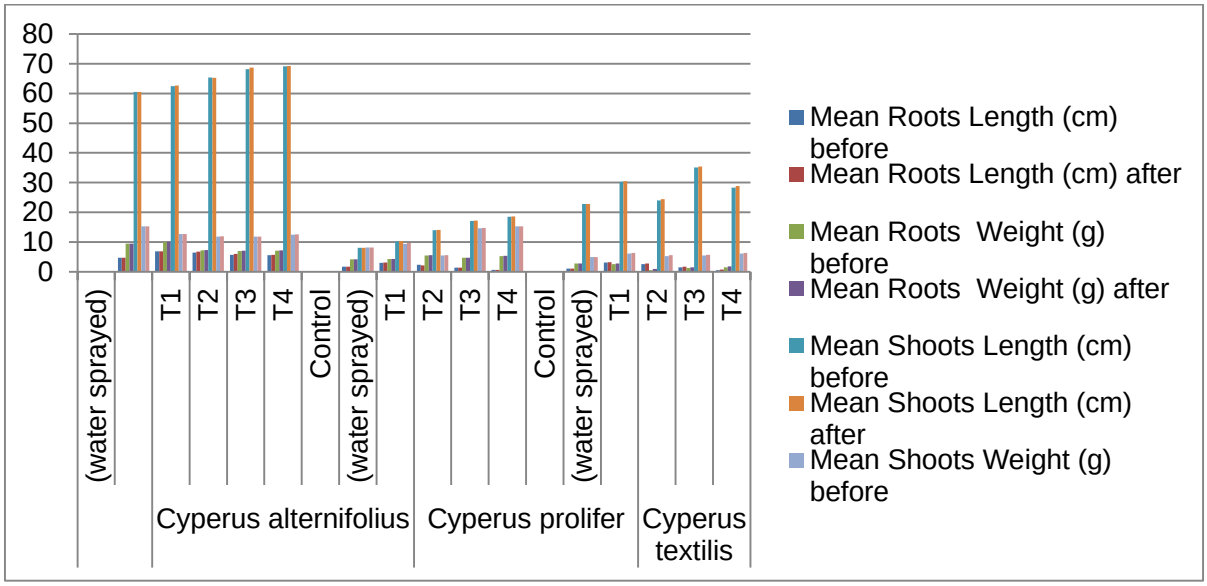

Figure 5.10: Mean weight and lengths of *Cyperus* roots and shoots before and after Fe treatment at day 7 (168 h)

5.2 Comparison of Al and Fe uptake and tolerance among *Cyperus* and other wetland plants

Aluminium (Al) is a non-essential element and is generally toxic to plants when it is in a form that is bioavailable. Biologically it has no known function in plants (Sun *et al.,* 2010:347). Information on Al toxicity is generally lacking. Inhibition of root elongation is a primary effect and has been used as a parameter for screening for Al resistance in many studies (Kochian, 1995:237). Some freshwater macrophytes including *Potamogeton lucens*, *Salvinia hergozi*, *Eichhornia crassipes*, *Myriophyllum spicatum*, *Cabomba* spp. and *Cratophyllum demersum* have been investigated for their potential in heavy-metal and colour removal (Priya & Selvan, 2014). Aluminium inhibits root cell division and elongation thus reducing water and nutrient uptake and inducing poor plant growth and yield (Xing *et al.,* 2013:6999).

The effect of Al and Fe concentrations in the roots and shoots of three selected *Cyperus* aquatic macrophytes investigated during this study is shown in Tables and Figs. 5.1-5.10. In spite of many years of research aimed at resolving Al toxicity and resistance, little is known about the fundamental mechanisms responsible (De la Fuente & Herrera-Estrella, 1999:103). Many scientists (Akinola & Ekiyoyo, 2006:597; Pandey, 2006:381; Umebese & Motajo, 2008:197) have reported that toxicity thresholds of plants are highly variable. A number of plants have the ability to accumulate metals in their shoots and show an exceptionally high tolerance to some while showing phytotoxic effects to others.

Aluminium is the most abundant metal in nature (De la Fuente & Herrera-Estrella, 1999:103). It is considered to be a micronutrient and Al metabolism interferes with cell division in root tips and lateral roots. It is also known to increase cell wall rigidity. In humans, Al has been implicated in oxidative and inflammatory events leading to tissue damage (Becaria *et al.,* 2002:309; Rao *et al.,* 2010:333).

Aluminium is known to inhibit plant growth as it influences redox state and various other biochemical, physiological and growth responses. Literature cites the deleterious effects of Al toxicity to include impairment of root development, which is often manifested in crops as poor growth. It subsequently delays maturity through interference with cell division in plant roots, fixation of phosphorus in less available forms in the soil and plant roots, reduction in root respiration and interference in the activity of many enzymes (Rao *et al.,* 2010:333). Aluminium-induced inhibition of root elongation has been recently been demonstrated in *Arabidopsis*, (Sun *et al.,* 2010:347). In the roots of *C. alternifolius* (Fig. 5.11) as the Al

was applied in increasing concentrations, thereby increasing the Al activity in solution, significant differences (P>0.05) were observed at each level.

(ppm)

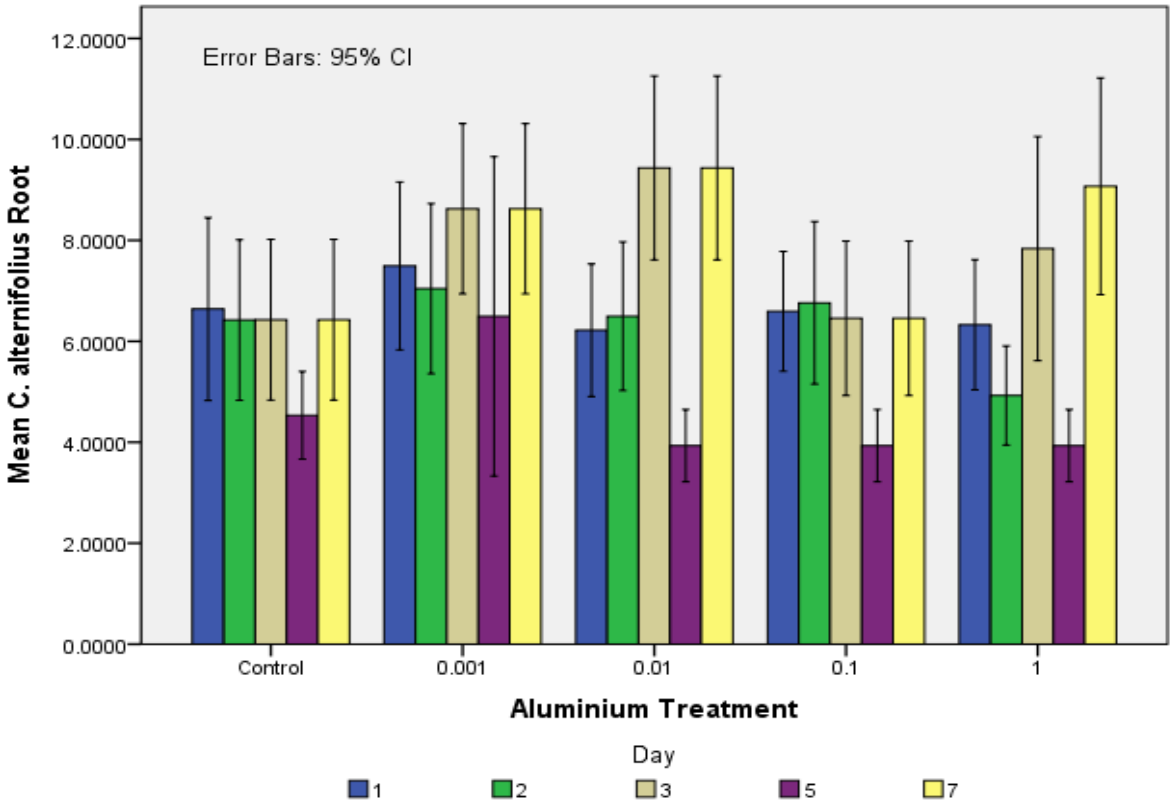

Figure 5.11: Mean root concentration (ppm) of Al in *C. alternifolius* over 7 days of exposure

Different concentrations of Al exert different effects on the growth of plants. Commonly referred to as Umbrella Sedge or Umbrella Palm, *C. alternifolius* can be grown in humid soil or in marshy areas. The plant has robust underground roots and an erect aerial unbranched stem. *Cyperus alternifolius* can be easily propagated by using seeds and plant fragments (Kyambadde *et al*., 2004:475). It has been used in different studies as a wetland plant. Like *Miscanthidium violaceum, C. alternifolius* is known to eliminate nutrients from wastewater (Kyambadde *et al*., 2004:475).

The *Cyperus* spp for this study were obtained from a pristine environment and were examined after hydroponic exposure to different concentrations of Al and Fe over a 20 week period (August - December).

Total biomass yield per treatment increased significantly (P≤0.05) over the course of the experiment for all studied plants. The total Fe/Al content in the different plants samples was determined using methods described by Odendaal and Reinecke (1999:64) by using Inductively Coupled Plasma-Mass Spectrophotometer (ICP-MS) (7500CE, Agilent, England). Results are shown in Figs. 5.1-5.10. Seedling growth was recorded as the elongation of roots and shoots of young seedlings of *C. alternifolius, C. prolifer* and *C. textilis* during the application of Al and Fe. The heavy metals decreased the elongation of roots and shoots as a function of increase in Al and Fe supply (Tables 5.1-5.10).

The toxic effect of metals on the physiological functioning of plants is related to their accumulation in different plant tissues. Scientific evidence has shown that plant species will concentrate certain metals in their roots and shoots to varying degrees and, hence, critical levels may vary among species (Liphadzi & Kirkham, 2006:737). Micromolar concentrations of Al^{3+} can inhibit root growth within minutes or hours in many agriculturally important plant species. This suggests that Al-accumulating plants must possess effective mechanisms to detoxify Al^{3+} internally.

In this study, the analysis of Al and Fe suggested that *C. textilis* plays an important role in metal retention. The most important structure of the plant showing heavy metal retention was the root system followed by the shoot (Figs. 5.16 and 5.17). Total biomass yield per test plant increased significantly (P≤0.05) over the course of the experiment for all plants (Figs. 5.1-5.10). After applying the unilateral F-Test for the accumulation of each metal separately in roots and shoots of the investigated aquatic macrophytes, it was clear that the accumulation of Al is more pronounced than was the case for Fe.

Morphological changes induced by enzymes such as hexokinase, phosphodiesterase, alkaline phosphatase and phosphoxidase are inhibited by aluminium since it has a greater affinity for DNA and RNA. Thus reduction in size was observed in root growth, which is the combination of cell division and elongation that showed highest metal concentration, followed by shoots. This is in agreement with findings by Ashraf *et al.* 2011:1. Research presented in the latter study also revealed that Al tended to accumulate in roots rather than in shoots. Similarly, a study by Kabata-Pendias and Pendias (1992:1) showed that Al is likely to be concentrated in the plant root. Root staining techniques showed that Al accumulates principally in the root tips of the main root and lateral root tissue (Matsumoto *et al.* 1996:99). An explanation of the higher metal accumulation in the roots of the three wetland plants studied here might be that robust root development and complexation of metals with the sulphydryl group (-SH) of soil

constituents reduced translocation of metals to the upper parts of these wetland plants (Sinha & Gupta, 2005:1204).

The accumulation of Al and Fe in the three *Cyperus* spp. over the duration of the experimental period is shown in Figs. 5.11-5.22. Total macrophyte biomass (roots plus shoots) showed an increase over time in all treatments. Iron addition induced a different response in the three *Cyperus* spp. studied (Tables 5.1-5.10). Long-lived plants such as *Cyperus* have countless meristems present and each meristem is able to produce shoots, which are better suited to the environment and adapted to the stress of excess metals. Inhibition of growth of aquatic plants was measured as reduction in fresh biomass due to toxic effects created by metals.

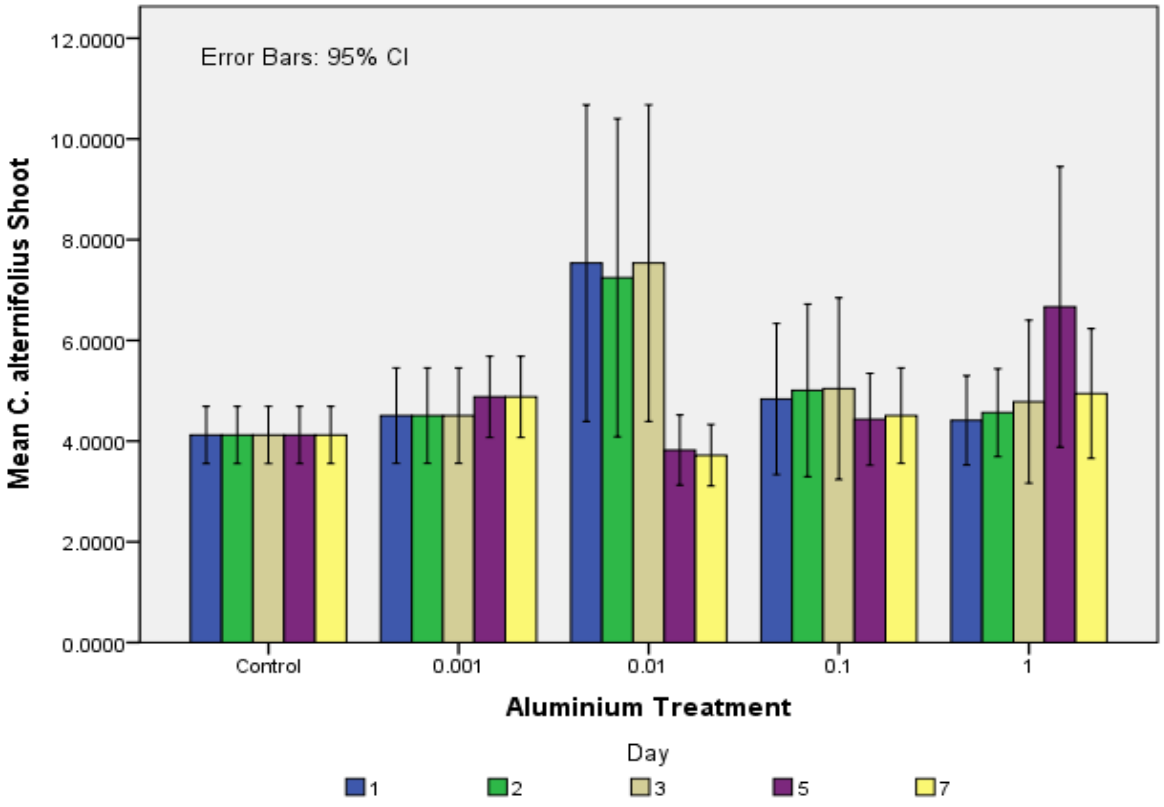

Figure 5.12: Mean shoot concentration (ppm) of Al in *C. alternifolius* during exposure over a 7 day period

Many have reported on the inhibitory effects of Al on plants (Blamey *et al.,* 2004:76; 2005:708; Simon *et al.,* 1994:307). For example, aluminium-induced inhibition of root elongation can be measured within hours or less after the roots have been exposed to excess Al supply (Blamey *et al.,* 2004:76). Doncheva *et al.* (2005:1213), Zobel *et al.* (2007:243), and Silva (2012:1),

discuss root growth inhibition, ROS production, alterations in root cell wall and plasma membrane, nutrient imbalances, callose accumulation and disturbances of cytoplasmic homeostasis caused by exposure to Al. Aluminium can directly affect cell elongation by inhibiting the nucleic of cells in the root tips (Silva *et al.,* 2000:1), and hinders the physiology of rice during germination (Kikui *et al.,* 2005:1837).

High values for Al concentrations were noticeable by day 5 (120 h) when exponential increases in shoot and root elongation were evident (Fig. 5.4). This could be due to many factors including metal tolerance and bioaccumulation. Figure 5.4 shows that there was a linear pattern in metal dosage and exposure time in all treatments. Delhaize *et al.* (1993:685) and Al-Qahtani *et al.* (2012:384) have both indicated that shoots accumulate less Al than do the roots. This could due to different adaptation strategies as reported by Cronk and Fennessy, (2001:1).

Analysis of *C. prolifer* showed that metal accumulated preferably in the root. This is in agreement with work done elsewhere (Baldantoni *et al.,* 2004:149; Aksoy *et al.,* 2005:241; Nabulo *et al.,* 2008:65) where it was found that similar uptake trends were also recorded in both the root and shoot. The current study on the three *Cyperus* spp. demonstrated that there was a relationship between high metal value in plants and their modified suppressed growth (Figs. 5.11- 5.22).

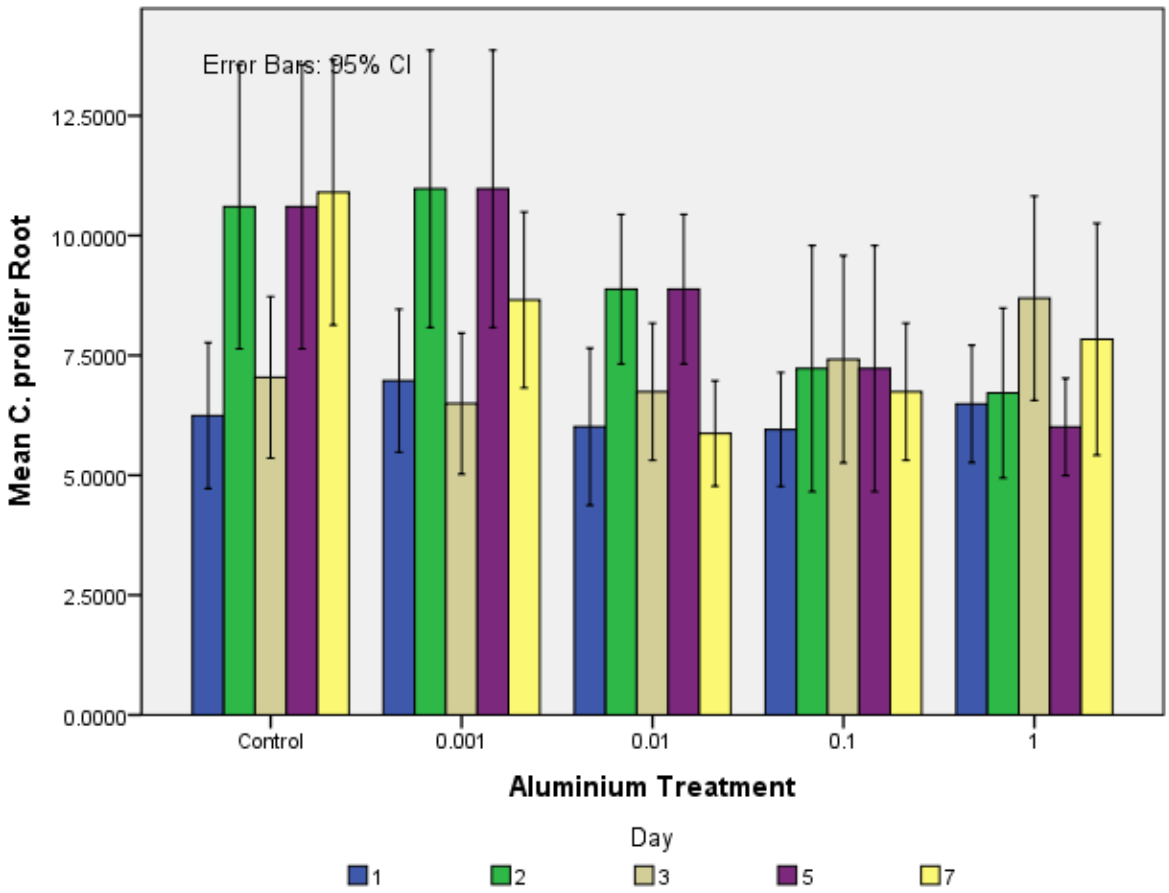

Figure 5.13: Mean root concentration (ppm) of Al in *Cyperus prolifer* over 7 days.

Differential uptake of Al into roots could possibly be accounted for by differences in tolerance between genotypes. The variations in Al uptake by roots representing three different *Cyperus* genotypes (Tables 5.1-5.5) is similar to those noted by Kabata-Pendias and Pendias (2001:1). The latter authors concluded that plant species and cultivars even within the same species differ considerably in their ability to take up and translocate aluminium to above-ground parts. The effect of Al application on *Cyperus* shoots is shown in Fig.5.14.

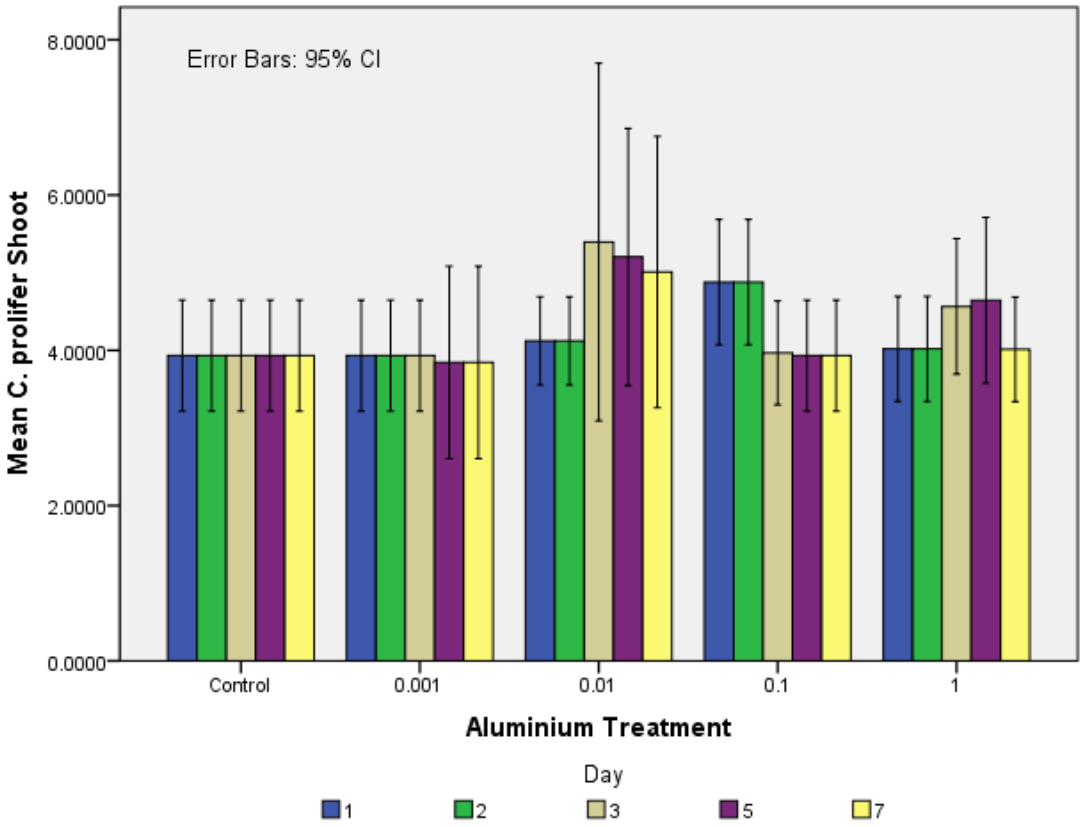

Figure 5.14: Mean shoot concentration (ppm) of Al treatment of *C. prolifer* over 7 days

The ability of wetlands plants to transform and store organic matter and nutrients has resulted in their widespread use in wetlands for wastewater treatment worldwide. The concept of using wetland environments for the removal of heavy metals such as Al and Fe has been examined in both field and bench scale studies. Aquatic macrophytes improve water quality through the accumulation of toxic nutrients and heavy metals. Many macrophytes have been used because of these capabilities. Furthermore they grow rapidly and are easy to maintain. Examples of macrophytes used include *Phragmites australis, Phalaris arundinacea, Iris pseudacorus, Typha* spp., *Scirpus* spp., and *Cyperus* spp. (Calheiros *et al.,* 2007:1790). As emphasised in the foregoing, the selection of appropriate wetlands plants as phytoremediants is important as selected plants must be tolerant of metal toxicity and changes in the properties of wastewater flowing into the system.

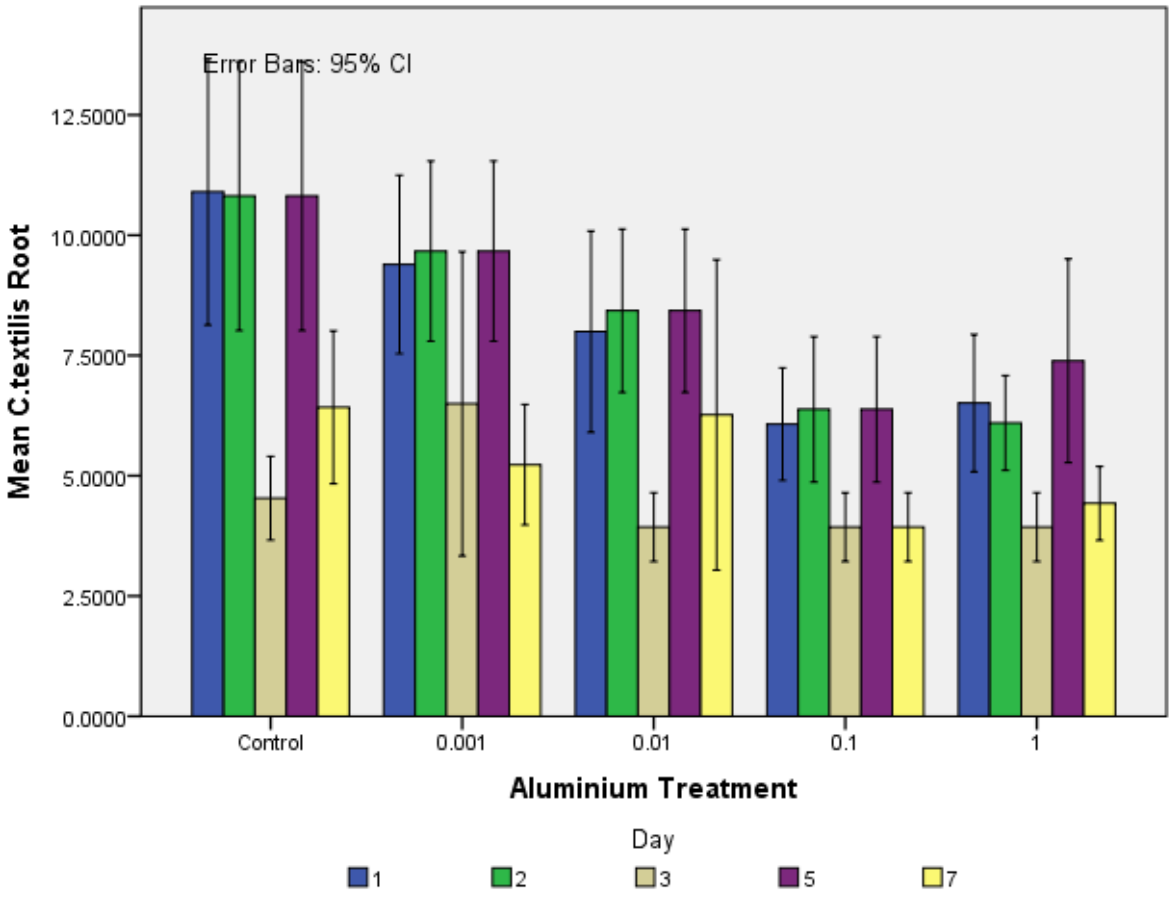

Figure 5.15: Mean root concentration (ppm) of Al in *Cyperus textilis* over 7 days

Bhalerao and Prabhu (2013:447) and Rout *et al.* (2001:3) observed the inhibitory effects of Al on plant growth. The most apparent symptom of Al toxicity, which could be detected within 30 m to 2 h, even at micromolar concentrations of Al, is root growth inhibition (Llugany *et al.,* 1995:265) which affects growth and developmental processes. In studies done elsewhere, the maximum rate of Al uptake into the root-cell cytoplast was observed after the first 30 m following application of 100 µm $AlCl_3$ solution after calculations based on Al-lumogallion calibration. An increase in the Al uptake rate after the initial 30 m was relatively small when compared to the internal Al concentration which increased to 0.35 ± 0.03 µm Al during the first 3 h of exposure to 100µm of $AlCl_3$ (Babourina & Rengel, 2009:189). There is limited entry of Al^{3+} through the cortex and epidermis cells of the mature root zone.

Research into bioaccumulation of various metals by aquatic plants has been well documented (Aksoy *et al.,* 2005:241; Deng *et al.,* 2009:29). In most of these studies it was found that the

roots of the aquatic plants were able to bioaccumulate metals more readily than the shoots. This study of three *Cyperus* spp showed that there was a relationship between high metal content in plants and their modified suppressed growth (Figs. 5.15 and 5.16).

Studies by Kelly *et al.* (1990:172) and Harrington *et al.* (1996:1742) indicated that under conditions of elevated metal supply, generally the majority of metals are restricted to the plant roots. Kochian *et al.* (2005:175) stated that Al, after being absorbed by root cells tends to accumulate preferentially in the root apex, promoting inhibition of root elongation and cell division. A similar trend in activity could be observed with the accumulation of Al in the shoots of both *C. alternifolius* and *C. prolifer* (Figs. 5.11-5.16) after 7 days, exposure to Al.

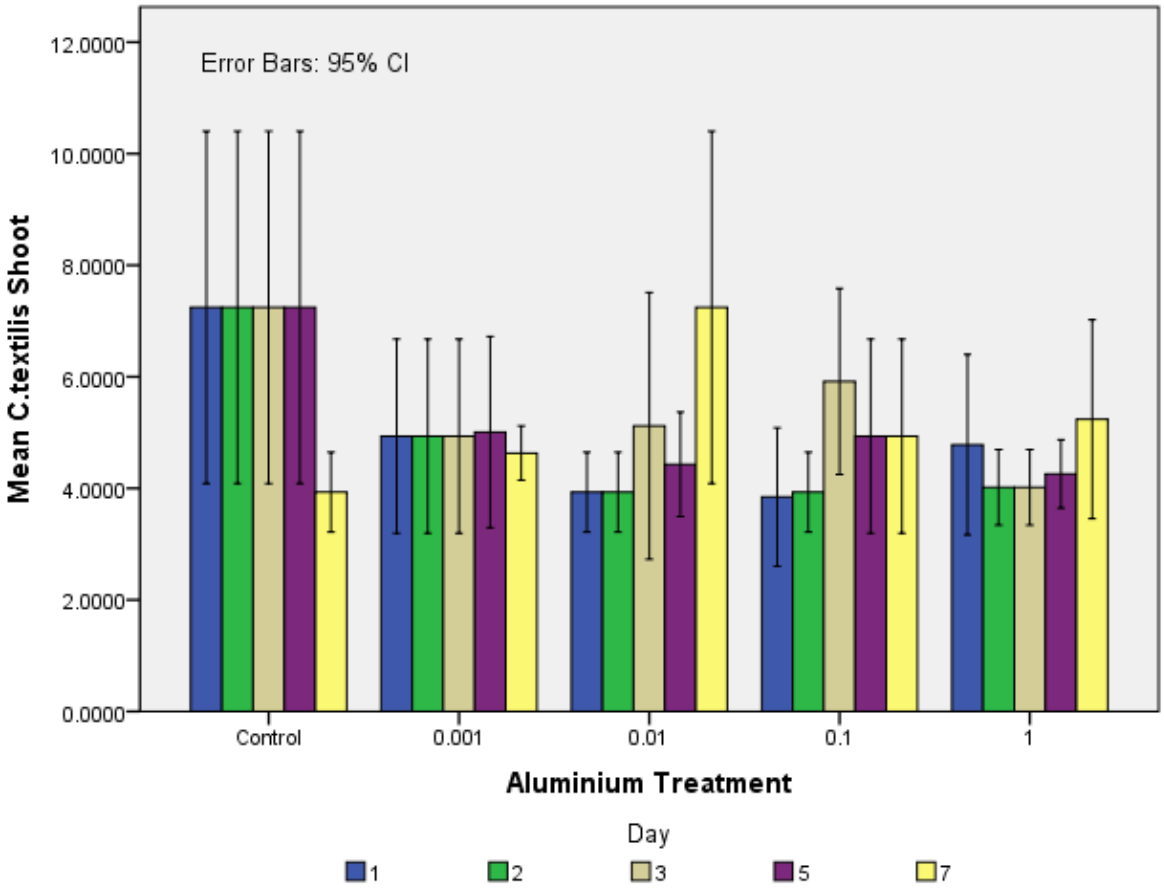

Figure 5.16: Mean shoot concentration (ppm) of Al recorded for C*. textilis* during a 7 day exposure

The accumulation of high concentrations of toxic metals may result in irreversible damage to the plant as these accumulate in plants organs (Sheoran *et al.,* 2011:168; Klos *et al.,*

2012:1829). Analysis of Al and Fe contained in the plants showed that *C. alternifolius* plays an important role in metal retention particularly in the root system. Shoots were also able to accumulate the metals but in far smaller quantities. However, it was apparent that the three *Cyperus* spp. investigated exhibited different elemental concentrations, depending on the plant organ and the treatment exposure periods.

Plants exposed to Al undergo damage to root systems as Al interferes with the uptake and transport of essential nutrients (Ca, Mg, K, and P). In the case of P, transport between roots and shoots diminishes as Al concentration in the roots increases (Bhalerae & Prabhu 2013:447). As Al toxicity occurs in strongly acidic soils, plants may also show deficiency symptoms of other essential nutrients (Ca, Mg, K, Mn) in these soils. Aluminium toxicity is influenced by soil pH as the latter influences the amount of soluble Al, rather than the total Al concentration. Even though there are no generally accepted critical levels of exchangeable Al, it has been found to be toxic to most plants when the concentration is greater than 2-3 ppm with a soil pH < 5.5 (Silva, 2012:8). Soils at a site with a pH greater than 5.5 can generally be considered non-toxic with regard to Al.

Aluminium toxicity leads to inhibition of root growth by altering root architecture and disrupting root elongation but Al toxicity is manifested only in acid conditions where the phytotoxic form of Al^{3+} predominates (Komarek *et al.,* 2010:138). Delhaize *et al.* (1993:685) reported on the uptake and tolerance of Al in the root apices of wheat (*Triticum aestivum* L). The results of the present work are in agreement with observations of others particularly with regard to the low metal concentrations recorded in the shoot systems of the plants (Baldantoni *et al.,* 2005:48; Nirmal Kumar, 2009:10).

Metal concentrations in plants vary with plant species (Alloway *et al.,* 1990:223). Babourina & Rengel (2009:189) and Kabata-Pendias & Pendias (1992:1) assumed that accumulation of a particular metal in wetland plants is affected by immobilization and uptake from the soil, compartmentalization and sequestration within the root, efficiency of xylem loading and transport, distribution between metal sinks in the aerial parts and sequestration and storage in leaf cells.

An understanding of the abilities of different *Cyperus* species to absorb, transport, tolerate and accumulate trace elements under different concentrations is important. Such understanding will assist in developing plants as agents of phytoremediation. Results obtained during this study showed that there were differences in the uptake levels of heavy metals related to the

plant species. Most metal tolerance and uptake was shown by *C. alternifolius* when compared with *C. prolifer* and *C. textilis.*

5.3 Comparison of Fe uptake and tolerance among the studied *Cyperus* spp

The cumulative effect of 21st century developments such as intensified mining and rapid industrialisation has caused extensive environmental pollution by organic compounds and inorganic metals (Babourina & Rengel, 2009:189; Sheoran *et al.,* 2011:168; Klos *et al.,* 2012:1829). Metals are principally introduced into the aquatic environments due to weathering of soils and rocks, volcanic activity and human activities that involve metals. Following mobilization, metals are absorbed by root cells. Metals bind to the cell wall which may be considered as an ion exchange system of comparatively low affinity and low selectivity. Transport systems and intracellular high affinity binding sites then mediate and drive uptake across the cell plasma membrane. Uptake of metal ions is likely to take place through secondary transporters such as carrier proteins and/or H^+ complex carrier proteins (Hanikenne *et al.,* 2005:428).

In many cases once a metal has entered a plant in excessive quantities, plants are able to store these unwanted substances within their vacuoles in the cytosol, where they will have least effect on plant metabolism. There are various ways in which a plant avoids metal build up within its organs but each plant species has specific mechanisms in place to reduce the uptake of excess metals into the cytosol (Hall, 2002:1). Maestri *et al.* (2010:1) reviewed the mechanisms for metal sequestration and chelation and stated that plant cell walls constitute a vast extension of material which can bind and effectively sequester metal ions.

Bioavailability depends on factors such as the biological parameters, and physico-chemical properties of the metallic elements and their compounds (Duffus, 2002:793). Many studies have shown that some plants are able to overcome the phytotoxic effects of excess metal accumulation and are able to survive in a contaminated aquatic environment which makes them valuable resources for phytoremediation of metal polluted water bodies (Gratão *et al.,* 2005b:53; Basumatary *et al.,* 2013:977). Steinberg (2012:131) reported hyper-accumulating plants survive in environments contaminated by heavy metals. Therefore plants must have developed an adaptive evolution of stress tolerance. It may be assumed that this stress tolerance is associated with constitutive over-expression of stress genes.

Iron is an essential micronutrient for plants and animals but when it occurs at high concentrations in the environment, Fe can exert marked toxic effects and is regarded as an

environmental pollutant. Results obtained from the current study suggest that higher concentrations of Fe in the roots of the investigated species could be due to Fe precipitation in iron plaques on the root surface as suggested by Tanner (1996:59) and Batty *et al.* (2002:443). The results obtained from plant analyses indicated that roots of all three plants were found to be very efficient in Fe accumulation (Figs. 5.17, 5.19 & 5.21)**.** The highest concentrations were recorded from the roots of *C. alternifolius* (109 758 µg.g-1 d.w) followed by *C. prolifer* (9 953 µg.g-1 d.w). According to Allen (1989:1) and Kloke (1980:9), Fe concentrations above 40-500 µg.g-1 d.w are reported to be toxic in plants. Tiffin (1977:315) also reported that roots tend to absorb Fe^{2+} more readily than Fe^{3+}. Thus, the ability of roots to reduce Fe^{3+} to Fe^{2+} is believed to be fundamental in the absorption of this cation by most plants (Tinker, 1996:41; Nirmal Kumar, 2009:10). Of interest is that certain bacterial species (e.g. *Metallogenium* spp.) are involved in Fe reduction and are known to accumulate this metal on the surface of living cells (Weinberg, 1977:492; Italiya & Shah, 2013:26;).

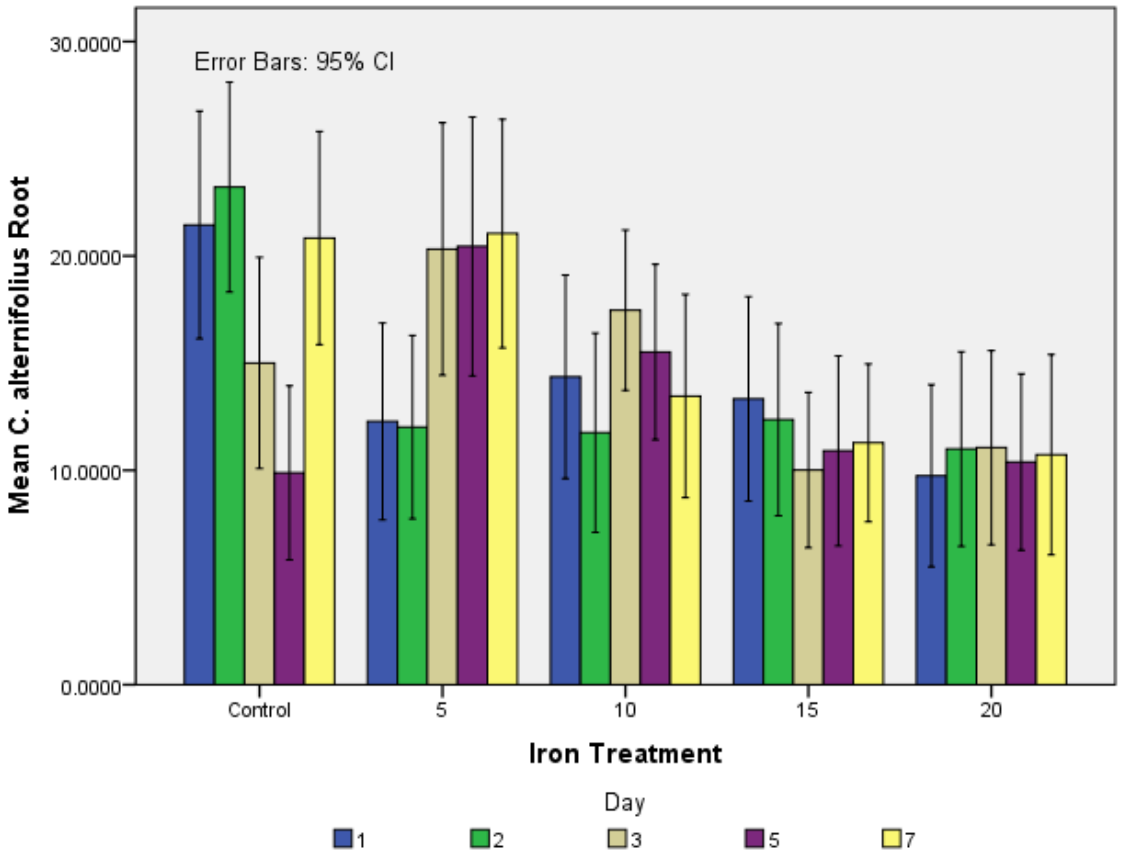

Figure 5.17: Mean root concentration (ppm) of Fe in *C. alternifolius* over 7 days.

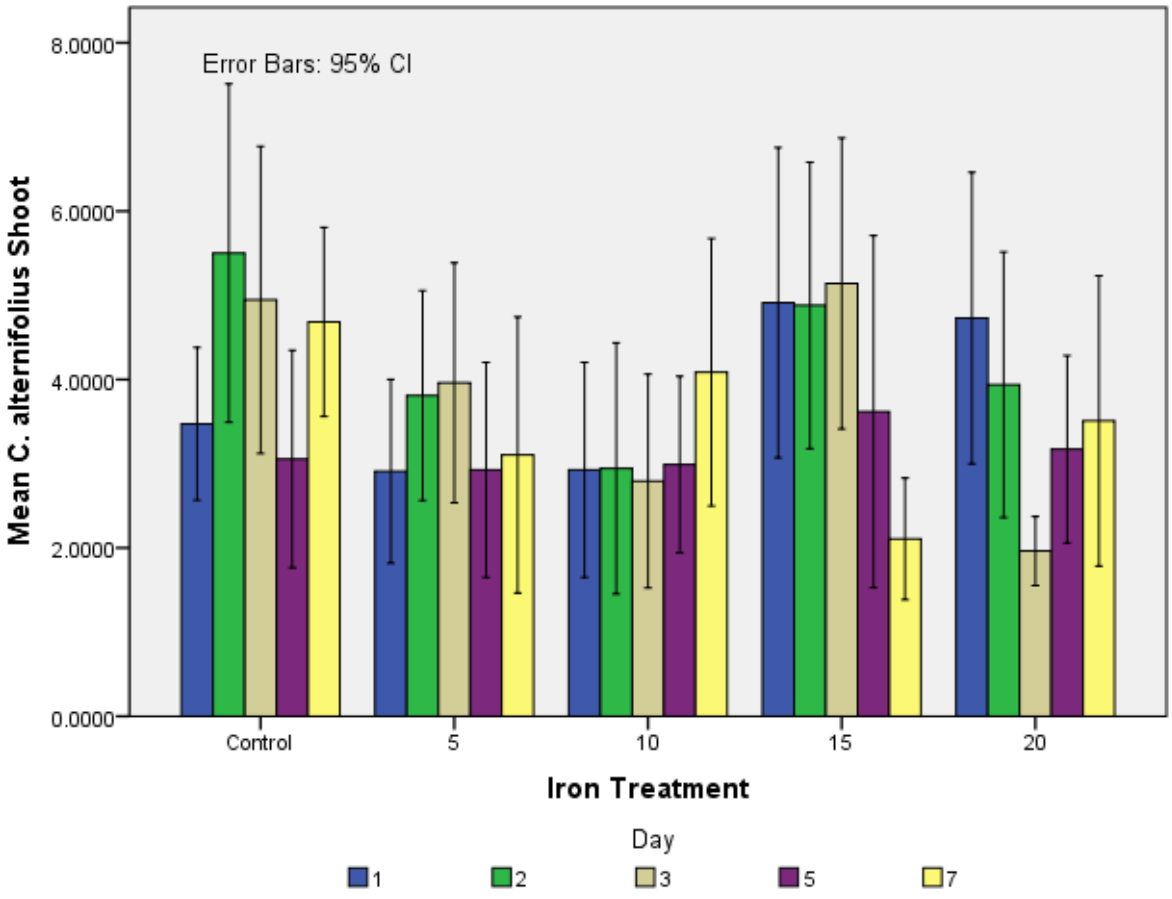

Figure 5.18: Mean shoot concentration (ppm) of Fe treatment in *C. alternifolius* over 7 days

The above Fig. 5.18 shows that there were moderate increases in the accumulation of Fe over the duration of exposure period of 7 days with maximum peaks seen on day 5 (120 h) in *C. alternifolius* shoots and the accumulation progressed as the concentration increased with the exception being the control. Results from this study agree with work done elsewhere which indicated that the growth of plants can be inhibited by high iron concentration by the manifestation of both necrotic leaf spots and plaques on roots. This could cause a decline in biomass (Van der Welle *et al.,* 2006:1592).

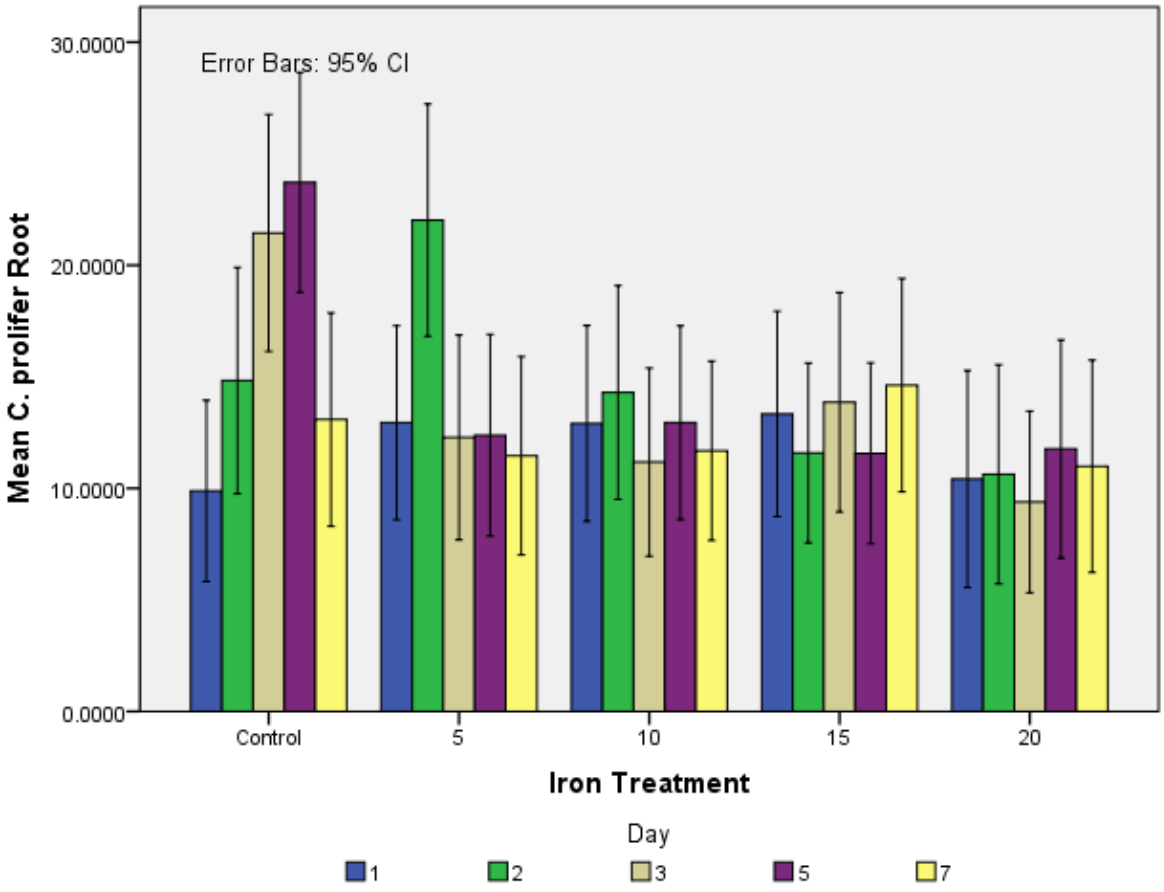

Figure 5.19: Mean root concentration (ppm) of Fe in *C. prolifer* over 7 days

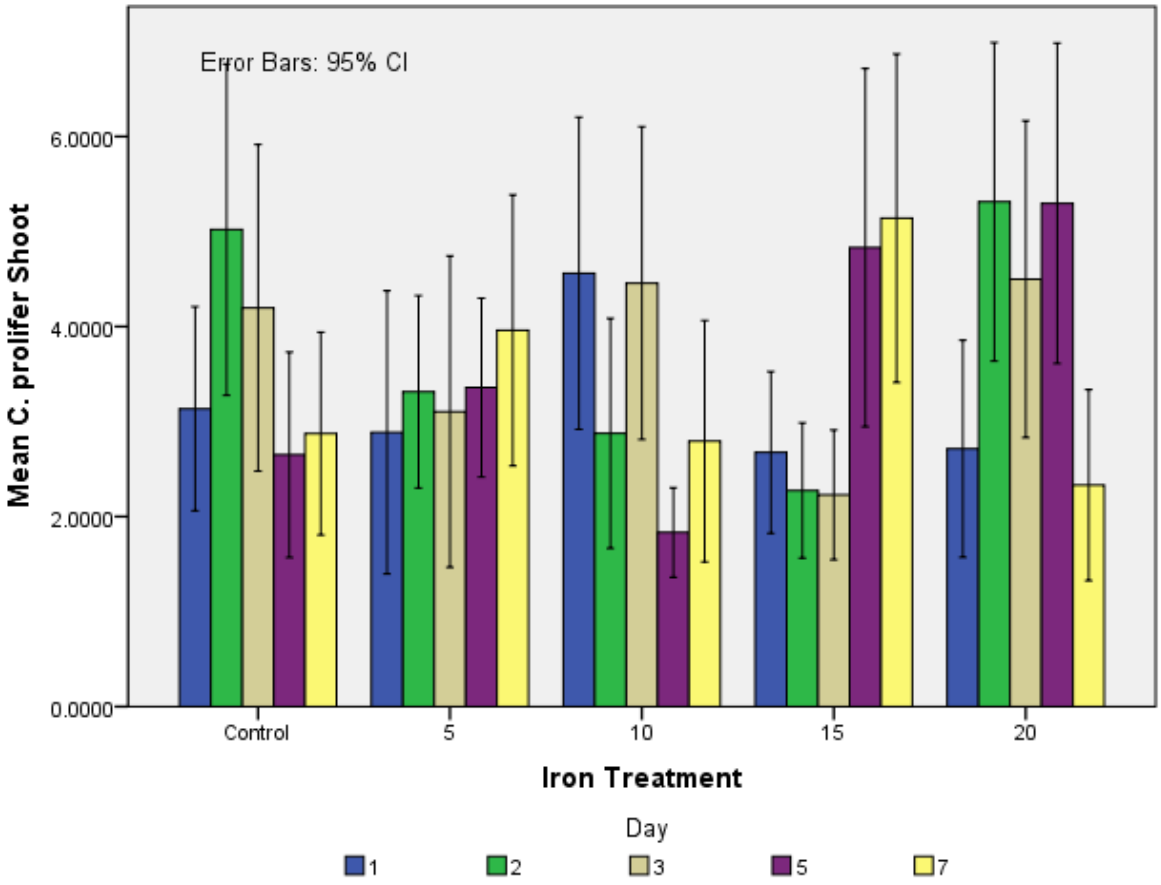

Figure 5.20: Mean shoot concentration (ppm) of Fe of *C. prolifer* over 7 days.

Plants may follow two different strategies of metal uptake and translocation. Metals are unable to be broken down into less harmful components and may be absorbed by plants through the roots (from sediment or soil) or leaves (via water through stomata). Bioaccumulation is the result of the accumulation of a contaminant in living organisms. Some metals are required by plants in small quantities as nutrients to sustain metabolic processes within the plant. However at large dosages these metals become toxic to the plant and have detrimental effects on plant growth (Brankovic *et al.,* 2011:11956; Singh *et al.,* 2011:246).

Ashraf *et al.* (2011:401) reported that morphological changes such as reduction in plant size, and changes in colour were observed in plants containing high metal concentrations. Similar trends were observed during this study on *Cyperus*. Iron accumulation in seedlings increased in the hydroponic system used for the current study. The decrease noted in plant biomass (Appendix) as iron concentrations increased could be related to iron toxicity. Iron toxicity can exert indirect and direct effects on plants (Lucassen *et al.,* 2000:321).

Indirect negative effects of iron act predominantly by limiting the phosphorus availability in plants. This is because in the presence of iron, phosphate will precipitate (Wheeler *et al.,* 1985:653).

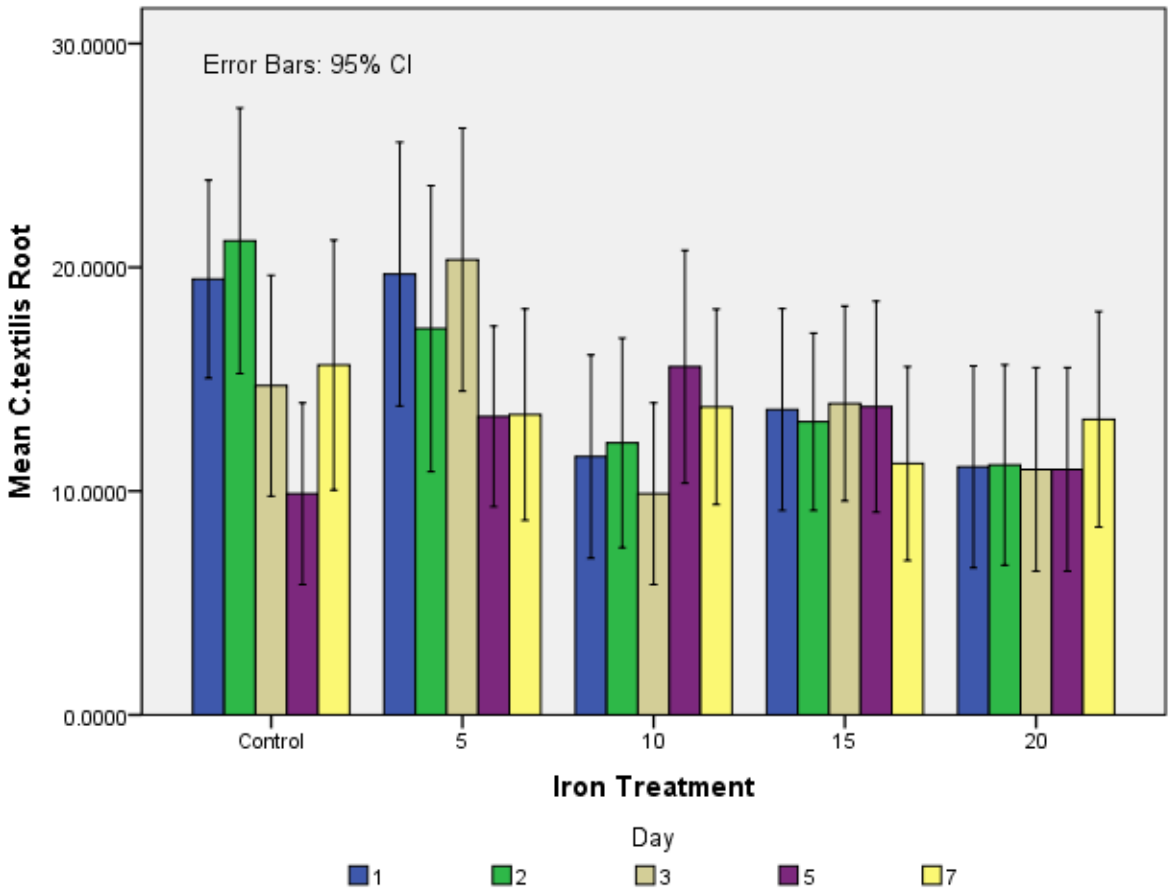

Figure 5.21: Mean root concentration (ppm) of Fe in *C. textilis* over 7 days

Accumulation and distribution of heavy metals by a plant depends on the plant species, the type of element, chemical bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature and secretion by roots. Dhir and Srivastava (2011:893) reported that the efficacy of heavy metal uptake varied for each metal present in an environment and reported on *Salvinia* spp, an aquatic plant which exhibits a high potential for the removal of a wide range of heavy metals from water bodies.

The current study showed that metal accumulation by the three *Cyperus* spp investigated differed among the species and even within the various tissues in a given plant (Figures 5.11-5.22).

Studies elsewhere have similarly shown that different plants vary in the quantities of metals stored and also that metal accumulation within these plants varies among the different tissues (Intawongse & Dean, 2006:36). Examples include the leaves where metals accumulate in concentrations higher than those recorded for the stems as leaves are more temporary than stems, and senesce. In this way the metal burden on the plant is reduced (Windham *et al.,* 2003:63). It is also known that metals in plants generally accumulate more in plant tissues directly in contact with the source of metals. In rootless aquatic submerged plants, the leaves are more exposed to the water source of metal contaminants than are the stems and will accumulate more of the metal (Intawongse & Dean, 2006:36). Prasad (2004:482) gave an account of the transportation and distribution of metals within plants and reported that during metal transportation through the plant, the metal is bound largely on the cell walls. This could explain why most metals accumulate in roots and to a lesser extent in the shoots

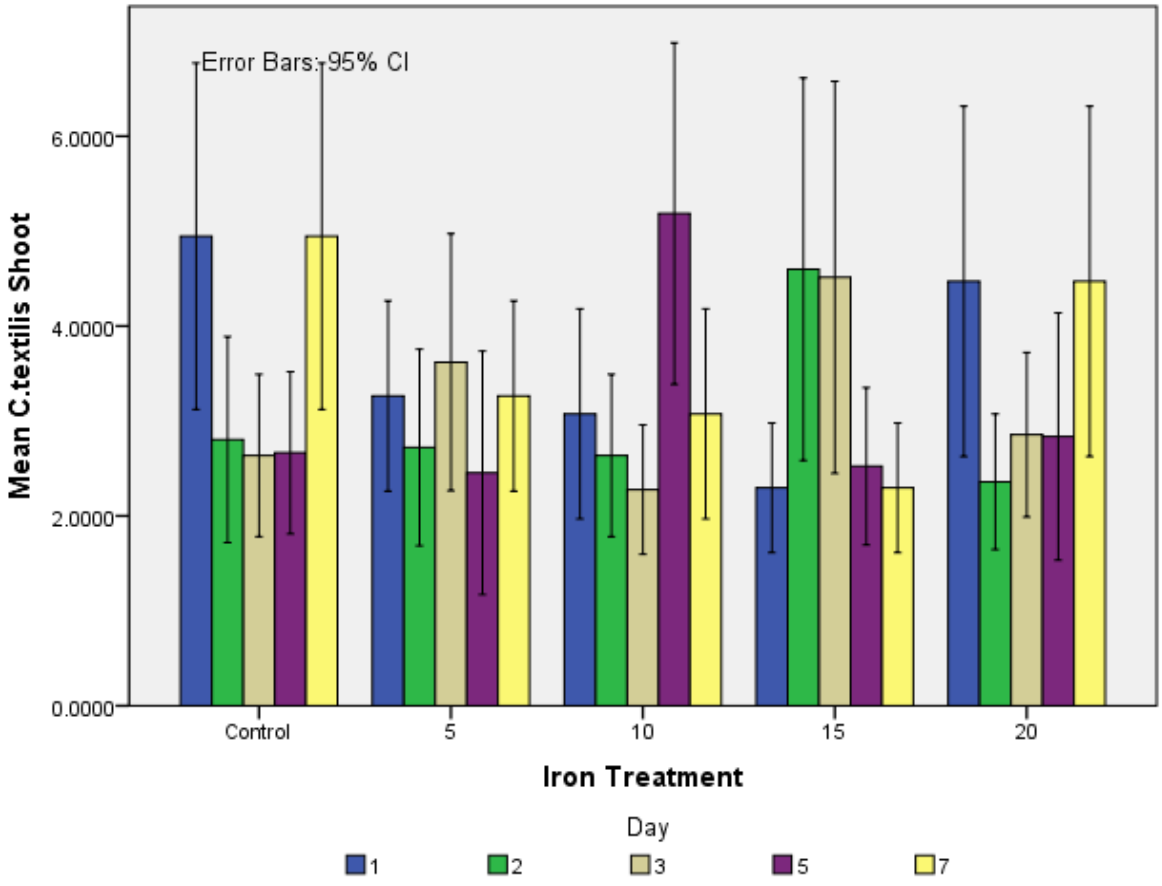

Figure 5.22: Mean shoot concentration (ppm) of Fe in *C. textilis* over 7 days

Aquatic plants (macrophytes) such as sedges play an important role in the aquatic ecosystem, filtering out pollutants in the water (Lee & Scholz, 2007:87; Brankovic *et al.,* 2011:11956). In order to evaluate the phytoextraction ability of any plant species, whole plant biomass, the metal concentration in the growth media and the metal concentration in the selected plant tissue must be taken into consideration. Many (Weis & Weis, 2004:685; Hallberg & Johnson, 2005:53; Tack & Van decasteele, 2008:283; Maestri *et al*., 2010:1) have stated that knowledge about differences in trace metal accumulation among plant species is essential to both evaluate the effect of plant growth on toxic metal cycling within marshes and to develop appropriate management options. To our knowledge, research presented here on their suitably as phytoremediants is the first study to investigate the effects of varied concentrations of Al and Fe exposure/accumulation and their effects on *C. alternifolius, C. prolifer* and *C. textilis* morphology and physiology (growth, chlorophyll and photosynthesis).

5.4 Varietal differences in Al and Fe tolerance and effects on growth

5.4.1 Bioaccumulation of Al in seedlings of Cyperus species: Al tolerance and plant growth

Metals are often toxic to life and differ from organic substances in that they cannot be decomposed by bacteria, and can only be absorbed and removed from the environment by organisms (Cheng, 2002:317). Studies on heavy metal tolerance by plants, especially in the case of grasses, have illustrated the power of natural selection and the manner in which plants are selected according to the metal tolerance of the genotypes (Bradshaw, 1976:1).

In this study, the assay for Al in *Cyperus* in the different plants samples was determined using methods described by Odendaal and Reinecke (1999:64) using ICP-MS (Inductively Coupled Plasma-Mass Spectrophotometer- 7500CE, Agilent, England). It was apparent from this study that the accumulation of metals by *Cyperus* affected the normal processes of plant metabolism. A relationship between high metal concentrations in plants and modified suppressed growth was found (Figs. 5.11-5.16). Results indicated that increasing Al concentrations caused reduction in root and shoot dry matter in all of the three plants studied. This was in agreement with the findings of Pereira *et al.* 2010:1496 where Al toxicity triggered alterations in the physiological status of cucumber seedlings (*Cucumis sativus*). In that study *Cucumis sativus* seedlings were grown in different concentrations of Al ranging from 1 to 2000 µM for 10 days.

During the initial seven days of growth, plants exposed to Al when compared with untreated controls, showed reduced growth and biomass production. Of the three species studied, *C. alternifolius* showed the most promising adaptation to exposure to Al. This was noted from the

growth and biomass production at five and seven days during the treatments. Over time, the plant height increased significantly (P≤0.05). The inhibition of growth of aquatic plants was measured as a reduction in fresh biomass due to toxic effects created by the Al. The average plant heights of *C. alternifolius* measured were 53.7, 51.7, 50.2, 48.4, and 46.7 cm in the 0.001, 0.01, 0.1, and 1 Al µM treatments respectively. In the control, a height of 59 cm was measured after seven days. Hence plant height decreased as Al concentration increased i.e. was inversely related (Tables 5.11-5.16). Throughout the course of the experiment, all *Cyperus* spp. continued to grow. This was seen by a significant increase (P=0.05) in total biomass yield recorded for the plants (Figs. 5.11-5.16).

5.4.2 Iron tolerance and effects on plant growth

Tolerance may be defined as the ability of organisms to cope with stress, either natural or anthropogenic, due to the chemical input of many different classes of contaminants into the environment (Atafar *et al.,* 2010:83). Resistance is frequently used in scientific literature as a synonym for tolerance. Determination of quality thresholds of aquatic pollutants is one of the outcomes of ecotoxicity studies; however determining a critical threshold to use as a guideline for total iron is difficult. Usually quality thresholds are determined for every pollutant separately, on the basis of the results of controlled experiments. Many have used root extension tests to determine the effects of metal tolerance (Wilkins, 1978:481; McCain & Davies, 1983:425), even though there is some evidence that these may be unsuitable for screening for iron tolerance (Al-Farraj, 1983:1).

Ideally, concentration-effect relationships should be established from *in-situ* studies, in order to integrate pollutant interactions within complex environments. However, this is extremely difficult when the effects are a result of exposure to mixtures, a common situation in nature. A concentration of total iron might represent a range of the directly biologically important dissolved iron (generally in the Fe^{2+} form) from a negligible percentage to a high percentage as part of the measurement of total iron. Total iron concentration and the relative proportion of ferrous iron may have a biological consequence depending on pH, temperature, dissolved oxygen concentration, humic acid concentration, chloride concentration or even solar radiation. Hussein et al. (2012:4032) reported that accumulation of iron recorded maximum values ranged between (105.5 and 900 µg/g d.wt.).

The idea of a critical or threshold tissue concentration of Fe relating to the commencement of a significant growth decrease as a result of toxicity is regularly used for diagnosis of toxicity

and to compare tolerances between plant species and metals. From a comparison of Fe concentrations corresponding to a 10% and a 50% decrease in yield of harvest, a qualitative estimate of the rate of toxicity can be determined. While the idea of critical concentration for toxicity is valuable, it relegates toxicity effects to one point along the response curve.

Tolerance to concentrations of Fe seems beneficial for environmental conservation because it contributes to the protection of biodiversity, which allows normal functioning of ecosystems. In any contaminated ecosystem, tolerance may be responsible for high burdens of toxicants in certain species with a subsequent risk of biomagnification. Therefore, it is important to assess carefully the health and ecological consequences of Fe tolerance. Analysing contaminants in biota instead of in water or sediment gives access to the bio-available fraction of the pollutant load in the medium that is the component which has the potential to induce toxic effects (Kabata-Pendias, 2011:98).

Plant roots show highest metal concentration followed by the shoots (Ashraf *et al.,* 2011:163). In this study, the effect of iron accumulation within *Cyperus* spp. caused morphological changes which included reduction in plant size and changes in colour when Fe concentration was high. Iron content of the three *Cyperus* spp in the different plants samples was determined using methods described by Odendaal and Reinecke (1999:64) using ICP-MS (Inductively Coupled Plasma-Mass Spectrophotometer- 7500CE, Agilent, England). Iron was most abundant in the plant root system with values ranging from (5.203 mg/kg^{-1}-6.031 mg/kg^{-1}) (Figs. 5.11-5.16). These observations confirm the view that wetland plants roots contain appreciable quantities of Fe (Aremu *et al.,* 2010:351).

Recently there has been an upsurge of research into the clean-up and remediation of heavy metal contaminated soil (Dushenkov, 2003:167; Meuser, 2010:1). Plants can only exert significant effects on the availability of metal through the release of exudates from the roots and in most cases most of the total contents of an element will not be available for immediate uptake by plants (Alloway, 2013:11).

From the results of this study (Figs. 5.11-5.16) it was found, with the exception of *C. textilis,* that high iron concentrations significantly ($P<0.05$) reduced the growth when compared with the control samples. The reason why iron tolerant species show little change in shoot: root ratio in response to increased iron concentration may be due to accumulation of ochreous deposits on roots which could increase root mass.

Lambers *et al.* (2008b:95) reported that different plant species are able to acquire different nutrients from various environments, where one plant will suffer nutrient deficiencies or even die and another will thrive or be able to sustain a healthy existence due to genetic adaptations. According to Sharma and Dhiman (2013:20), plant mechanisms of tolerance to toxic effects include restriction of entry of Fe into the cell through the plasma membrane and chelation of the metal by phytochelatins, metallothiones and nicotinamide making it less toxic for the plants.

A number of studies have reported iron toxicity is a problem in aquatic plants (Haese, 2006:241). Many have reported varied Fe concentrations for many species such as rice (*Oryza sativa* L.) (Howeler, 1973:898; Snowden & Wheeler 1993:35). Wang & Greger (2004:1779) found that in Duckweed (*Lemna minor*) Fe concentration was 3.7 mg/L and suggested a maximum permissible concentration of 0.37 mg/L. Den Dooren de Jong (1965:301) reported that at a concentration of 6 mg/L of iron ($FeCl_3$) the growth of *Chlorella vulgaris* was inhibited. Sinha *et al.* 1997:286 showed that in the aquatic plant *Hydrilla verticillata* 0.5 to 5.0 mg/L Fe was inhibitory. Batty and Younger (2003:801) reported on the effects of external iron concentration upon seedling growth and uptake of Fe (added as $FeSO_4.7H_2O$) and phosphate by the common reed *Phragmites australis.* They found an inhibition of growth above a concentration of 1 mg/L total Fe; however they suggested that the Fe alone might not directly explain the reduction in growth.

The accumulation of high concentrations of toxic metals may result in irreversible damage to the plant as they accumulate in plants organs (Sheoran *et al.,* 2011:168; Klos *et al.,* 2012:1829). Results recorded for *Cyperus* indicated that excessive Fe uptake occurred in the roots of *C. alternifolius* thereby producing toxic effects.

Uptake of metals into plant roots is a complex process involving transfer of metals from the soil solution to the root surface and inside the root cells. According to Berry & Wallace (1981:13) plant responses to metals are dose dependent.

The results obtained for this study from plant analysis asserted that roots of all three *Cyperus* spp effectively accumulated iron (Figs. 5.11-5.16). The highest concentrations (6.031 mg/kg^{-1}) were recorded from the roots of *C. alternifolius*, followed by *C. prolifer* (5.203 mg/kg^{-1}). The latter value agrees with that reported elsewhere for *Cyperus laevigatus* (27 398 $ug.g^{-1}$d.w) (Al-Qahtani, 2012:384). Similarly, Allen (1989:1) reported that Fe concentrations above 40-500 $ug.g^{-1}$d.w are toxic to plants.

Many scientists (Deng *et al.,* 2004:29, Rai *et al.,* 2004:697, Sinha & Gupta, 2005:1204) showed the maximum concentration of Fe to occur in plant roots and shoots. The result indicated that the metal accumulation on the root surface could be due to the co-precipitation in the iron oxyhydrate plaque layers. Kumar *et al.* (2006:193) used Energy Dispersive Analysis of X-rays (EDAX) to investigate elemental contents of the aquatic plants *Vallisneria spiralis*, *Hydrilla verticillata* and *Azolla pinnata.* They recorded high levels of heavy metals such as Al, Si, Mn and Fe.

Jacq *et al.* (2014) discovered that *Crocosphaera watsonii* WH8501 growing under a range of dissolved Fe concentrations (from 3.3 to 403 nM) suffered severe Fe limitation which led to significant decreases in: growth rate (2.6-fold), C, N and chlorophyll a contents per cell (up to 4.1-fold), N_2 and CO_2 fixation rates per cell (17- and 7-fold) as well as bio-volume (2.2-fold).

It has been recorded that that the use of metal tolerant plant species which can act as metal indicators and the ability of the same plants to accumulate metals are of immense use for biogeochemical prospecting (Basile *et al.,* 2012:374).

In the present study, exposure to heavy metals (Fe and Al) in the growth medium resulted in *Cyperus alternifolius* being able to assimilate and tolerate Fe as well as Al within the plant more efficiently than did either *C. textilis* or *C. prolifer*.

Total biomass yield per experiment in *Cyperus* spp increased significantly ($P \leq 0.05$) over the course of the experiment. Metal concentrations in the shoots were found to be higher than in the roots 6.031 mg/kg^{-1} Results showed that metals were higher in the roots which according to Jabeen *et al.* (2009:339) makes *C. alternifolius* an ideal plant for phytoremediation.

CHAPTER SIX

RESULTS AND DISCUSSION (PART 2): THE EVALUATION OF GROWTH PARAMETERS

Photosynthesis in shoots (leaf and stem) differs considerably and contributes to carbon budget. Plant and the environment, as well as the position of the leaf on the plant for the

interception of radiation, modulate leaf photosynthesis. Ageing affects net partitioning. Another important environmental factor is increased carbon dioxide levels which enhance the photosynthetic rate resulting in more dry matter response (Pettaralskli, 2009:152)

6.1 Chlorophyll concentration in *Cyperus* as influenced by exposure to Al and Fe

As a main component of the photosynthetic apparatus, chlorophyll molecules play major roles in the development and maintenance of life (Schoefs & Bertrand, 1997:47). Aluminium inhibits CO_2 assimilation in many plant species (Pereira *et al.,* 2010b:1496) as a result of both stomatal and non-stomatal factors (Simon *et al.,* 1994:307). The metal is also associated with structural damage to the thylakoids (Pereira *et al.,* 2010a:683) while Lidon *et al.* 1999:1 infer that Al induced a decrease in photosynthetic electron transport associated with photosystem 1 (PS1) (or plastocyanin: ferredoxin oxidoreductase). Moustakas *et al.* (1995:1) stated that the decline in photosynthesis caused by Al is a result of the closure of photosystem 11 (PSII) reaction centres and concomitant reduction in PSII electron transport rate. Work by Peixoto *et al.* (2002) showed that a combination of factors which include reduced pigment content, impaired PSII photochemistry and the distribution of enzymatic machinery could account for an observed Al-induced decrease in CO_2 assimilation.

In plants, iron is involved in chlorophyll synthesis, and it is essential for the maintenance of chloroplast structure and function. The involvement of iron in chlorophyll synthesis is the reason for the chlorosis (yellowing) associated with Fe deficiency. Pena-Olmos and Casierra-Posada, (2013:1) reported that excess Fe toxicity was related to the behaviour of the photosynthetic apparatus in broccoli plants which was principally based on measuring the fluorescence of chlorophyll a. Typically, approximately 80% of iron is found in photosynthetic cells where it is essential for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport system, and the construction of Fe-S clusters (Briat *et al*., 2007:276; Jeong & Connolly, 2009:709).

In this study, where the Al- and Fe-induced effects on three *Cyperus* spp were studied, chlorophyll content in the shoot and roots was determined by portable version of an imaging-PAM chlorophyll fluorometer (CCM-200) OPTI-SCIENCES, Hudson. Results demonstrated that excess Al caused toxic effects in the plants.

Biologically Fe, being the most abundant transition metal in the earth's crust and the most important nutrient for most living creatures as it is the cofactor for many vital proteins and enzymes therefore Fe toxicity manifests in plant as Chlorosis. These effects included a

reduction in both chlorophyll content, and therefore also possibly the rate of photosynthesis. When compared with the untreated controls, it was observed that chlorophyll content was significantly ($P<0.05$) reduced in treated seedlings and this reduction became more marked as the concentrations of Al and Fe in nutrient solutions increased (Tables 6.1-6.10). Once these metals are accumulated in shoots, they are thought to intervene in chlorophyll synthesizing pathways. The maximum reduction in total chlorophyll content was observed at 50 % µM of Al and Fe concentration. These results confirm the concept that the chloroplast is completely autonomous in the performance of steady state photosynthesis.

Table 6.1: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Al (day 1= 24 h)

Species	**Common Name**	**Treatment mg/litre**	**Chlorophyll (CCI)**		**Photosynthesis**	
			Before	**After**	**Before**	**After**
Cyperus alternifolius	Umbrella sedge	0	0.7	0.7	0.8	0.8
		0.001	1.1	0.9	0.8	0.6
		0.01	0.8	0.7	0.5	0.6
		0.1	0.7	0.6	0.7	0.7
		1	0.7	0.6	0.8	0.6
Cyperus prolifer	Flat sedge	0	0.8	0.8	1.2	1.2
		0.001	0.8	0.8	0.8	0.7
		0.01	1.0	1.0	1.0	0.6
		0.1	0.9	0.8	0.8	0.6
		1	1.6	1.5	1.5	1.3
Cyperus textilis	Mat sedge	0	0.9	0.9	0.8	0.8
		0.001	0.8	0.7	0.9	0.6
		0.01	0.8	0.8	0.8	0.6
		0.1	0.7	0.6	0.9	0.8
		1	1.0	0.9	1.2	1.0

Table 6.2: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Al (day 2= 48 h)

Species	**Common Name**	**Treatment mg/litre**	**Chlorophyll (CCI)**		**Photosynthesis**	
			Before	After	Before	After
Cyperus alternifolius	Umbrella sedge	0	0.7	0.7	0.7	0.7
		0.001	0.9	0.8	0.9	0.9
		0.01	0.7	0.7	0.7	0.6
		0.1	0.8	0.5	0.8	0.7
		1	0.6	0.6	0.6	0.5

Cyperus prolifer	Flat sedge	0	1.0	1.0	1.0	1.0
		0.001	0.8	0.7	0.8	0.6
		0.01	0.9	0.7	0.9	0.8
		0.1	0.8	0.8	0.8	0.7
		1	0.6	0.6	0.6	0.6
Cyperus textilis	Mat sedge	0	0.7	0.7	0.7	0.7
		0.001	1.1	1.0	1.3	1.1
		0.01	0.8	0.6	0.9	0.6
		0.1	0.9	0.8	0.9	0.8
		1	1.0	0.8	0.8	0.8

Table 6.3: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots and roots treated with Al (day 3= 72 h)

Species	**Common Name**	**Treatment mg/litre**	**Chlorophyll (CCI)**		**Photosynthesis**	
			Before	**After**	**Before**	**After**
Cyperus alternifolius	Umbrella sedge	0	0.7	0.7	0.8	0.8
		0.001	0.9	0.9	1.0	0.9
		0.01	0.7	0.7	0.8	0.8
		0.1	0.8	07	0.7	0.6
		1	0.6	0.6	0.7	0.6
Cyperus prolifer	Flat sedge	0	1.0	1.0	0.9	0.9
		0.001	0.8	0.8	0.9	0.6
		0.01	0.9	0.9	0.9	0.8
		0.1	0.8	0.7	0.9	0.7
		1	0.6	0.6	0.9	0.8
Cyperus textilis	Mat sedge	0	0.7	0.7	0.9	0.9
		0.001	1.1	1.0	0.9	0.8
		0.01	0.6	0.6	0.8	0.7
		0.1	0.8	0.7	1.0	0.9
		1	0.8	0.8	0.7	0.6

Table 6.4: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Al (day 5= 120 h)

Species	**Common Name**	**Treatment mg/litre**	**Chlorophyll (CCI)**		**Photosynthesis**	
			Before	**After**	**Before**	**After**
Cyperus alternifolius	Umbrella sedge	0	1.3	1.3	1.0	1.0
		0.001	0.9	0.8	1.3	1.1
		0.01	0.8	0.8	1.0	0.9
		0.1	0.6	0.6	0.9	0.8
		1	0.7	0.6	0.7	0.6
Cyperus prolifer	Flat sedge	0	1.1	1.1	0.9	0.9
		0.001	1.0	1.0	0.8	0.8

		0.01	0.9	0.7	0.8	0.6
		0.1	0.9	0.8	0.8	0.7
		1	0.8	0.7	1.3	1.1
Cyperus textilis	Mat sedge	0	1.3	1.3	1.2	1.2
		0.001	1.0	0.9	1.3	1.0
		0.01	0.9	0.8	1.0	0.9
		0.1	0.8	0.6	0.9	0.8
		1	1.2	0.9	0.8	0.7

Table 6.5: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Al (day 7= 168 h)

Species	**Common Name**	**Treatment mg/litre**	**Chlorophyll (CCI)**		**Photosynthesis**	
			Before	**After**	**Before**	**After**
Cyperus alternifolius	Umbrella sedge	0	0.9	0.9	0.7	0.7
		0.001	0.8	0.8	0.7	0.6
		0.01	0.6	0.5	1.1	1.0
		0.1	0.9	0.8	1.0	0.8
		1	0.8	0.7	1.0	0.9
Cyperus prolifer	Flat sedge	0	0.6	0.6	0.9	0.9
		0.001	0.7	0.7	0.6	0.5
		0.01	1.1	0.9	0.9	0.8
		0.1	1.0	0.9	0.8	0.7
		1	1.0	0.8	0.6	0.5
Cyperus textilis	Mat sedge	0	0.9	0.9	0.8	0.8
		0.001	0.8	0.7	0.6	0.5
		0.01	0.7	0.7	0.9	0.7
		0.1	0.9	0.8	0.8	0.7
		1	1.1	0.9	0.6	0.6

Total chlorophyll concentration is a unifying parameter for indicating the effect of specific interventions. Aluminium is well known for its toxic effects on plant growth and metabolism (Mihailovic *et al.,* 2008:159) but the threshold at which the metal becomes toxic varies markedly depending on species.

Monitoring the exposure and probable intervention for reducing additional exposure to heavy metals in the environment and in humans can become a momentous step towards prevention.

Results (Table 6.1-6.5) indicated that prolonged exposure of plants to high concentrations of Al significantly reduced the chlorophyll content in the *Cyperus* spp. investigated ($P \leq 0.05$). Thus it is probable that *Cyperus* plants had absorbed Al even to toxic levels. Aluminium toxicity has also been indicated to reduce the quantity of chlorophyll pigments and is therefore

accompanied by a marked decrease in overall photosynthesis and photosynthetic rate (Moustakas *et al.,* 1995:1; Pereira *et al.,* 2010:683).

Based on available data, it can be assumed that Al may inhibit chlorophyll synthesis and/or stimulate chlorophyll damage due to increased chlorophyllase activity. The inhibition in photosynthetic pigment accumulation in response to Al stress might be also a consequence of peroxidation of chloroplast membranes by enhancing the rate of H_2O_2 production and lipid peroxidation in chloroplast membranes.

Table 6.6: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Fe (day 1= 24 h)

Species	Common Name	Treatment mg/litre	Chlorophyll (CCI)		Photosynthesis	
			Before	After	Before	After
Cyperus alternifolius	Umbrella sedge	0	0.7	0.7	0.8	0.8
		5	1.1	0.9	0.8	0.6
		10	0.8	0.7	0.5	0.6
		15	0.7	0.6	0.7	0.7
		20	0.7	0.6	0.8	0.6
Cyperus prolifer	Flat sedge	0	0.8	0.8	1.2	1.2
		5	0.8	0.8	0.8	0.7
		10	1.0	1.0	1.0	0.6
		15	0.9	0.8	0.8	0.6
		20	1.6	1.5	1.5	1.3
Cyperus textilis	Mat sedge	0	0.9	0.9	0.8	0.8
		5	0.8	0.7	0.9	0.6
		10	0.8	0.8	0.8	0.6
		15	0.7	0.6	0.9	0.8
		20	1.0	0.9	1.2	1.0

Table 6.7: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Fe (day 2= 48 h)

Species	Common Name	Treatment mg/litre	Chlorophyll (CCI)		Photosynthesis	
			Before	After	Before	After
Cyperus alternifolius	Umbrella sedge	0	0.7	0.7	0.7	0.7
		5	0.9	0.8	0.9	0.9
		10	0.7	0.7	0.7	0.6
		15	0.8	0.5	0.8	0.7
		20	0.6	0.6	0.6	0.5
Cyperus prolifer	Flat sedge	0	1.0	1.0	1.0	1.0
		5	0.8	0.7	0.8	0.6
		10	0.9	0.7	0.9	0.8
		15	0.8	0.8	0.8	0.7
		20	0.6	0.6	0.6	0.6
Cyperus textilis	Mat sedge	0	0.7	0.7	0.7	0.7
		5	1.1	1.0	1.3	1.1
		10	0.8	0.6	0.9	0.6
		15	0.9	0.8	0.9	0.8
		20	1.0	0.8	0.8	0.8

Table 6.8: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Fe (day 3= 72 h)

Species	Common Name	Treatment mg/litre	Chlorophyll (CCI)		Photosynthesis	
			Before	After	Before	After
Cyperus alternifolius	Umbrella sedge	0	0.7	0.7	0.8	0.8
		5	0.9	0.9	1.0	0.9
		10	0.7	0.7	0.8	0.8
		15	0.8	07	0.7	0.6
		20	0.6	0.6	0.7	0.6
Cyperus prolifer	Flat sedge	0	1.0	1.0	0.9	0.9
		5	0.8	0.8	0.9	0.6
		10	0.9	0.9	0.9	0.8
		15	0.8	0.7	0.9	0.7
		20	0.6	0.6	0.9	0.8
Cyperus textilis	Mat sedge	0	0.7	0.7	0.9	0.9
		5	1.1	1.0	0.9	0.8
		10	0.6	0.6	0.8	0.7
		15	0.8	0.7	1.0	0.9
		20	0.8	0.8	0.7	0.6

Table 6.9: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Fe (day 5= 120 h)

Species	Common Name	Treatment mg/litre	Chlorophyll (CCI)		Photosynthesis	
			Before	After	Before	After
Cyperus alternifolius	Umbrella sedge	0	1.3	1.3	1.0	1.0
		5	0.9	0.8	1.3	1.1
		10	0.8	0.8	1.0	0.9
		15	0.6	0.6	0.9	0.8
		20	0.7	0.6	0.7	0.6
Cyperus prolifer	Flat sedge	0	1.1	1.1	0.9	0.9
		5	1.0	1.0	0.8	0.8
		10	0.9	0.7	0.8	0.6
		15	0.9	0.8	0.8	0.7
		20	0.8	0.7	1.3	1.1
Cyperus textilis	Mat sedge	0	1.3	1.3	1.2	1.2
		5	1.0	0.9	1.3	1.0
		10	0.9	0.8	1.0	0.9
		15	0.8	0.6	0.9	0.8
		20	1.2	0.9	0.8	0.7

Table 6.10: Chlorophyll and photosynthetic pigments content in *Cyperus* shoots/roots treated with Fe (day 7= 168 h)

Species	Common Name	Treatment mg/litre	Chlorophyll (CCI)		Photosynthesis	
			Before	After	Before	After
Cyperus alternifolius	Umbrella sedge	0	0.9	0.9	0.7	0.7
		5	0.5	0.4	0.7	0.6
		10	0.6	0.5	1.1	1.0
		15	0.9	0.8	1.0	0.8
		20	0.8	0.7	1.0	0.9
Cyperus prolifer	Flat sedge	0	0.6	0.6	0.9	0.9
		5	0.7	0.7	0.6	0.5
		10	1.1	0.9	0.9	0.8
		15	1.0	0.9	0.8	0.7
		20	1.0	0.8	0.6	0.5
Cyperus textilis	Mat sedge	0	0.9	0.9	0.8	0.8
		5	0.8	0.7	0.6	0.5
		10	0.7	0.7	0.9	0.7
		15	0.9	0.8	0.8	0.7
		20	1.1	0.9	0.6	0.6

Various studies have indicated that the leaf greening is the visible symptom of chlorophyll accumulation in developing chloroplasts (Peixoto *et al.,* 2002:1; Pereira *et al.,* 2010b: 1496). The actual process of photosynthesis is carried out within a specific cytoplasmic organelle viz.

the chloroplast. Photosynthesis is a primary physiological process and is greatly affected by a deficiency or toxicity of Fe. The effects on plant growth processes are seen at subcellular, cellular and also at the level of the entire plant. Sinha *et al.* (1997:286) studied the effect of iron ($FeCl_3$) on chlorophyll content in *Hydrilla verticillata* and found that a decrease in chlorophyll content is part of the overall expression of iron toxicity. It has been recommended that to avoid toxic effects the maximum total Fe concentration should not exceed 1.0 mg/L to protect aquatic systems from detrimental effects of iron (Phippen *et al.,* 2008:3).

Cyperus species accumulated heavy metals in a dose- and exposure-dependent manner (Tables 6.1-6.10). Biologically, Fe is the most important nutrient for most living creatures as it is the cofactor for many vital proteins and enzymes. This makes it soluble and readily available to organisms. Iron affected plants exhibit discolored leaves, diminished photosynthetic activity and decreased root respiration. Deaths of plants often occur if the concentration of reduced iron is high (Otte, 2001).

Prasad *et al.* (2006) reported that phytoextraction by accumulation of metals in harvestable plant parts becomes effective only in the presence of plants that either hyper-accumulate metals. Reduced photosynthetic pigment content can be used to monitor the heavy metal-induced damage in *C. vulgaris* cells (Teuchies *et al*., 2013:146).

Based on available data, it can be assumed that heavy metals may inhibit chlorophyll synthesis and/or stimulate chlorophyll damage due to increased chlorophyllase activity. The inhibition in photosynthetic pigment accumulation in response to heavy metal stress might be also a consequence of peroxidation of chloroplast membranes by enhancing the rate of H_2O_2 production and lipid peroxidation in chloroplast membranes.

Heavy metals accumulation which causes a decrease in total chlorophyll concentration invariably affects the Chla/Chlb ratio (Bragato *et al.,* 2006:967). However, loss in pigment content in heavy metal exposed plants could be the due to the:

(i) reduced efficiency of enzymes involved in chlorophyll biosynthesis viz. ð-aminolevulinic acid dehydratase;
(ii) decreased availability of ions such as Fe and Mn (heavy metals outcompete uptake of essential metal ions);
(iii) peroxidation of chloroplast membranes resulting from heavy metal induced oxidative stress; and
(iv) formation of metal substituted chlorophylls (Dhir *et al.,* 2011:1678).

The photosynthetic pigments namely chlorophyll a, chlorophyll b and total chlorophyll were isolated from leaves (Arnon, 1949.) and then the plant samples were homogenized in methanol. The absorbance of the extract was spectrophotometrically measured at 645, 663 and 750 nm. The amounts of chlorophyll-a + b present in the extract were calculated according to the equations of Wellburn (1994:307). Results from this study revealed that the accumulation of Al and Fe at high concentrations within the plants affected production of photosynthetic pigments (Tables 6.1-6.10). Chlorophyll content was reduced after exposure to both metals. Reduced plant growth was also observed. This was probably due to reduction in photosynthetic and metabolic rates caused by the presence of excessive levels of Al and Fe.

6.2 The influence of heavy metals on photosynthesis

Metal toxicity is known to reduce photosynthesis and it also apparent that different plant species appear to elicit different responses.

It is generally assumed that the major sites of metal sequestration are the vacuoles of root cells. Plant roots contribute to making metal ions more available to the uptake proteins as they not only acidify the rhizosphere through plasma membrane-localized proton pumps, but actively secrete low-molecular weight (LMW) compounds that can function as metal chelators and the production of phytochelatins. In the current study on the effects of Fe and Al on *Cyperus* there was a severe adverse response in photosynthetic pigments when levels of these metals became toxic. Similar results were reported elsewhere for *Lycopersicon esculentum*, and *Elsholtzia splendens* (Sytar *et al.,* 2013:985).

The idea of critical or threshold toxicity is often used to establish the point at which metals cause a yield decrease of 10%. Critical concentrations vary across metals and plant species. Although Al is thought to be nonessential and not beneficial to plants, many species will absorb and incorporate the metal into their biomass. This often occurs together with the uptake of other metals. Li *et al.* (2012:1) reported the mechanism by which Al affects PSII photochemical activity and these authors characterized the target site of Al in the photosynthetic electron transport chain.

Photosynthetic pigments in the leaves of the *Cyperus* seedlings investigated were sensitive to both Al and Fe toxicity (Tables 6.1-6.10). Photosynthesis is considered to be sensitive to most abiotic stresses. Both Al and Fe exposure have been reported to decrease the chlorophyll

contents in *Triticum aestivum* (Latif, 2008:4832), and *B. juncia* (Ebs & Unchil, 2008:49). The reduction of biomass caused by Cd toxicity was considered as a direct consequence of inhibition in chlorophyll biosynthesis and photosynthesis (Hasan *et al.,* 2009:165).

Mihailovic *et al.* (2008:21) describe how plant productivity in acid soil is affected by Al toxicity which causes photosynthetic damage associated with pigment degradation, carbon assimilation inhibition and decreased photosynthetic electron transport. This occurs when Al enters plant cells, and accumulates in the chloroplast, where the metal reacts with or replaces the non-heme iron between chlorophyll a and chlorophyll b binding sites. It also blocks PSII electron transport, resulting in PSII photochemical damage and inhibition of photosynthesis.

Dhir *et al.* (2011:1678) reported that heavy metal accumulation affected the physiological status of *Salvinia* by altering photosynthetic potential. This was seen by changes in the carbon assimilation efficiency with slight variations in primary photochemical activities and also in photophosphorylation potential. The report found PSII activity declined in Ni, Co, Cd, Pb, Zn and Cu-exposed plants, while PSI activity appeared to be enhanced under conditions of heavy metal stress. An increase in PSI activity supported build-up of the transthylakoidal proton gradient (*Δ*pH), which subsequently assisted in maintaining the photophosphorylation potential. Ribulose 1,5 dicarboxylase/oxygenase (Rubisco) activity declined.

Naumann *et al.* (2008:402) reported that leaf chlorophyll content gives an indication of photosynthetic ability, mutations and stress condition. It has been confirmed that inhibition of growth and photosynthesis are the basic reflex of the toxic effects of pollutants on wetland plants (Dhir *et al.,* 2011:1678).

Xing *et al.* (2010:103) have reported on the effect of excessive heavy metal concentrations on production of photosynthetic pigments viz. chlorophyll *a*, chlorophyll *b* and total chlorophyll as its acts by slowing down the transport of electrons in the photosynthesis process. In contrast, exposure of Fe promoted chlorophyll synthesis in *Salvinia natans* according to finding by Dhir *et al.* (2011:1678).

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Concluding remarks

This study reports that the degradation and destruction of wetlands is a continuing concern in most parts of the world. Globally, human populations are suffering socially, economically and environmentally from the destruction and mismanagement of natural resources, particularly the wetlands and water resources (Pereira *et al.,* 2010b:1496). Wetlands provide substantial economic benefits in areas that include the following; water supply (quantity and quality), fisheries (over two thirds of the world's fish harvest is linked to the health of coastal and inland wetland areas); agriculture, through the maintenance of water tables and nutrient retention in floodplains; timber production; energy resources (peat and plant matter); wildlife resources; transport and recreation and tourism (Naidoo *et al.,* 2008:9495). Although wetlands are amongst the richest life-supporting ecosystems on earth, they are amongst the most threatened and are being destroyed. Wetlands are cradles of biological diversity, providing the water and primary productivity, upon which countless species of plants and animals depend for survival. Rice, for example, which is a common wetland plant, is the staple diet of more than half of humanity (Pereira *et al.,* 2010b:1496). Wetlands are also important storehouses of plant genetic material.

Although some heavy metals are important and essential trace elements, at high concentrations, as occurs in many environments, many can become toxic. Scientific studies have concluded that heavy metal contamination is prevalent in the environment due to human activities. It is common knowledge that metal concentrations within aquatic plants vary considerably according to the plant tissue involved as well as to the type of elements.

One of the most important design considerations for research into the phytoremediation of wetlands is the selection of appropriate candidate plant species and varieties. *Cyperus* were found capable of achieving the desired treatment objectives and readily adapted to the chemical treatments and greenhouse conditions. Once the plant species were selected, phytoremediation studies which included growth rates and evidence of plant stress, such as adverse effects on chlorophyll were used to evaluate the suitability of the selected *Cyperus.*

Plants were obtained from commercial nurseries located as close as possible to the experimental site, as endemic species generally ensure some resistance to local pests and diseases.

Greenhouse studies have shown that the primary factors that determine the amount of water removed from a closed system via evapotranspiration are the total aerial plant biomass of transpiring tissue and its surface area. Sedges and Rushes such as *Spartina alterniflora,* (Saltwater Cordgrass) can be ideal for volume reduction. In the current study suitable experimental design was ensured, to accurately quantify the effects of the various experimental conditions including the choice of plants and substrates.

Water is becoming a scarce resource. The conservation of water resources is high on the agenda of the world leaders. Water is required to meet ever-increasing domestic, industrial and agricultural demands. Wetland and wetland plants offer a means to continually treat wastewater for reuse to meet increasing shortages of water resources of good quality in many parts of the world.

The present study showed that a systematic improvement in phytoremediation can be better understood through processes involved in plant heavy metal uptake, transport, accumulation and resistance. Plants, already established as a major renewable resources exploited by humans to supply food, energy, construction material natural fibres and medicines, may also offer a means of removing pollutants from the environment. Some plant species present suitable options for phytoextraction and phytostabilization. Growth factors important for successful phytoremediation can provide a basis for the genetic modification of plants for improved performance. Biotechnological and genetic engineering based approaches could be used to enhance naturally occurring plants such that they detoxify hazardous compounds.

Wetland plants have adapted to tolerate the presence of metals and many grow actively in their presence. Thus the interactions between these plants and metals have important environmental and ecosystem implications. Today, wetland plants are being used internationally for the remediation of metal-contaminated sites.

Macrophytes are exposed to elevated levels of heavy metals in aquatic ecosystems. Extensive proliferation of both industrial and metallurgical activities in recent years has caused the appearance of large quantities of heavy metals in the environment (Wood & Mcatamney, 1994:653; Kamal *et al.,* 2004:1029).

The heavy metals are readily available and easily absorbed by wetland plants from environmental media such as soil, water and sediment. Heavy metals generally reduce chlorophyll content in the plant, decrease the chlorophyll a/b ratio and enhance chlorophyllase activity which causes degradation of chlorophyll. Aluminium, a non-essential heavy metal, exerts strong phytotoxicity at elevated concentrations. This toxicity is seen as interference in physiological parameters such as photosynthesis by lowering chlorophyll content. Thus net photosynthetic rate (P_N) is decreased as discussed in the foregoing.

Past research shows that macrophytes can be used for heavy metal removal from contaminated water bodies (Arora *et al.,* 2006:97; Umali *et al.,* 2006:45). Aquatic macrophytes have much potential to accumulate heavy metals from the environment (Khan *et al.,* 2006:223; Maine *et al.,* 2009:355). The role of plants in metal removal is through filtration, adsorption and cation exchange and through plant-induced chemical changes in the rhizosphere. Aluminium and Fe are metals that are naturally included in the clay mineral structure (Ashman & Puri 2002:1). A lack of oxygen in wetlands is common and constitutes a problem for plant growth. Anaerobiosis is due to constant bacterial activities decomposing the organic matter that accumulates on the bottom of the system. Many research studies have reported that plants play significant roles in the wetland treatment processes, particularly when oxygen gas is introduced to the rhizome system which serves as a substrate for microbial biofilm and uptake of nutrients (Karathanasis *et al.,* 2003:157).

Environmental restoration and management of contaminated wetlands are major problems faced by industries and countries. Although ecological restoration can generally be viewed as positive, its impact on inorganic and organic contaminants in the environment must be understood for risk assessment, long-term management, and potential cost-effective mitigating measures. The risks associated with accumulated contamination are especially pronounced if pollutants are recalcitrant to degradation or persistent and bio-accumulative.

Macrophytes are important members of the emergent plant community and are sensitive to various abiotic stresses (Choudhary *et al.,* 2007:204). Wetlands rely on supplies of clean water. Whenever the water is diverted for irrigation or polluted, the wetland or any component therein may never recover (Cabrera *et al.,* 2006:1). A plant has a natural range of conditions under which it can thrive, but beyond that its growth will be poor and it may die (Milner & Kochian, 2008).

Excess accumulation of Fe in plant tissues is a rare phenomenon; but Fe increases in leaves may lead to severe cellular damage. However, Fe toxicity in soil is often caused by an acidic pH, cation exchange capacity, base status, levels of K, PO_4, Zn, and easily reducible Mn. Excessive Fe often causes deficiencies in Zn and Mn which is marked by imbalances of nutrients the presence of H_2S. Increased Fe concentration in plant shoots is generally identified by the appearance of bronze spots caused by increased uptake of Fe in the chloroplasts and is followed by a dramatic impairment of total photosynthetic electron transport capacity. Iron toxicity may cause the stimulation of photorespiration (Merkl *et al.,* 2005:86; Almeida *et al.,* 2006:424). Importantly, an understanding of photosynthetic efficiency underlines the biological activity on our planet. This is targeted toward increasing plant productivity. Photosynthesis forms the basis on which all ecosystems on earth function.

Many studies have demonstrated the potential of some grasses and other hyper-accumulating plants with high production of biomass, a deep root system, rapid growth and high tolerance to metals as suitable for use in environmental biotechnology research for the decontamination of sites contaminated with heavy metals (Merkl *et al.,* 2005:86; Almeida *et al.,* 2006:424). The techniques for applying biological processes for decontamination are daunting. In order to remove these contaminants, heavy metals must be extracted and concentrated by a unique technique for proper disposal in designated secure landfills areas. Hyper-accumulation of various metals by different plant species wherein many of these metals are partitioned in the shoots and roots is of considerable importance in the environment. For plants to qualify as useful for phytoremediation, they must be able to yield high biomass and withstand the metal stress.

Emergent wetland plants are sessile; and only their roots are in an anoxic environment. Normally, wetland plants roots are so prolific that the oxygen supply rapidly decreases. This terminates aerobic metabolism of the roots, impairs the energy status of the cells, and reduces nearly all metabolically-mediated activities such as cell extension and division, and nutrient absorption. When cell metabolism shifts to anaerobic glycolysis, adenosine triphosphate (ATP) production is reduced. Under these conditions, toxic metabolic end products of fermentation may accumulate, causing cytoplasmic acidosis and eventually death. Anoxia is soon followed by pathological changes in mitochondrial structure. The complete destruction of mitochondria and other organelles occurs within 24 h. Anoxia further alters the chemical environment of the root, increasing the availability of reduced forms of Fe, S and Mn, which may accumulate to toxic levels in the root.

Metabolic problems encountered by wetland plants deprived of oxygen are associated with the loss of the electron acceptor that enables normal energy metabolism through ATP formation and use.

Excessive amounts of certain heavy metals can be toxic through the direct action of the metal or through their inorganic salts or via organic compounds from which the metal can become easily detached and introduced into the cell. Exposure to different metals may commonly occur under many environmental circumstances particularly those associated with industrial activity. Accidents in some environments can result in acute, high levels of metal exposure. Metal uptake rates vary according to the organism and the metal in question. Accumulation usually occurs in plant roots, but may also occur throughout the plant (Ahalya *et al.,* 2005:258). Some of the heavy metals are toxic to aquatic organisms even at low concentrations. Faced with these problems, wetland plants have several physiological adaptations that attempt to solve the problem of anoxic conditions in root system.

The contamination of soils and water with metals has been an increasing major environmental problem throughout the world and emerging phytoremediation technologies with their lower cost and environmentally friendly nature offer solutions. Phytoremediation presents a unique opportunity to treat wastes in wetland ecosystems. Plants contribute significantly to contaminant removal by accumulating pollutants, altering hydrology and sequestering particulates.

By 2030 the world will need 40% more water than it is currently using (Abira *et al.,* 2005:173). Furthermore statistics produced by the United Nations show that 770 million of the world's seven billion people already lack access to safe drinking water.

Field studies involving wetlands, such as those containing *Cyperus* spp. provide information on the extent of Al and Fe removal that could be expected in natural or constructed wetlands. The results of this study provided insight into the potentially important removal mechanisms that could influence the fate of Al and Fe in the wetland rhizosphere. However, because the conditions used in the greenhouse/laboratory are artificial and different from those encountered in wetland ecosystems, further research is required before accurate predictions can be made about the fate of Al and Fe in ground water that discharges into wetlands.

The quantities of Al and Fe may be even more marked in a natural wetland than the values used in this study. The uptake rates could be seasonal. For example during the summer months *Cyperus* spp. are considerably larger than the plant sizes used in the current study.

Hence in summer in a wetland higher levels of Al and Fe phytovolatilization might occur. This would be accompanied by increased oxidation of Al and Fe by microorganisms through increased delivery of oxygen to the rhizosphere. Additionally, summer time temperatures will be higher than those used in the study under discussion. Higher temperatures could further increase microbial activity including the extent of microbial-mediated oxidation of Al and Fe. Conversely, during the winter months, when there are no active growing shoots and microbial activity is decreased, there may be less removal of Al and Fe than was observed in this study. The effects of these environmental changes would have to be factored into any assumptions about Al and Fe removal in either natural or constructed wetlands.

In the present study, where Al and Fe toxic responses in roots and stems were investigated in *Cyperus* it was clear that Al toxicity reduced plant growth and chlorophyll content which agrees with the report of Guo *et al.* (2012:207).

The recent use of plants or plant products, collectively known as phytoremediation, takes advantage of the natural abilities of plants to take up, accumulate, store, or degrade organic and inorganic substances. This is seen as an innovative and cost-effective option to address recalcitrant environmental contaminants and aims to restore or stabilize contaminated sites (Matthew *et al.,* 2004:39; Lesage *et al.,* 2007:102; Vymazal *et al.,* 2007:154; Maine *et al.,* 2009:355).

Wetlands occur on all of the continents and are depository areas composed of highly organic sediments and dominated by large aquatic macrophytes representative of diverse groups which could be emergent, submerged, floating or rooted (ARS-GRIN, 2013). These macrophytes have the ability to survive adverse conditions and exhibit high colonization rates. Hence they are excellent tools for phytoremediation (Gratão *et al.,* 2005b:53).

The presence of one metal can significantly affect the impact of another metal on an organism. These could be synergistic, additive or antagonistic (Batty *et al.,* 2002:443). For example, Fe^{3+} will occur in acidic solutions but a slight increase in hydroxyl ion concentration would result in its precipitation as a hydroxide thereby rendering it unavailable. However, if the solution contained Fe^{2+} and another metal such as $Mn^{3+,}$ then, the following reaction is possible:

$$Fe^{2+} \rightarrow Mn^{3+} \leftrightarrow Fe^{3+} + Mn^{2+}$$

The problem of heavy metal pollution in water and aquatic organisms needs continuous monitoring and surveillance as these elements do not degrade and tend to be biomagnified in

man through the food chain (Singh *et al.,* 2011:246; Wu *et al*., 2012:1991). Hence, there is a need to remove heavy metals from aquatic ecosystems. It should be borne in mind that what humans put into the environment has many implications and a multiplicity of consequences for all life forms. Any plants found with genetic potential for uptake, extraction, degradation, metabolization and immobilization of pollutants present a promising means of cleaning up of contaminated sites by phytoremediation.

The current study has shown the duration of exposure to Al is critical when assessing a threshold concentration for Al toxicity because wetland plants can adapt to Al concentrations previously considered toxic. Moreover, an inter-related network of physiological and molecular mechanisms governs plant tolerance to a particular metal. An understanding of these mechanisms and their genetic basis is an important aspect for the development of plants as agents of phytoremediation (Seregin & Kozhevnikova, 2006:257).

As wetland vegetation responds to changes in water supply, measurement of any changes in the plant distribution and composition of species will give an indication as to the viability of such an area. This could provide environmental managers with a means of determining, implementing and monitoring the ecological reserve for wetlands. The extensive use of rapidly growing *Cyperus alternifolius* as a phytoremdiant in wetlands could be cost effective.

Becker (2005:1) and Prasad (1999:1; 2004:1) stated that wetland plants do not undergo Al stress at low metal concentrations as do terrestrial plants. An assessment and thorough understanding of the toxicity threshold and toxic effects of trace elements on wetlands plants is highly desirable as this would enable assessment of the ecological viability status and environmental risks of contaminated sites. While ideally wetland plants to be considered for any revegetation and tolerant of a contaminant should be done *in situ*, results obtained in the artificial conditions used for the current study have provided reliable data, supporting the importance of the selection of suitable plants.

In this era of climate change, the ability of wetland plants in accumulating toxic metals and their use in bioremediation is of great interest. The screening of *Cyperus* spp in this study which are often considered to be weeds has shown the immense potential of these plants to hyper-accumulate metals for bioremediation of contaminated sites.
Kyambadde *et al.* (2004:475), in their comparative study of *C. papyrus* and *Miscanthidius violaceum* as phytoremediants in constructed wetlands for wastewater treatments in a tropical country, reported that *C. papyrus* was more suitable. Climate-sustainable water resource

management should be part of a long-term strategy of the conservation community to assist economies and terrestrial and freshwater ecosystems to adjust to an uncertain future (Matthew *et al.,* 2011:1).

Climate change appears to be exacerbating the variability and intensity of weather patterns and events. According to a report titled 'Climate change 2014: Impacts, adaptation and vulnerability' from working Group 11 of the Intergovernmental Panel on Climate Change (IPCC) which details the impact of climate change to date, effects of climate change are already occurring on all continents and across the oceans. The need for effective action can reduce risks. The report noted that observed impacts of climate change have already affected agriculture, human health, ecosystems on land and oceans.

Humans have always interacted with the environment. Wetlands, formerly taken as wastelands are now appreciated as vital to the continued existence of aquatic ecosystems In order to increase the ability to manage and protect wetland ecosystems, as well as obtain the maximum benefits therefrom, wetlands and wetland plants are receiving international attention. This is attributable to the fact that wetland plants have been found vigorously growing in different metal-contaminated sites (Deng *et al.,* 2004:29; Jiang & Wang, 2008:697).

Functioning wetlands have benefits for all life forms. Some of these benefits particularly those categorised as indirect are not obvious and can be easily overlooked. For this reason, many of the wetlands in South Africa particularly, have been destroyed through development and degradation. Unless action is taken to positively influence the activities of people affecting wetlands, the results could be disastrous. In a water-scarce country like South Africa, continued destruction of wetlands will result in lower agricultural productivity, decreases in potable water supplies, increases in downstream flooding; and increasingly threatened plant and animal resources.

To diminish environmental pollutants and to establish sustainable living on a global scale, there is a need to address three important issues: changing life styles to prevent or decrease emission of pollutants, develop technologies to avoid or greatly decrease emission of pollutants; and decontaminating pollutants that are already in the environment. The development of a regional monitoring programme of ecosystem health could be linked to effective monitoring for the impacts of climate change and associated increases in sea levels. Furthermore, this may assist in curbing seasonal storms in various areas. Finally, a

comprehensive understanding of the uptake, tolerance and transport of Al and Fe by plants will be an essential contribution to the development of phytoremediation technologies.

Adequate knowledge of wetland ecosystems is a fundamental prerequisite for effective policies and management. In accordance with Hanjra and Qureshi (2010:1) there is need for better understanding among stakeholders of how biodiversity, water resources, climate change and environmental integrity interact with food security. The stakeholders involved with protection of fragile ecosystems such as wetlands should work together to prevent the impacts of high doses of metals on these environments which can have an immediate or a delayed detrimental effect on human health.

As conservation of water resources with regard to the quantity and quality is being increasingly emphasized across the globe, it becomes imperative for all stakeholders to safeguard and restore wetlands which are vital for water security, biodiversity, climate regulation, and sustainable development. Water resources are sensitive to climate, and future climate change will have an effect on the hydrology of rivers and lakes. This in turn impacts on the plant diversity, plant structure and competition for resources.

In summary, heavy metals have been described as “the most common pollutants in wastewater” (Prasad, 1999:1; Li, *et al.,* 2008:553). Heavy metals are particularly destructive because they can bio-accumulate and bio-magnify within an ecosystem (Bradl, 2005:1). There is a variety of anthropogenic sources of heavy metal contamination such as: mining and smelting operations, road and transportation runoff, landfill sites, agriculture, and fossil fuel combustion (Bradl, 2005:1). Sources of heavy metal contamination can be of gaseous, particulate, aqueous, solid of diffuse or point origin (Bradl, 2005:1).

A key understanding of the transportation mechanisms of heavy metal contaminants provides insight into designing a targeted management strategy, policy or remediation method. Granted that there are many uncertainties in predicting patterns of contamination, scientific research plays a central role in establishing effective policies. Treatment with macrophytes such as *Cyperus* would be cost effective. A comprehensive understanding of the uptake, tolerance and transport of Al and Fe by all wetland plants in their environments will be essential for the development of phytoremediation technologies (Cheng *et al.,* 2002:335; Basile *et al.,* 2012:374).

The conclusions drawn from the study were:

Results indicated that during the experimental period *C. alternifolius* and *C. textilis* were able to accumulate high levels of Al and Fe, thus removing these two elements from the aqueous environment. This accumulation did not prevent an increase in plant biomass i.e. the two species continued to grow in size. The greater accumulation was demonstrated by *C. alternifolius and C. prolifer* which accumulated both metals in the roots and shoots. The study also provided an insight into the mechanisms of Al and Fe accumulation, tolerance and resistance in *C. alternifolius*, *C. prolifer*, and *C. textilis*.

Plant metal uptake depends on the specific metal element and plant species. The results showed that during the test period, shoots of *Cyperus* spp accumulated Al and Fe in lower concentrations than did the roots. This could be due to the fact that the shoots have a shorter life span than do the rhizomes.

That an individual stressor such as the exposure to one metal provokes not only stress-specific but also a general stress-response in *Cyperus*.

Cyperus can take up heavy metals by their roots, or even via their stems and leaves, and accumulate them.

Cyperus take up elements selectively.

Cyperus responses to metals are dose dependent.

Accumulation and distribution of heavy metals in *Cyperus* depends on the plant species, element species, and chemical bioavailability.

During the test period, none of the screened *Cyperus* showed any reduction in growth and physical deterioration. Over the test period adverse effects such as chlorosis, necrosis, or whitish-brown discolouration were not observed. The hydroponic studies conducted indicated that the three *Cyperus* spp. survived in the presence of Al and Fe. Further extensive studies are required, but it is possible that the species could be used for phytoremediation of wetlands contaminated with Al and Fe.

7.2 Recommendations

1. The ability of three *Cyperus* spp. to accumulate the selected metals and survive throughout the experiment demonstrated the potential of the selected macrophytes to remediate waters polluted with Al and Fe. This should be pursued further.

2. This systematic study showed that it is important to select for species that could be used for extracting heavy metals. There is a need to develop coordinated research collaborations to evaluate the wetland ecological status for the future.

3. Phytoremediation is an interdisciplinary technology requiring research in many disciplines. One of the disciplines recommended for further investigation would be a study of the biological processes that underlie a plant's ability to detoxify and accumulate pollutants**.**

7.3 Future research

Wetland plants such as *Typha latifolia* and *Phragmites australis* have been identified in previous studies as important in relation to water treatment (Vymazal *et al.,* 2007:154; 2009:303; Brisson & Chazarenc, 2009:3923). This study has established that *Cyperus* has properties that enhance its potential for use in phytoremediation. These properties include a rapid growth rate associated with high biomass production, a moderately extensive root system, ease of harvest and tolerance to a wide range of heavy metals. Information with respect to the molecular mechanisms involved in phytoremediation in *Cyperus* were not investigated during the current study. It is likely that such an in-depth molecular analysis on *Cyperus* would be required to ascertain whether the species is actually an ideal phytoremediant.

An integrated approach should be developed such that *Cyperus* biomass produced during phytoremediation could be used as a source of bioenergy or bio-ore for the recovery of marketable amounts of precious heavy metals. Plant residue (cake) remaining after any such extraction of heavy metals could be used as protein-rich feed for animals or for use as a green manure.

Much research on metal transporters and their regulatory genes in *Cyperus* is still required. This will provide effective strategies to utilize *Cyperus* for the treatment of wastewater with multi-element contamination. The impact of heavy metal uptake on the overall physiological/biochemical metabolism and its regulation at the genetic level represent further areas for key future research.

Wetlands are ecosystems that perform important ecological and socioeconomic functions for water resources management and therefore should be preserved. The solution to environmental problems will involve the co-operation of multidisciplinary teams comprised of scientists, engineers, sociologists, and lawyers. These groups need to collaborate to design

and implement processes or procedures to solve or prevent a real or perceived environmental problem. New technologies and stricter environmental sanctions/laws are expected to improve enforcement and compliance on climate change. Van Dam *et al.* (2014:469) stated that the importance and significance of wetland ecosystems are better understood through integrating wetland policy with other sectors such as water resources management and agriculture. There is little information about the response of wetland plant communities to changes in water depth and human exploitation. It is important to use endemic plants for phytoremediation because plants that mediate the clean-up should be adapted to the soil properties, toxicity level, and climate of the contaminated site. Climate-sustainable water resource management should be part of the long-term strategy of the conservation community to assist economies and terrestrial and freshwater ecosystems to adjust to an uncertain future.

When applying a metal pollutant to a wetland plant, the duration of the application should be sufficiently long to allow the metal concentration range to be increased until plant mortality is observed (Fonkou *et al.,* 2005:459). The mortality of the plants should occur before the highest dose is applied. Plant mortality may be a better indicator of genotype variability than growth parameters. Test concentrations of metals should be consistently increased in arithmetic or logarithmic increments. The dose should be increased at every time interval to prevent plants from becoming acclimatised to one concentration. Overall, extensive research is needed to expand our knowledge base of the sources of metal contaminations, their transportation into the aquatic environments, and management of their use to prevent severe contamination in the environment.

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Aquatic ecosystem health and management, 17(2):122-128.

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International Journal of Ecosystems and Ecology Science, 3(4):819-828.

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APPENDIX A: Classification of key words and concepts applicable to this study

Bio-assessment	A tool for the assessment of metal pollution in aquatic ecosystems. The bioindicators could be algae, macrophytes, zooplankton, insects, bivalves, molluscs, fish etc. (Zhou *et al.* 2008:135). An evaluation of the biological condition of a water body that uses biological surveys and other direct measurements of resident biota in surface waters.
Biodiversity	The variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological

	complexes of which they are part; this includes diversity within species, between species and of ecosystems.(Convention on Biological Diversity, [CBD], 1992; National Environmental Management Biodiversity Act [NEMBA], 2004).
Biological resources	Includes genetic resources, organisms or parts thereof, populations, or any other biotic component of ecosystems with actual or potential use or value for humanity (CBD, 1992).
Ecosystem	Means a dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit (CBD, 1992).
Environmental pollutants	The build-up and concentration of toxic levels of chemicals in the air, water, and land, which reduces the ability of the affected area to support life (Gleick, 2001:1; 1998:23).
Oxidative stress	A persistent imbalance between antioxidants and pro-oxidants in favour of the latter, resulting (often) in irreversible cellular damage. Oxidative stress is the ability of a biological system to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells (Singh *et al.* 2011:246).
Reactive Oxygen Species (ROS)	Reactive oxygen species present in high concentrations in a medium can cause toxic effects due to their ability to coordinate various organic compounds resulting in an inhibition of some metalloenzymes systems. The ROS can be viewed as the cellular and secondary messengers involved in all aspects of plant metabolism such as gene expression; translation to enzymes chemistry. It may also lead to membrane lipid damage, protein, pigment and nucleic acid damage which ultimately can result in dramatic growth retardation and productivity finally causing plant death (Luhua *et al.* 2008:280; Tsukagoshi, 2012:30, Lopez-Alarcon & Denicola, 2013:10).
Toxicity	Adverse effect on any system such as wetland ecosystem resulting from exposure to toxic substances (Environmental Protection Agency United States of America [US EPA, 2000).
Wetlands	The International Union for the Conservation of Nature and Natural Resources (IUCN) defines wetlands as “areas of marsh, fen, peat-land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tides does not exceed ten meters”(Ewart-Smith *et al.* 2006:10);or land where an excess of water is the dominant factor determining the nature of soil development and the types of animals and plant communities living at the soil surface. It spans a continuum of environments where terrestrial and aquatic systems intergraded (Cowardin *et al.* 1979: 31).Tables 1.2 and 1.3 are examples of different types of wetland classification and definitions.
Wetland plants	Defined as those plant species normally found growing in wetlands, either in or on the water, or where soils are flooded or saturated long enough for anaerobic conditions to develop in the root zone (Ewart-Smith *et al.* 2006:10) as shown in Table 1.4.

Xenobiotics	These are important sources of oxidative stress which are reduced in living cells and form oxygen (O_{2^-}) upon oxidation. Examples include compounds which inhibit electron transport, and cause an increase in oxygen production in the chloroplasts

APPENDIX B: Selected terms and types of wetlands based primarily on Mitsch & Gosselink (1993:582)

Types of wetlands	**Definition**
Bog	Peat accumulation usually dominated by moss. Receives only direct precipitation; characterized by acid water, low alkalinity, and low nutrients.
Fen	Peat accumulation; may be dominated by sedge, reed, shrub or forest. Receives some surface runoff and/or ground water, which has neutral pH and moderate to high nutrients.
Mire	Used mainly in Europe to include any peat-forming wetland (bog or fen).
Marsh	Permanently or periodically inundated site characterized by nutrient-rich water. In Europe, this must have a mineral substrate and lack peat accumulation.
Playa	Shallow, ephemeral ponds or lagoons that experience significant seasonal changes in semi-arid to arid climates. Often have high salinity or may be completely dry.
Slough	Widely used term for wetland environment in a channel or series of shallow lakes. Water is stagnant or may flow slowly on a seasonal basis. Synonym--bayou.
Swamp	Characterized by forest, shrub, or reed cover (fen). Particularly a forested wetland in North America. Depends on nutrient-rich ground water derived from mineral soils.
Wet meadow	Open prairie, grassland or savannah with waterlogged soils but without standing water for most of the year.
Open water	Deeper, normally perennial pools within wetlands and shallow portions of lakes and rivers. Typically home to submerged macrophytes.

APPENDIX C: Commonly used wetlands terms and their definitions, based on US EPA (2000)

Types of wetlands	Definitions
Marsh	A type of wetland ecosystem characterized by poorly drained mineral soils and by plant life dominated by grasses.
Tidal marsh	Marshes are common at the mouths of rivers, especially where extensive deltas have been built. The marsh plants retard the flow of water and allow for the nutrient- enriched sediments to be deposited, thus providing conditions for the further development of the marsh.
Swamp	A wetland ecosystem characterized by mineral soils with poor drainage and by plant life dominated by trees Swamps are found throughout the world, most often in low-lying regions (with poor drainage) next to rivers, which supply the swamp with water. Some swamps develop from marshes that fill in slowly, thus allowing trees and woody shrubs to grow.
Bog	A type of wetland ecosystem characterized by wet, spongy, poorly drained peaty soil, dominated by the growth of bog mosses, *Sphagnum,* and heaths, particularly *Chamae daphne* Bogs are usually acid areas, frequently surrounding a body of open water. Bogs receive water exclusively from rainfall.
Fen	A type of wetland ecosystem characterized by peaty soil, dominated by grass-like plants, grasses, sedges, and reeds. Fens are alkaline rather than acid areas, receiving water mostly from surface and groundwater sources.

```
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Descriptives

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Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
RA1	60	3.26	12.90	6.6545	.29456	2.28164
RP1	60	3.26	11.63	6.3338	.28360	2.19675
RT1	60	3.26	19.97	8.1745	.44726	3.46447
RA2	60	2.37	11.63	6.3295	.30743	2.38137
RP2	60	2.37	18.13	8.8807	.52514	4.06769
RT2	60	4.36	19.97	8.2783	.43732	3.38746
RA3	60	2.37	14.86	7.7557	.38309	2.96744
RP3	60	3.26	14.86	7.2788	.36758	2.84724
RT3	60	2.33	17.35	4.5650	.33353	2.58348

RA5	60	1.35	21.88	5.8933	.49108	3.80390
RP5	60	2.37	18.13	8.7390	.52085	4.03446
RT5	60	4.36	19.97	8.5368	.45132	3.49593
RA7	60	2.37	14.86	8.0020	.38614	2.99099
RP7	60	2.58	19.97	8.0023	.45347	3.51255
RT7	60	2.33	17.35	5.2547	.36941	2.86142
SA1	61	1.35	17.35	5.0710	.36347	2.83879
SP1	61	2.33	7.95	4.1743	.14531	1.13493
ST1	61	1.35	17.35	4.9452	.40042	3.12741
SA2	61	1.35	17.35	5.0805	.36401	2.84302
SP2	61	2.33	7.95	4.1743	.14531	1.13493
ST2	61	1.35	17.35	4.7995	.36782	2.87277
SA3	61	1.35	17.35	5.1905	.39285	3.06823
SP3	61	2.33	15.90	4.3621	.25031	1.95501
ST3	61	1.35	17.35	5.4234	.42890	3.34979
SA5	61	2.33	17.35	4.8161	.32279	2.52111
SP5	61	1.35	11.83	4.3170	.23320	1.82133
ST5	61	1.35	17.35	5.1603	.38430	3.00145
SA7	61	1.35	9.02	4.4426	.18755	1.46480
SP7	61	1.35	11.83	4.1441	.22138	1.72901
ST7	61	1.35	17.35	5.1974	.38883	3.03683
Valid N (listwise)	60					

TITLE Aluminium.

Aluminium

```
GLM RA1 RA2 RA3 RA5 RA7 BY Treatment
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 /MEASURE=RootA
 /METHOD=SSTYPE(3)
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 /EMMEANS=TABLES(OVERALL)
 /EMMEANS=TABLES(Treatment) COMPARE ADJ(BONFERRONI)
 /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
 /EMMEANS=TABLES(Treatment*Day) COMPARE (Treatment) ADJ(BONFERRONI)
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 /WSDESIGN=Day
 /DESIGN=Treatment.
```

General Linear Model

Within-Subjects Factors

Measure: RootA

Day	Dependent Variable
1	RA1
2	RA2
3	RA3
4	RA5
5	RA7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	0.001	12
	2	0.01	12
	3	0.1	12
	4	1	12

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value
Day	Pillai's Trace	.289	5.275[b]	4.000	52.000	.001
	Wilks' Lambda	.711	5.275[b]	4.000	52.000	.001
	Hotelling's Trace	.406	5.275[b]	4.000	52.000	.001
	Roy's Largest Root	.406	5.275[b]	4.000	52.000	.001
Day * Treatment	Pillai's Trace	.374	1.419	16.000	220.000	.134
	Wilks' Lambda	.650	1.507	16.000	159.500	.103
	Hotelling's Trace	.500	1.578	16.000	202.000	.077
	Roy's Largest Root	.414	5.698[c]	4.000	55.000	.001

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: RootA

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.519	35.081	9	.000	.799	.916	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + Treatment
Within Subjects Design: Day

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: RootA

Source		Type III Sum of Squares	df	Mean Square	F	p-value
Day	Sphericity Assumed	200.523	4	50.131	7.120	.000
	Greenhouse-Geisser	200.523	3.196	62.744	7.120	.000
	Huynh-Feldt	200.523	3.663	54.745	7.120	.000
	Lower-bound	200.523	1.000	200.523	7.120	.010
Day * Treatment	Sphericity Assumed	202.137	16	12.634	1.794	.033
	Greenhouse-Geisser	202.137	12.783	15.812	1.794	.048
	Huynh-Feldt	202.137	14.652	13.796	1.794	.039
	Lower-bound	202.137	4.000	50.534	1.794	.143
Error(Day)	Sphericity Assumed	1549.072	220	7.041		
	Greenhouse-Geisser	1549.072	175.773	8.813		
	Huynh-Feldt	1549.072	201.458	7.689		
	Lower-bound	1549.072	55.000	28.165		

Tests of Within-Subjects Contrasts

Measure: RootA

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value
Day	Linear	30.614	1	30.614	4.524	.038
	Quadratic	10.683	1	10.683	2.105	.152
	Cubic	29.566	1	29.566	3.156	.081
	Order 4	129.660	1	129.660	18.643	.000
Day * Treatment	Linear	129.728	4	32.432	4.793	.002
	Quadratic	9.810	4	2.453	.483	.748
	Cubic	44.340	4	11.085	1.183	.328
	Order 4	18.258	4	4.564	.656	.625
Error(Day)	Linear	372.195	55	6.767		
	Quadratic	279.124	55	5.075		
	Cubic	515.228	55	9.368		
	Order 4	382.525	55	6.955		

Tests of Between-Subjects Effects

Measure: RootA
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Intercept	14394.999	1	14394.999	1230.063	.000
Treatment	147.937	4	36.984	3.160	.021
Error	643.646	55	11.703		

Estimated Marginal Means

1. Grand Mean

Measure: RootA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
6.927	.198	6.531	7.323

2. Treatment

Estimates

Measure: RootA

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	5.969	.442	5.084	6.854
0.001	7.486	.442	6.600	8.371
0.01	7.766	.442	6.880	8.651
0.1	6.240	.442	5.355	7.125
1	7.175	.442	6.290	8.060

Pairwise Comparisons

Measure: RootA

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	0.001	-1.517	.625	.185	-3.343	.310
	0.01	-1.797	.625	.057	-3.623	.030
	0.1	-.271	.625	1.000	-2.098	1.555
	1	-1.206	.625	.587	-3.032	.621
0.001	Control	1.517	.625	.185	-.310	3.343
	0.01	-.280	.625	1.000	-2.107	1.547
	0.1	1.245	.625	.512	-.582	3.072
	1	.311	.625	1.000	-1.516	2.138
0.01	Control	1.797	.625	.057	-.030	3.623
	0.001	.280	.625	1.000	-1.547	2.107
	0.1	1.525	.625	.179	-.302	3.352

	1	.591	.625	1.000	-1.236	2.418
0.1	Control	.271	.625	1.000	-1.555	2.098
	0.001	-1.245	.625	.512	-3.072	.582
	0.01	-1.525	.625	.179	-3.352	.302
	1	-.934	.625	1.000	-2.761	.892
1	Control	1.206	.625	.587	-.621	3.032
	0.001	-.311	.625	1.000	-2.138	1.516
	0.01	-.591	.625	1.000	-2.418	1.236
	0.1	.934	.625	1.000	-.892	2.761

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootA

	Sum of Squares	df	Mean Square	F	p-value
Contrast	29.587	4	7.397	3.160	.021
Error	128.729	55	2.341		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

3. Day

Estimates

Measure: RootA

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	6.655	.299	6.055	7.254
2	6.329	.303	5.723	6.936
3	7.756	.363	7.028	8.483
4	5.893	.472	4.948	6.838
5	8.002	.359	7.282	8.722

Pairwise Comparisons

Measure: RootA

					95% Confidence Interval for Difference[b]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	Lower Bound	Upper Bound
1	2	.325	.454	1.000	-1.003	1.653
	3	-1.101	.476	.244	-2.493	.291
	4	.761	.564	1.000	-.887	2.410
	5	-1.347	.464	.053	-2.705	.010
2	1	-.325	.454	1.000	-1.653	1.003
	3	-1.426*	.419	.013	-2.652	-.200
	4	.436	.568	1.000	-1.225	2.097
	5	-1.672*	.434	.003	-2.941	-.404
3	1	1.101	.476	.244	-.291	2.493
	2	1.426*	.419	.013	.200	2.652
	4	1.862*	.538	.010	.290	3.435

	5	-.246	.279	1.000	-1.064	.571
4	1	-.761	.564	1.000	-2.410	.887
	2	-.436	.568	1.000	-2.097	1.225
	3	-1.862*	.538	.010	-3.435	-.290
	5	-2.109*	.572	.005	-3.783	-.434
5	1	1.347	.464	.053	-.010	2.705
	2	1.672*	.434	.003	.404	2.941
	3	.246	.279	1.000	-.571	1.064
	4	2.109*	.572	.005	.434	3.783

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value
Pillai's trace	.289	5.275[a]	4.000	52.000	.001
Wilks' lambda	.711	5.275[a]	4.000	52.000	.001
Hotelling's trace	.406	5.275[a]	4.000	52.000	.001
Roy's largest root	.406	5.275[a]	4.000	52.000	.001

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

4. Treatment * Day

Estimates

Measure: RootA

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	6.638	.669	5.298	7.978
	2	6.421	.677	5.065	7.777
	3	6.427	.812	4.800	8.054
	4	3.932	1.055	1.819	6.046
	5	6.427	.804	4.816	8.037
0.001	1	7.494	.669	6.154	8.834
	2	7.043	.677	5.687	8.399
	3	8.626	.812	6.999	10.253
	4	5.638	1.055	3.525	7.752
	5	8.626	.804	7.015	10.237
0.01	1	6.219	.669	4.879	7.559
	2	6.496	.677	5.140	7.852
	3	9.434	.812	7.807	11.061
	4	7.244	1.055	5.131	9.358
	5	9.434	.804	7.823	11.045
0.1	1	6.594	.669	5.254	7.934
	2	6.762	.677	5.406	8.118
	3	6.455	.812	4.828	8.082
	4	4.936	1.055	2.822	7.049
	5	6.455	.804	4.844	8.066
1	1	6.327	.669	4.987	7.667

2	4.926	.677	3.570	6.282
3	7.837	.812	6.210	9.464
4	7.716	1.055	5.602	9.829
5	9.068	.804	7.458	10.679

Pairwise Comparisons

Measure: RootA

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
1	Control	0.001	-.856	.946	1.000	-3.621	1.910
		0.01	.419	.946	1.000	-2.346	3.185
		0.1	.044	.946	1.000	-2.721	2.810
		1	.312	.946	1.000	-2.454	3.077
	0.001	Control	.856	.946	1.000	-1.910	3.621
		0.01	1.275	.946	1.000	-1.491	4.041
		0.1	.900	.946	1.000	-1.866	3.666
		1	1.168	.946	1.000	-1.598	3.933
	0.01	Control	-.419	.946	1.000	-3.185	2.346
		0.001	-1.275	.946	1.000	-4.041	1.491
		0.1	-.375	.946	1.000	-3.141	2.391
		1	-.107	.946	1.000	-2.873	2.658
	0.1	Control	-.044	.946	1.000	-2.810	2.721
		0.001	-.900	.946	1.000	-3.666	1.866
		0.01	.375	.946	1.000	-2.391	3.141
		1	.268	.946	1.000	-2.498	3.033
	1	Control	-.312	.946	1.000	-3.077	2.454
		0.001	-1.168	.946	1.000	-3.933	1.598
		0.01	.107	.946	1.000	-2.658	2.873
		0.1	-.268	.946	1.000	-3.033	2.498
2	Control	0.001	-.622	.957	1.000	-3.421	2.176
		0.01	-.075	.957	1.000	-2.874	2.724
		0.1	-.341	.957	1.000	-3.139	2.458
		1	1.495	.957	1.000	-1.304	4.294
	0.001	Control	.622	.957	1.000	-2.176	3.421
		0.01	.547	.957	1.000	-2.251	3.346
		0.1	.282	.957	1.000	-2.517	3.080
		1	2.118	.957	.311	-.681	4.916
	0.01	Control	.075	.957	1.000	-2.724	2.874
		0.001	-.547	.957	1.000	-3.346	2.251
		0.1	-.266	.957	1.000	-3.064	2.533
		1	1.570	.957	1.000	-1.229	4.369
	0.1	Control	.341	.957	1.000	-2.458	3.139
		0.001	-.282	.957	1.000	-3.080	2.517
		0.01	.266	.957	1.000	-2.533	3.064
		1	1.836	.957	.602	-.963	4.634
	1	Control	-1.495	.957	1.000	-4.294	1.304
		0.001	-2.118	.957	.311	-4.916	.681
		0.01	-1.570	.957	1.000	-4.369	1.229
		0.1	-1.836	.957	.602	-4.634	.963
3	Control	0.001	-2.199	1.148	.607	-5.557	1.159
		0.01	-3.008	1.148	.114	-6.366	.351
		0.1	-.028	1.148	1.000	-3.387	3.330
		1	-1.410	1.148	1.000	-4.768	1.948
	0.001	Control	2.199	1.148	.607	-1.159	5.557

		0.01	-.808	1.148	1.000	-4.167	2.550
		0.1	2.171	1.148	.639	-1.187	5.529
		1	.789	1.148	1.000	-2.569	4.147
	0.01	Control	3.008	1.148	.114	-.351	6.366
		0.001	.808	1.148	1.000	-2.550	4.167
		0.1	2.979	1.148	.121	-.379	6.337
		1	1.598	1.148	1.000	-1.761	4.956
	0.1	Control	.028	1.148	1.000	-3.330	3.387
		0.001	-2.171	1.148	.639	-5.529	1.187
		0.01	-2.979	1.148	.121	-6.337	.379
		1	-1.382	1.148	1.000	-4.740	1.977
	1	Control	1.410	1.148	1.000	-1.948	4.768
		0.001	-.789	1.148	1.000	-4.147	2.569
		0.01	-1.598	1.148	1.000	-4.956	1.761
		0.1	1.382	1.148	1.000	-1.977	4.740
4	Control	0.001	-1.706	1.491	1.000	-6.068	2.656
		0.01	-3.312	1.491	.305	-7.674	1.050
		0.1	-1.003	1.491	1.000	-5.365	3.359
		1	-3.783	1.491	.141	-8.145	.579
	0.001	Control	1.706	1.491	1.000	-2.656	6.068
		0.01	-1.606	1.491	1.000	-5.968	2.756
		0.1	.702	1.491	1.000	-3.659	5.064
		1	-2.078	1.491	1.000	-6.439	2.284
	0.01	Control	3.312	1.491	.305	-1.050	7.674
		0.001	1.606	1.491	1.000	-2.756	5.968
		0.1	2.308	1.491	1.000	-2.054	6.670
		1	-.472	1.491	1.000	-4.834	3.890
	0.1	Control	1.003	1.491	1.000	-3.359	5.365
		0.001	-.702	1.491	1.000	-5.064	3.659
		0.01	-2.308	1.491	1.000	-6.670	2.054
		1	-2.780	1.491	.677	-7.142	1.582
	1	Control	3.783	1.491	.141	-.579	8.145
		0.001	2.078	1.491	1.000	-2.284	6.439
		0.01	.472	1.491	1.000	-3.890	4.834
		0.1	2.780	1.491	.677	-1.582	7.142
5	Control	0.001	-2.199	1.137	.582	-5.524	1.125
		0.01	-3.008	1.137	.106	-6.332	.317
		0.1	-.028	1.137	1.000	-3.353	3.296
		1	-2.642	1.137	.238	-5.966	.683
	0.001	Control	2.199	1.137	.582	-1.125	5.524
		0.01	-.808	1.137	1.000	-4.133	2.516
		0.1	2.171	1.137	.614	-1.154	5.495
		1	-.442	1.137	1.000	-3.767	2.882
	0.01	Control	3.008	1.137	.106	-.317	6.332
		0.001	.808	1.137	1.000	-2.516	4.133
		0.1	2.979	1.137	.113	-.345	6.304
		1	.366	1.137	1.000	-2.959	3.690
	0.1	Control	.028	1.137	1.000	-3.296	3.353
		0.001	-2.171	1.137	.614	-5.495	1.154
		0.01	-2.979	1.137	.113	-6.304	.345
		1	-2.613	1.137	.253	-5.938	.711
	1	Control	2.642	1.137	.238	-.683	5.966
		0.001	.442	1.137	1.000	-2.882	3.767
		0.01	-.366	1.137	1.000	-3.690	2.959
		0.1	2.613	1.137	.253	-.711	5.938

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootA

Day		Sum of Squares	df	Mean Square	F	p-value
1	Contrast	12.071	4	3.018	.562	.691
	Error	295.076	55	5.365		
2	Contrast	32.431	4	8.108	1.476	.222
	Error	302.153	55	5.494		
3	Contrast	84.469	4	21.117	2.670	.042
	Error	435.067	55	7.910		
4	Contrast	119.675	4	29.919	2.242	.076
	Error	734.033	55	13.346		
5	Contrast	101.427	4	25.357	3.271	.018
	Error	426.389	55	7.753		

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment * Day

Estimates

Measure: RootA

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	6.638	.669	5.298	7.978
	2	6.421	.677	5.065	7.777
	3	6.427	.812	4.800	8.054
	4	3.932	1.055	1.819	6.046
	5	6.427	.804	4.816	8.037
0.001	1	7.494	.669	6.154	8.834
	2	7.043	.677	5.687	8.399
	3	8.626	.812	6.999	10.253
	4	5.638	1.055	3.525	7.752
	5	8.626	.804	7.015	10.237
0.01	1	6.219	.669	4.879	7.559
	2	6.496	.677	5.140	7.852
	3	9.434	.812	7.807	11.061
	4	7.244	1.055	5.131	9.358
	5	9.434	.804	7.823	11.045
0.1	1	6.594	.669	5.254	7.934
	2	6.762	.677	5.406	8.118
	3	6.455	.812	4.828	8.082
	4	4.936	1.055	2.822	7.049
	5	6.455	.804	4.844	8.066
1	1	6.327	.669	4.987	7.667
	2	4.926	.677	3.570	6.282
	3	7.837	.812	6.210	9.464

	4	7.716	1.055	5.602	9.829
	5	9.068	.804	7.458	10.679

Pairwise Comparisons

Measure: RootA

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	.218	1.016	1.000	-2.753	3.188
		3	.212	1.064	1.000	-2.901	3.324
		4	2.706	1.260	.362	-.981	6.392
		5	.212	1.038	1.000	-2.824	3.247
	2	1	-.218	1.016	1.000	-3.188	2.753
		3	-.006	.937	1.000	-2.747	2.735
		4	2.488	1.270	.551	-1.225	6.202
		5	-.006	.970	1.000	-2.842	2.831
	3	1	-.212	1.064	1.000	-3.324	2.901
		2	.006	.937	1.000	-2.735	2.747
		4	2.494	1.202	.428	-1.023	6.011
		5	.000	.625	1.000	-1.827	1.827
	4	1	-2.706	1.260	.362	-6.392	.981
		2	-2.488	1.270	.551	-6.202	1.225
		3	-2.494	1.202	.428	-6.011	1.023
		5	-2.494	1.280	.565	-6.238	1.250
	5	1	-.212	1.038	1.000	-3.247	2.824
		2	.006	.970	1.000	-2.831	2.842
		3	.000	.625	1.000	-1.827	1.827
		4	2.494	1.280	.565	-1.250	6.238
0.001	1	2	.451	1.016	1.000	-2.519	3.421
		3	-1.132	1.064	1.000	-4.244	1.981
		4	1.856	1.260	1.000	-1.831	5.542
		5	-1.132	1.038	1.000	-4.167	1.904
	2	1	-.451	1.016	1.000	-3.421	2.519
		3	-1.583	.937	.970	-4.324	1.159
		4	1.405	1.270	1.000	-2.308	5.118
		5	-1.583	.970	1.000	-4.419	1.254
	3	1	1.132	1.064	1.000	-1.981	4.244
		2	1.583	.937	.970	-1.159	4.324
		4	2.988	1.202	.160	-.529	6.504
		5	-1.776E-15	.625	1.000	-1.827	1.827
	4	1	-1.856	1.260	1.000	-5.542	1.831
		2	-1.405	1.270	1.000	-5.118	2.308
		3	-2.988	1.202	.160	-6.504	.529
		5	-2.988	1.280	.233	-6.731	.756
	5	1	1.132	1.038	1.000	-1.904	4.167
		2	1.583	.970	1.000	-1.254	4.419
		3	1.776E-15	.625	1.000	-1.827	1.827
		4	2.988	1.280	.233	-.756	6.731
0.01	1	2	-.277	1.016	1.000	-3.247	2.693
		3	-3.215*	1.064	.038	-6.327	-.103

		4	-1.025	1.260	1.000	-4.711	2.661
		5	-3.215*	1.038	.031	-6.250	-.180
	2	1	.277	1.016	1.000	-2.693	3.247
		3	-2.938*	.937	.028	-5.679	-.197
		4	-.748	1.270	1.000	-4.462	2.965
		5	-2.938*	.970	.037	-5.775	-.102
	3	1	3.215*	1.064	.038	.103	6.327
		2	2.938*	.937	.028	.197	5.679
		4	2.190	1.202	.740	-1.327	5.707
		5	-1.776E-15	.625	1.000	-1.827	1.827
	4	1	1.025	1.260	1.000	-2.661	4.711
		2	.748	1.270	1.000	-2.965	4.462
		3	-2.190	1.202	.740	-5.707	1.327
		5	-2.190	1.280	.927	-5.934	1.554
	5	1	3.215*	1.038	.031	.180	6.250
		2	2.938*	.970	.037	.102	5.775
		3	1.776E-15	.625	1.000	-1.827	1.827
		4	2.190	1.280	.927	-1.554	5.934
0.1	1	2	-.167	1.016	1.000	-3.138	2.803
		3	.139	1.064	1.000	-2.973	3.252
		4	1.658	1.260	1.000	-2.028	5.345
		5	.139	1.038	1.000	-2.896	3.174
	2	1	.167	1.016	1.000	-2.803	3.138
		3	.307	.937	1.000	-2.434	3.048
		4	1.826	1.270	1.000	-1.888	5.539
		5	.307	.970	1.000	-2.530	3.143
	3	1	-.139	1.064	1.000	-3.252	2.973
		2	-.307	.937	1.000	-3.048	2.434
		4	1.519	1.202	1.000	-1.998	5.036
		5	-8.882E-16	.625	1.000	-1.827	1.827
	4	1	-1.658	1.260	1.000	-5.345	2.028
		2	-1.826	1.270	1.000	-5.539	1.888
		3	-1.519	1.202	1.000	-5.036	1.998
		5	-1.519	1.280	1.000	-5.263	2.225
	5	1	-.139	1.038	1.000	-3.174	2.896
		2	-.307	.970	1.000	-3.143	2.530
		3	8.882E-16	.625	1.000	-1.827	1.827
		4	1.519	1.280	1.000	-2.225	5.263
1	1	2	1.401	1.016	1.000	-1.569	4.371
		3	-1.510	1.064	1.000	-4.622	1.602
		4	-1.389	1.260	1.000	-5.076	2.297
		5	-2.742	1.038	.107	-5.777	.294
	2	1	-1.401	1.016	1.000	-4.371	1.569
		3	-2.911*	.937	.030	-5.652	-.170
		4	-2.790	1.270	.322	-6.503	.923
		5	-4.142*	.970	.001	-6.979	-1.306
	3	1	1.510	1.064	1.000	-1.602	4.622
		2	2.911*	.937	.030	.170	5.652
		4	.121	1.202	1.000	-3.396	3.638
		5	-1.232	.625	.537	-3.059	.596
	4	1	1.389	1.260	1.000	-2.297	5.076

		2	2.790	1.270	.322	-.923	6.503
		3	-.121	1.202	1.000	-3.638	3.396
		5	-1.352	1.280	1.000	-5.096	2.391
	5	1	2.742	1.038	.107	-.294	5.777
		2	4.142*	.970	.001	1.306	6.979
		3	1.232	.625	.537	-.596	3.059
		4	1.352	1.280	1.000	-2.391	5.096

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

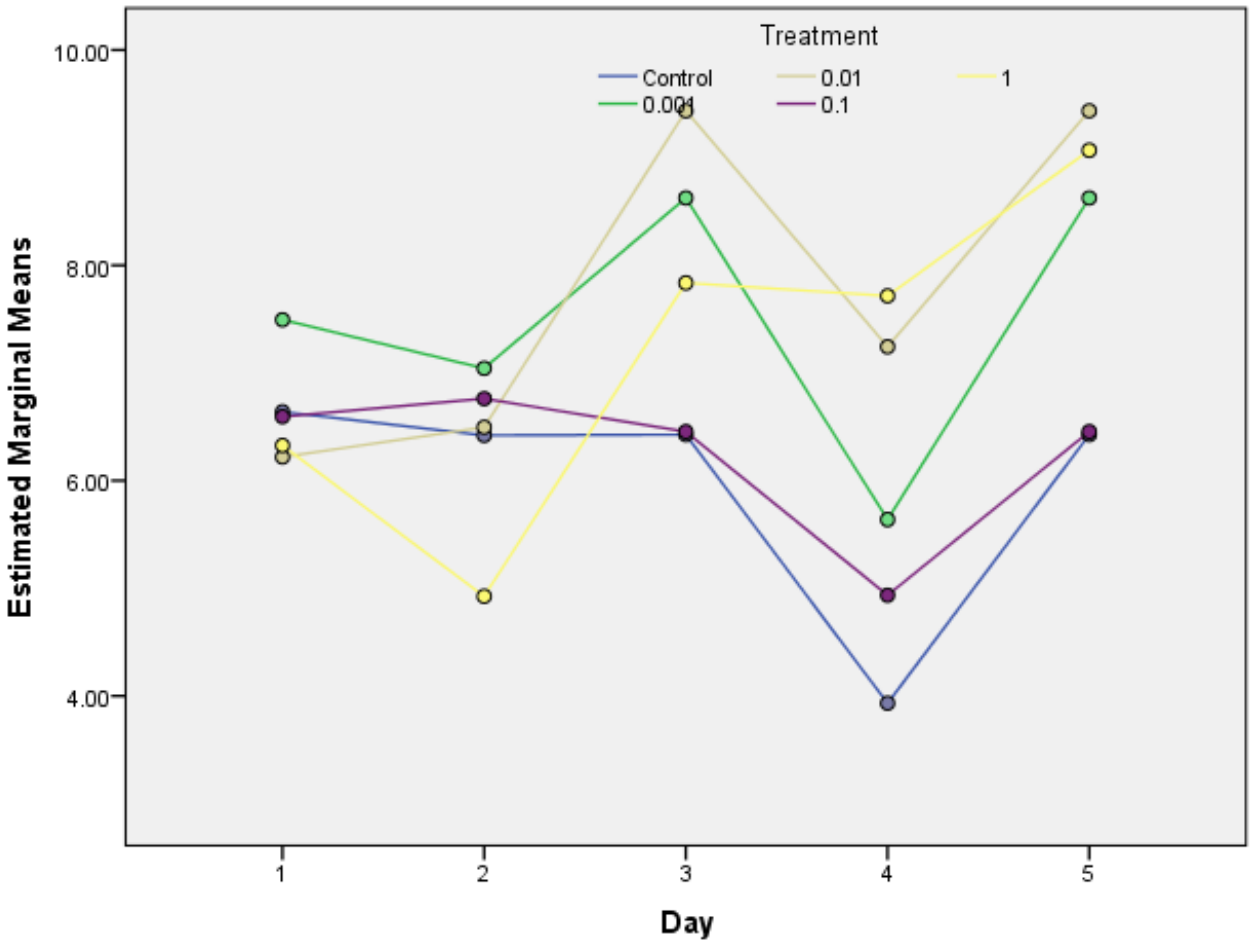
Multivariate Tests

Treatment		Value	F	Hypothesis df	Error df	p-value
Control	Pillai's trace	.092	1.313[a]	4.000	52.000	.278
	Wilks' lambda	.908	1.313[a]	4.000	52.000	.278
	Hotelling's trace	.101	1.313[a]	4.000	52.000	.278
	Roy's largest root	.101	1.313[a]	4.000	52.000	.278
0.001	Pillai's trace	.119	1.764[a]	4.000	52.000	.150
	Wilks' lambda	.881	1.764[a]	4.000	52.000	.150
	Hotelling's trace	.136	1.764[a]	4.000	52.000	.150
	Roy's largest root	.136	1.764[a]	4.000	52.000	.150
0.01	Pillai's trace	.211	3.477[a]	4.000	52.000	.014
	Wilks' lambda	.789	3.477[a]	4.000	52.000	.014
	Hotelling's trace	.267	3.477[a]	4.000	52.000	.014
	Roy's largest root	.267	3.477[a]	4.000	52.000	.014
0.1	Pillai's trace	.041	.554[a]	4.000	52.000	.697
	Wilks' lambda	.959	.554[a]	4.000	52.000	.697
	Hotelling's trace	.043	.554[a]	4.000	52.000	.697
	Roy's largest root	.043	.554[a]	4.000	52.000	.697
1	Pillai's trace	.264	4.668[a]	4.000	52.000	.003
	Wilks' lambda	.736	4.668[a]	4.000	52.000	.003
	Hotelling's trace	.359	4.668[a]	4.000	52.000	.003
	Roy's largest root	.359	4.668[a]	4.000	52.000	.003

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

Profile Plots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Control
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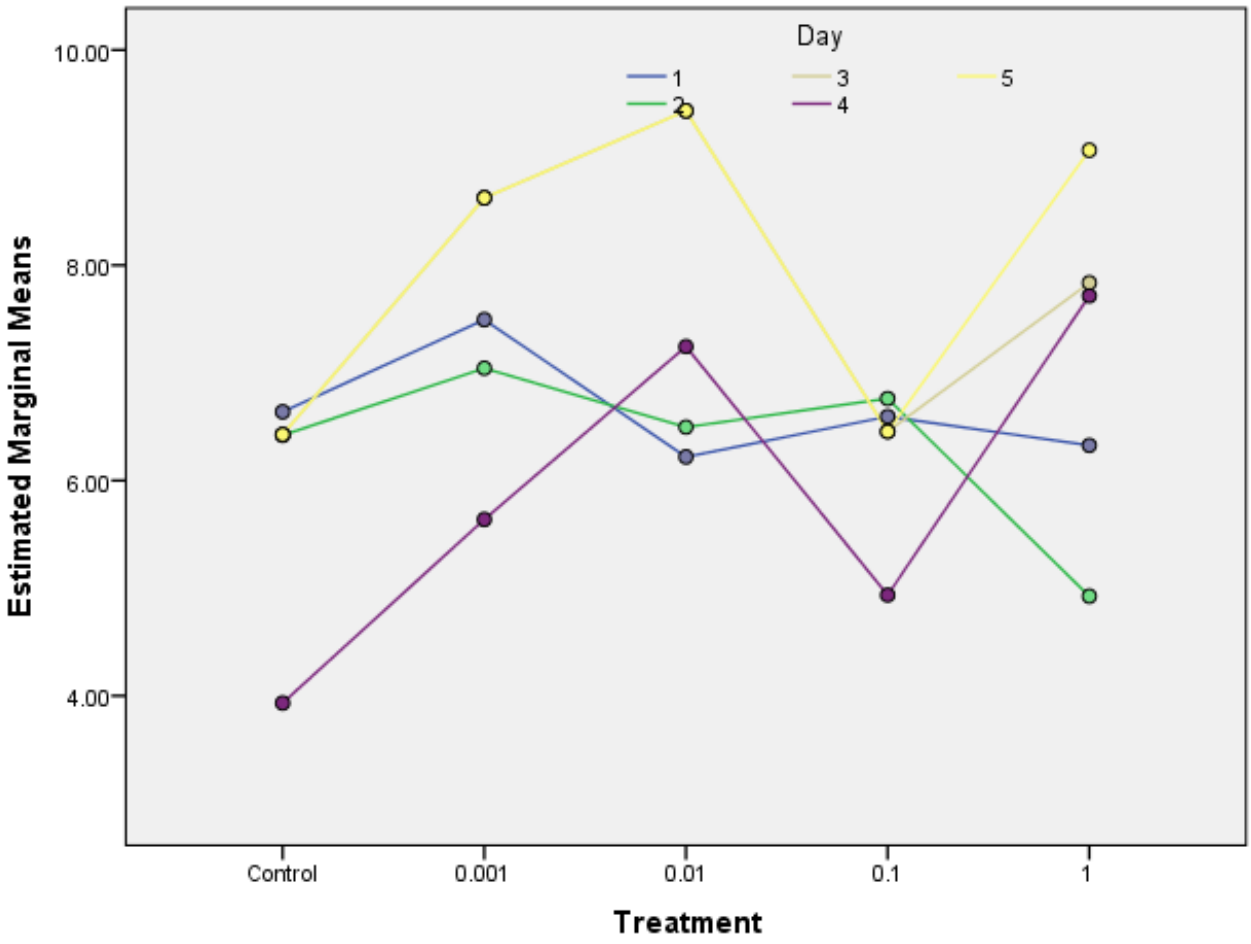
0.001
0.1
Estimated Marginal Means
10.00
8.00
6.00
4.00
1
2
3
4
5
Day



TITLE Aluminium.

Aluminium

```
GLM RP1 RP2 RP3 RP5 RP7 BY Treatment
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 /METHOD=SSTYPE(3)
 /PLOT=PROFILE(Day*Treatment Treatment*Day)
 /EMMEANS=TABLES(OVERALL)
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 /CRITERIA=ALPHA(.05)
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 /DESIGN= Treatment.
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General Linear Model

Within-Subjects Factors

Measure: RootP

Day	Dependent Variable
1	RP1
2	RP2
3	RP3
4	RP5
5	RP7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	0.001	12
	2	0.01	12
	3	0.1	12
	4	1	12

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
RP1	Control	6.2442	2.39885	12
	0.001	6.9725	2.34614	12
	0.01	6.0133	2.58290	12
	0.1	5.9525	1.86897	12
	1	6.4867	1.92616	12
	Total	6.3338	2.19675	60
RP2	Control	10.6033	4.66539	12
	0.001	10.9758	4.55314	12
	0.01	8.8825	2.45706	12

	0.1	7.2250	4.03866	12
	1	6.7167	2.78874	12
	Total	8.8807	4.06769	60
RP3	Control	7.0433	2.65175	12
	0.001	6.4967	2.31723	12
	0.01	6.7425	2.24739	12
	0.1	7.4192	3.40117	12
	1	8.6925	3.34939	12
	Total	7.2788	2.84724	60
RP5	Control	10.6033	4.66539	12
	0.001	10.9758	4.55314	12
	0.01	8.8825	2.45706	12
	0.1	7.2250	4.03866	12
	1	6.0083	1.59712	12
	Total	8.7390	4.03446	60
RP7	Control	10.9008	4.35376	12
	0.001	8.6567	2.88702	12
	0.01	5.8742	1.73035	12
	0.1	6.7425	2.24739	12
	1	7.8375	3.80890	12
	Total	8.0023	3.51255	60

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.353	7.097[b]	4.000	52.000	.000	28.386	.992
	Wilks' Lambda	.647	7.097[b]	4.000	52.000	.000	28.386	.992
	Hotelling's Trace	.546	7.097[b]	4.000	52.000	.000	28.386	.992
	Roy's Largest Root	.546	7.097[b]	4.000	52.000	.000	28.386	.992
Day * Treatment	Pillai's Trace	.443	1.713	16.000	220.000	.046	27.411	.924
	Wilks' Lambda	.603	1.795	16.000	159.500	.036	21.494	.815
	Hotelling's Trace	.582	1.838	16.000	202.000	.028	29.416	.942
	Roy's Largest Root	.404	5.561[c]	4.000	55.000	.001	22.244	.967

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: RootP

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.198	86.504	9	.000	.676	.766	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: RootP

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	270.045	4	67.511	7.755	.000	31.021	.997
	Greenhouse-Geisser	270.045	2.703	99.911	7.755	.000	20.961	.981

	Huynh-Feldt	270.045	3.063	88.172	7.755	.000	23.752	.989
	Lower-bound	270.045	1.000	270.045	7.755	.007	7.755	.781
Day * Treatment	Sphericity Assumed	371.605	16	23.225	2.668	.001	42.688	.994
	Greenhouse-Geisser	371.605	10.811	34.372	2.668	.004	28.845	.966
	Huynh-Feldt	371.605	12.251	30.333	2.668	.002	32.685	.979
	Lower-bound	371.605	4.000	92.901	2.668	.042	10.672	.706
Error(Day)	Sphericity Assumed	1915.133	220	8.705				
	Greenhouse-Geisser	1915.133	148.657	12.883				
	Huynh-Feldt	1915.133	168.449	11.369				
	Lower-bound	1915.133	55.000	34.821				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: RootP

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	61.261	1	61.261	8.645	.005	8.645	.823
	Quadratic	52.650	1	52.650	5.895	.018	5.895	.665
	Cubic	22.858	1	22.858	10.579	.002	10.579	.892
	Order 4	133.276	1	133.276	8.008	.006	8.008	.794
Day * Treatment	Linear	64.296	4	16.074	2.268	.073	9.074	.625
	Quadratic	22.613	4	5.653	.633	.641	2.532	.195
	Cubic	16.530	4	4.133	1.913	.121	7.650	.542
	Order 4	268.165	4	67.041	4.028	.006	16.113	.886
Error(Day)	Linear	389.728	55	7.086				
	Quadratic	491.197	55	8.931				
	Cubic	118.840	55	2.161				
	Order 4	915.368	55	16.643				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: RootP
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	18472.309	1	18472.309	1138.451	.000	1138.451	1.000
Treatment	248.355	4	62.089	3.827	.008	15.306	.868
Error	892.420	55	16.226				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
RP1, RP2, RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	50.000	1.000	.000	.050
RP1, RP2, RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.

	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP2, RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP2, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP2, RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP2, RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP2, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP2, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP2, RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP2, RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP2, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1, RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.

	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP2, RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP2, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP2, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
RP1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	276.467	55	5.027				
RP2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	798.841	55	14.524				
RP3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	442.623	55	8.048				
RP5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	741.352	55	13.479				
RP7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	548.270	55	9.969				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: RootP

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
7.847	.233	7.381	8.313

2. Treatment

Estimates

Measure: RootP

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	9.079	.520	8.037	10.121
0.001	8.816	.520	7.773	9.858
0.01	7.279	.520	6.237	8.321
0.1	6.913	.520	5.871	7.955
1	7.148	.520	6.106	8.190

Pairwise Comparisons

Measure: RootP

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Control	0.001	.264	.735	1.000	-1.887	2.414
	0.01	1.800	.735	.176	-.351	3.951
	0.1	2.166*	.735	.047	.015	4.317
	1	1.931	.735	.112	-.220	4.082
0.001	Control	-.264	.735	1.000	-2.414	1.887
	0.01	1.537	.735	.413	-.614	3.687
	0.1	1.903	.735	.124	-.248	4.054
	1	1.667	.735	.273	-.484	3.818
0.01	Control	-1.800	.735	.176	-3.951	.351
	0.001	-1.537	.735	.413	-3.687	.614
	0.1	.366	.735	1.000	-1.785	2.517
	1	.131	.735	1.000	-2.020	2.282
0.1	Control	-2.166*	.735	.047	-4.317	-.015
	0.001	-1.903	.735	.124	-4.054	.248
	0.01	-.366	.735	1.000	-2.517	1.785
	1	-.235	.735	1.000	-2.386	1.915
1	Control	-1.931	.735	.112	-4.082	.220
	0.001	-1.667	.735	.273	-3.818	.484

0.01	-.131	.735	1.000	-2.282	2.020
0.1	.235	.735	1.000	-1.915	2.386

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootP

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	49.671	4	12.418	3.827	.008	15.306	.868
Error	178.484	55	3.245				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

3. Day

Estimates

Measure: RootP

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	6.334	.289	5.754	6.914
2	8.881	.492	7.895	9.867
3	7.279	.366	6.545	8.013
4	8.739	.474	7.789	9.689
5	8.002	.408	7.185	8.819

Pairwise Comparisons

Measure: RootP

					95% Confidence Interval for Difference[b]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	Lower Bound	Upper Bound
1	2	-2.547*	.500	.000	-4.008	-1.086
	3	-.945	.496	.622	-2.397	.507
	4	-2.405*	.498	.000	-3.861	-.950
	5	-1.668*	.520	.022	-3.189	-.148
2	1	2.547*	.500	.000	1.086	4.008
	3	1.602	.647	.163	-.289	3.493
	4	.142	.195	1.000	-.429	.712
	5	.878	.619	1.000	-.931	2.688
3	1	.945	.496	.622	-.507	2.397
	2	-1.602	.647	.163	-3.493	.289
	4	-1.460	.606	.194	-3.233	.312
	5	-.723	.576	1.000	-2.409	.962
4	1	2.405*	.498	.000	.950	3.861
	2	-.142	.195	1.000	-.712	.429
	3	1.460	.606	.194	-.312	3.233
	5	.737	.591	1.000	-.993	2.466
5	1	1.668*	.520	.022	.148	3.189

2	-.878	.619	1.000	-2.688	.931
3	.723	.576	1.000	-.962	2.409
4	-.737	.591	1.000	-2.466	.993

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.353	7.097[a]	4.000	52.000	.000	28.386	.992
Wilks' lambda	.647	7.097[a]	4.000	52.000	.000	28.386	.992
Hotelling's trace	.546	7.097[a]	4.000	52.000	.000	28.386	.992
Roy's largest root	.546	7.097[a]	4.000	52.000	.000	28.386	.992

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: RootP

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	6.244	.647	4.947	7.541
	2	10.603	1.100	8.399	12.808
	3	7.043	.819	5.402	8.684
	4	10.603	1.060	8.479	12.727
	5	10.901	.911	9.074	12.727
0.001	1	6.972	.647	5.675	8.270
	2	10.976	1.100	8.771	13.181
	3	6.497	.819	4.856	8.138
	4	10.976	1.060	8.852	13.100
	5	8.657	.911	6.830	10.483
0.01	1	6.013	.647	4.716	7.310
	2	8.883	1.100	6.678	11.087
	3	6.743	.819	5.101	8.384
	4	8.883	1.060	6.759	11.006
	5	5.874	.911	4.048	7.701
0.1	1	5.952	.647	4.655	7.250
	2	7.225	1.100	5.020	9.430
	3	7.419	.819	5.778	9.060
	4	7.225	1.060	5.101	9.349
	5	6.743	.911	4.916	8.569
1	1	6.487	.647	5.190	7.784
	2	6.717	1.100	4.512	8.921
	3	8.693	.819	7.051	10.334
	4	6.008	1.060	3.884	8.132
	5	7.837	.911	6.011	9.664

Pairwise Comparisons

Measure: RootP

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	0.001	-.728	.915	1.000	-3.405	1.949
		0.01	.231	.915	1.000	-2.446	2.908
		0.1	.292	.915	1.000	-2.385	2.969
		1	-.243	.915	1.000	-2.919	2.434
	0.001	Control	.728	.915	1.000	-1.949	3.405
		0.01	.959	.915	1.000	-1.718	3.636
		0.1	1.020	.915	1.000	-1.657	3.697
		1	.486	.915	1.000	-2.191	3.163
	0.01	Control	-.231	.915	1.000	-2.908	2.446
		0.001	-.959	.915	1.000	-3.636	1.718
		0.1	.061	.915	1.000	-2.616	2.738
		1	-.473	.915	1.000	-3.150	2.204
	0.1	Control	-.292	.915	1.000	-2.969	2.385
		0.001	-1.020	.915	1.000	-3.697	1.657
		0.01	-.061	.915	1.000	-2.738	2.616
		1	-.534	.915	1.000	-3.211	2.143
	1	Control	.243	.915	1.000	-2.434	2.919
		0.001	-.486	.915	1.000	-3.163	2.191
		0.01	.473	.915	1.000	-2.204	3.150
		0.1	.534	.915	1.000	-2.143	3.211
2	Control	0.001	-.372	1.556	1.000	-4.923	4.178
		0.01	1.721	1.556	1.000	-2.830	6.271
		0.1	3.378	1.556	.342	-1.172	7.929
		1	3.887	1.556	.155	-.664	8.437
	0.001	Control	.372	1.556	1.000	-4.178	4.923
		0.01	2.093	1.556	1.000	-2.457	6.644
		0.1	3.751	1.556	.193	-.800	8.301
		1	4.259	1.556	.083	-.291	8.810
	0.01	Control	-1.721	1.556	1.000	-6.271	2.830
		0.001	-2.093	1.556	1.000	-6.644	2.457
		0.1	1.657	1.556	1.000	-2.893	6.208
		1	2.166	1.556	1.000	-2.385	6.716
	0.1	Control	-3.378	1.556	.342	-7.929	1.172
		0.001	-3.751	1.556	.193	-8.301	.800
		0.01	-1.657	1.556	1.000	-6.208	2.893
		1	.508	1.556	1.000	-4.042	5.059
	1	Control	-3.887	1.556	.155	-8.437	.664
		0.001	-4.259	1.556	.083	-8.810	.291
		0.01	-2.166	1.556	1.000	-6.716	2.385
		0.1	-.508	1.556	1.000	-5.059	4.042
3	Control	0.001	.547	1.158	1.000	-2.841	3.934
		0.01	.301	1.158	1.000	-3.086	3.688
		0.1	-.376	1.158	1.000	-3.763	3.011
		1	-1.649	1.158	1.000	-5.036	1.738
	0.001	Control	-.547	1.158	1.000	-3.934	2.841
		0.01	-.246	1.158	1.000	-3.633	3.141
		0.1	-.923	1.158	1.000	-4.310	2.465
		1	-2.196	1.158	.632	-5.583	1.191
	0.01	Control	-.301	1.158	1.000	-3.688	3.086

		0.001	.246	1.158	1.000	-3.141	3.633
		0.1	-.677	1.158	1.000	-4.064	2.711
		1	-1.950	1.158	.979	-5.337	1.437
	0.1	Control	.376	1.158	1.000	-3.011	3.763
		0.001	.923	1.158	1.000	-2.465	4.310
		0.01	.677	1.158	1.000	-2.711	4.064
		1	-1.273	1.158	1.000	-4.661	2.114
	1	Control	1.649	1.158	1.000	-1.738	5.036
		0.001	2.196	1.158	.632	-1.191	5.583
		0.01	1.950	1.158	.979	-1.437	5.337
		0.1	1.273	1.158	1.000	-2.114	4.661
4	Control	0.001	-.372	1.499	1.000	-4.756	4.011
		0.01	1.721	1.499	1.000	-2.663	6.104
		0.1	3.378	1.499	.282	-1.005	7.762
		1	4.595*	1.499	.034	.211	8.979
	0.001	Control	.372	1.499	1.000	-4.011	4.756
		0.01	2.093	1.499	1.000	-2.290	6.477
		0.1	3.751	1.499	.153	-.633	8.134
		1	4.968*	1.499	.016	.584	9.351
	0.01	Control	-1.721	1.499	1.000	-6.104	2.663
		0.001	-2.093	1.499	1.000	-6.477	2.290
		0.1	1.657	1.499	1.000	-2.726	6.041
		1	2.874	1.499	.604	-1.509	7.258
	0.1	Control	-3.378	1.499	.282	-7.762	1.005
		0.001	-3.751	1.499	.153	-8.134	.633
		0.01	-1.657	1.499	1.000	-6.041	2.726
		1	1.217	1.499	1.000	-3.167	5.600
	1	Control	-4.595*	1.499	.034	-8.979	-.211
		0.001	-4.968*	1.499	.016	-9.351	-.584
		0.01	-2.874	1.499	.604	-7.258	1.509
		0.1	-1.217	1.499	1.000	-5.600	3.167
5	Control	0.001	2.244	1.289	.873	-1.526	6.014
		0.01	5.027*	1.289	.003	1.257	8.796
		0.1	4.158*	1.289	.021	.389	7.928
		1	3.063	1.289	.210	-.706	6.833
	0.001	Control	-2.244	1.289	.873	-6.014	1.526
		0.01	2.782	1.289	.353	-.987	6.552
		0.1	1.914	1.289	1.000	-1.856	5.684
		1	.819	1.289	1.000	-2.951	4.589
	0.01	Control	-5.027*	1.289	.003	-8.796	-1.257
		0.001	-2.782	1.289	.353	-6.552	.987
		0.1	-.868	1.289	1.000	-4.638	2.901
		1	-1.963	1.289	1.000	-5.733	1.806
	0.1	Control	-4.158*	1.289	.021	-7.928	-.389
		0.001	-1.914	1.289	1.000	-5.684	1.856
		0.01	.868	1.289	1.000	-2.901	4.638
		1	-1.095	1.289	1.000	-4.865	2.675
	1	Control	-3.063	1.289	.210	-6.833	.706
		0.001	-.819	1.289	1.000	-4.589	2.951
		0.01	1.963	1.289	1.000	-1.806	5.733
		0.1	1.095	1.289	1.000	-2.675	4.865

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootP

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	8.249	4	2.062	.410	.800	1.641	.138
	Error	276.467	55	5.027				
2	Contrast	177.377	4	44.344	3.053	.024	12.212	.771
	Error	798.841	55	14.524				
3	Contrast	35.677	4	8.919	1.108	.362	4.433	.326
	Error	442.623	55	8.048				
4	Contrast	218.982	4	54.745	4.061	.006	16.246	.889
	Error	741.352	55	13.479				
5	Contrast	179.675	4	44.919	4.506	.003	18.024	.921
	Error	548.270	55	9.969				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: RootP

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	6.244	.647	4.947	7.541
	2	10.603	1.100	8.399	12.808
	3	7.043	.819	5.402	8.684
	4	10.603	1.060	8.479	12.727
	5	10.901	.911	9.074	12.727
0.001	1	6.972	.647	5.675	8.270
	2	10.976	1.100	8.771	13.181
	3	6.497	.819	4.856	8.138
	4	10.976	1.060	8.852	13.100
	5	8.657	.911	6.830	10.483
0.01	1	6.013	.647	4.716	7.310
	2	8.883	1.100	6.678	11.087
	3	6.743	.819	5.101	8.384
	4	8.883	1.060	6.759	11.006
	5	5.874	.911	4.048	7.701
0.1	1	5.952	.647	4.655	7.250
	2	7.225	1.100	5.020	9.430
	3	7.419	.819	5.778	9.060
	4	7.225	1.060	5.101	9.349
	5	6.743	.911	4.916	8.569
1	1	6.487	.647	5.190	7.784
	2	6.717	1.100	4.512	8.921
	3	8.693	.819	7.051	10.334
	4	6.008	1.060	3.884	8.132
	5	7.837	.911	6.011	9.664

Pairwise Comparisons

Measure: RootP

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	-4.359*	1.117	.003	-7.627	-1.092
		3	-.799	1.110	1.000	-4.046	2.448
		4	-4.359*	1.113	.002	-7.614	-1.105
		5	-4.657*	1.162	.002	-8.056	-1.257
	2	1	4.359*	1.117	.003	1.092	7.627
		3	3.560	1.446	.170	-.669	7.789
		4	-1.776E-15	.436	1.000	-1.276	1.276
		5	-.298	1.383	1.000	-4.343	3.748
	3	1	.799	1.110	1.000	-2.448	4.046
		2	-3.560	1.446	.170	-7.789	.669
		4	-3.560	1.355	.111	-7.524	.404
		5	-3.858*	1.289	.041	-7.626	-.089
	4	1	4.359*	1.113	.002	1.105	7.614
		2	1.776E-15	.436	1.000	-1.276	1.276
		3	3.560	1.355	.111	-.404	7.524
		5	-.297	1.322	1.000	-4.164	3.569
	5	1	4.657*	1.162	.002	1.257	8.056
		2	.298	1.383	1.000	-3.748	4.343
		3	3.858*	1.289	.041	.089	7.626
		4	.297	1.322	1.000	-3.569	4.164
0.001	1	2	-4.003*	1.117	.007	-7.271	-.736
		3	.476	1.110	1.000	-2.771	3.723
		4	-4.003*	1.113	.007	-7.258	-.749
		5	-1.684	1.162	1.000	-5.083	1.715
	2	1	4.003*	1.117	.007	.736	7.271
		3	4.479*	1.446	.031	.251	8.708
		4	.000	.436	1.000	-1.276	1.276
		5	2.319	1.383	.993	-1.726	6.365
	3	1	-.476	1.110	1.000	-3.723	2.771
		2	-4.479*	1.446	.031	-8.708	-.251
		4	-4.479*	1.355	.017	-8.443	-.516
		5	-2.160	1.289	.994	-5.929	1.609
	4	1	4.003*	1.113	.007	.749	7.258
		2	.000	.436	1.000	-1.276	1.276
		3	4.479*	1.355	.017	.516	8.443
		5	2.319	1.322	.850	-1.547	6.186
	5	1	1.684	1.162	1.000	-1.715	5.083
		2	-2.319	1.383	.993	-6.365	1.726
		3	2.160	1.289	.994	-1.609	5.929
		4	-2.319	1.322	.850	-6.186	1.547
0.01	1	2	-2.869	1.117	.130	-6.137	.398
		3	-.729	1.110	1.000	-3.976	2.518
		4	-2.869	1.113	.126	-6.124	.385
		5	.139	1.162	1.000	-3.260	3.538

	2	1	2.869	1.117	.130	-.398	6.137
		3	2.140	1.446	1.000	-2.089	6.369
		4	.000	.436	1.000	-1.276	1.276
		5	3.008	1.383	.340	-1.037	7.054
	3	1	.729	1.110	1.000	-2.518	3.976
		2	-2.140	1.446	1.000	-6.369	2.089
		4	-2.140	1.355	1.000	-6.104	1.824
		5	.868	1.289	1.000	-2.900	4.637
	4	1	2.869	1.113	.126	-.385	6.124
		2	.000	.436	1.000	-1.276	1.276
		3	2.140	1.355	1.000	-1.824	6.104
		5	3.008	1.322	.268	-.858	6.875
	5	1	-.139	1.162	1.000	-3.538	3.260
		2	-3.008	1.383	.340	-7.054	1.037
		3	-.868	1.289	1.000	-4.637	2.900
		4	-3.008	1.322	.268	-6.875	.858
0.1	1	2	-1.273	1.117	1.000	-4.540	1.995
		3	-1.467	1.110	1.000	-4.714	1.780
		4	-1.273	1.113	1.000	-4.527	1.982
		5	-.790	1.162	1.000	-4.189	2.609
	2	1	1.273	1.117	1.000	-1.995	4.540
		3	-.194	1.446	1.000	-4.423	4.034
		4	-8.882E-16	.436	1.000	-1.276	1.276
		5	.483	1.383	1.000	-3.563	4.528
	3	1	1.467	1.110	1.000	-1.780	4.714
		2	.194	1.446	1.000	-4.034	4.423
		4	.194	1.355	1.000	-3.769	4.158
		5	.677	1.289	1.000	-3.092	4.445
	4	1	1.273	1.113	1.000	-1.982	4.527
		2	8.882E-16	.436	1.000	-1.276	1.276
		3	-.194	1.355	1.000	-4.158	3.769
		5	.483	1.322	1.000	-3.384	4.349
	5	1	.790	1.162	1.000	-2.609	4.189
		2	-.483	1.383	1.000	-4.528	3.563
		3	-.677	1.289	1.000	-4.445	3.092
		4	-.483	1.322	1.000	-4.349	3.384
1	1	2	-.230	1.117	1.000	-3.497	3.037
		3	-2.206	1.110	.519	-5.453	1.041
		4	.478	1.113	1.000	-2.776	3.733
		5	-1.351	1.162	1.000	-4.750	2.048
	2	1	.230	1.117	1.000	-3.037	3.497
		3	-1.976	1.446	1.000	-6.204	2.253
		4	.708	.436	1.000	-.567	1.984
		5	-1.121	1.383	1.000	-5.166	2.925
	3	1	2.206	1.110	.519	-1.041	5.453
		2	1.976	1.446	1.000	-2.253	6.204
		4	2.684	1.355	.526	-1.279	6.648
		5	.855	1.289	1.000	-2.914	4.624
	4	1	-.478	1.113	1.000	-3.733	2.776
		2	-.708	.436	1.000	-1.984	.567
		3	-2.684	1.355	.526	-6.648	1.279

		5	-1.829	1.322	1.000	-5.696	2.037
	5	1	1.351	1.162	1.000	-2.048	4.750
		2	1.121	1.383	1.000	-2.925	5.166
		3	-.855	1.289	1.000	-4.624	2.914
		4	1.829	1.322	1.000	-2.037	5.696

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

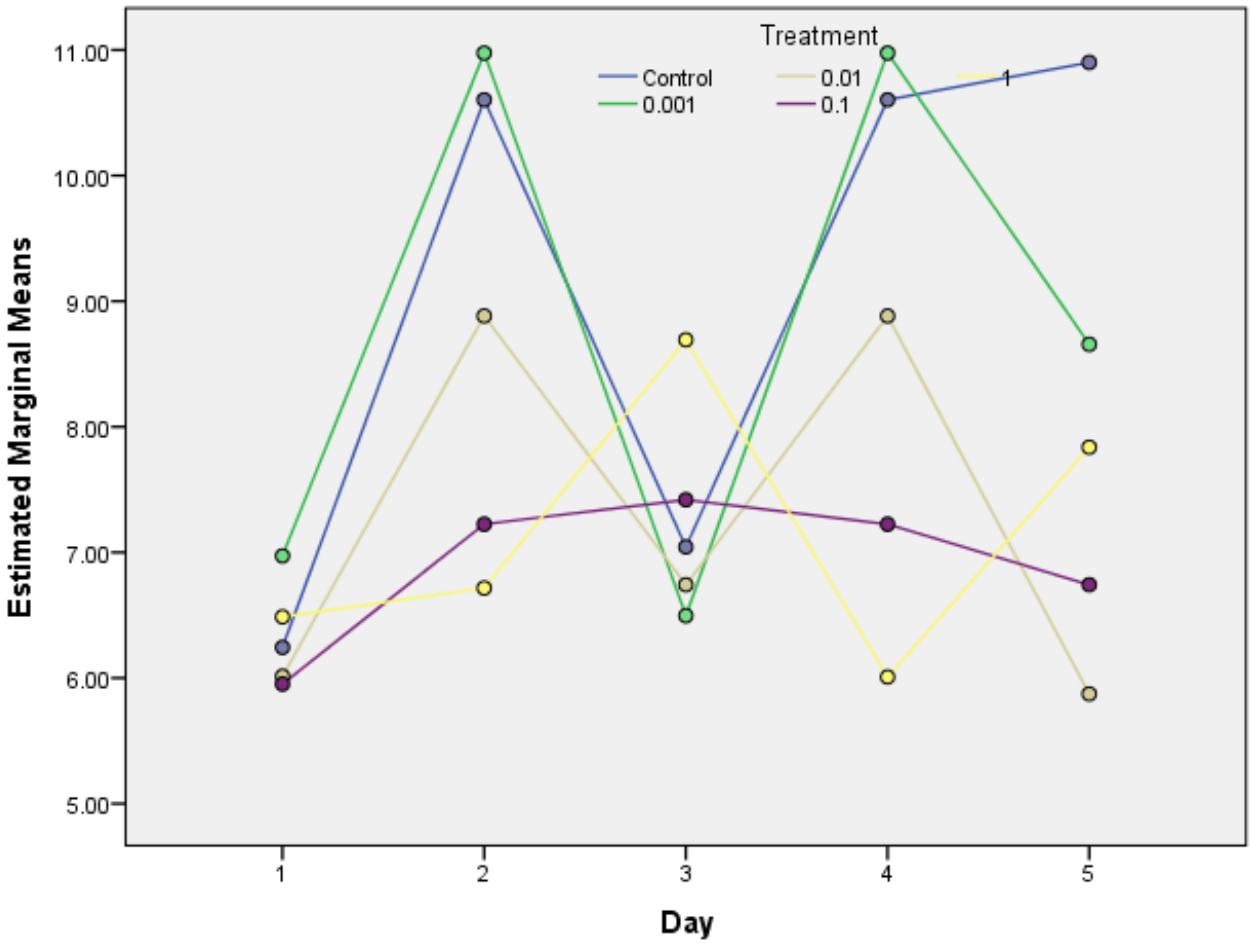
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.320	6.108[a]	4.000	52.000	.000	24.433	.979
	Wilks' lambda	.680	6.108[a]	4.000	52.000	.000	24.433	.979
	Hotelling's trace	.470	6.108[a]	4.000	52.000	.000	24.433	.979
	Roy's largest root	.470	6.108[a]	4.000	52.000	.000	24.433	.979
0.001	Pillai's trace	.221	3.696[a]	4.000	52.000	.010	14.783	.852
	Wilks' lambda	.779	3.696[a]	4.000	52.000	.010	14.783	.852
	Hotelling's trace	.284	3.696[a]	4.000	52.000	.010	14.783	.852
	Roy's largest root	.284	3.696[a]	4.000	52.000	.010	14.783	.852
0.01	Pillai's trace	.127	1.887[a]	4.000	52.000	.127	7.548	.533
	Wilks' lambda	.873	1.887[a]	4.000	52.000	.127	7.548	.533
	Hotelling's trace	.145	1.887[a]	4.000	52.000	.127	7.548	.533
	Roy's largest root	.145	1.887[a]	4.000	52.000	.127	7.548	.533
0.1	Pillai's trace	.046	.625[a]	4.000	52.000	.646	2.502	.192
	Wilks' lambda	.954	.625[a]	4.000	52.000	.646	2.502	.192
	Hotelling's trace	.048	.625[a]	4.000	52.000	.646	2.502	.192
	Roy's largest root	.048	.625[a]	4.000	52.000	.646	2.502	.192
1	Pillai's trace	.153	2.353[a]	4.000	52.000	.066	9.411	.641
	Wilks' lambda	.847	2.353[a]	4.000	52.000	.066	9.411	.641
	Hotelling's trace	.181	2.353[a]	4.000	52.000	.066	9.411	.641
	Roy's largest root	.181	2.353[a]	4.000	52.000	.066	9.411	.641

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots


Treatment
Control
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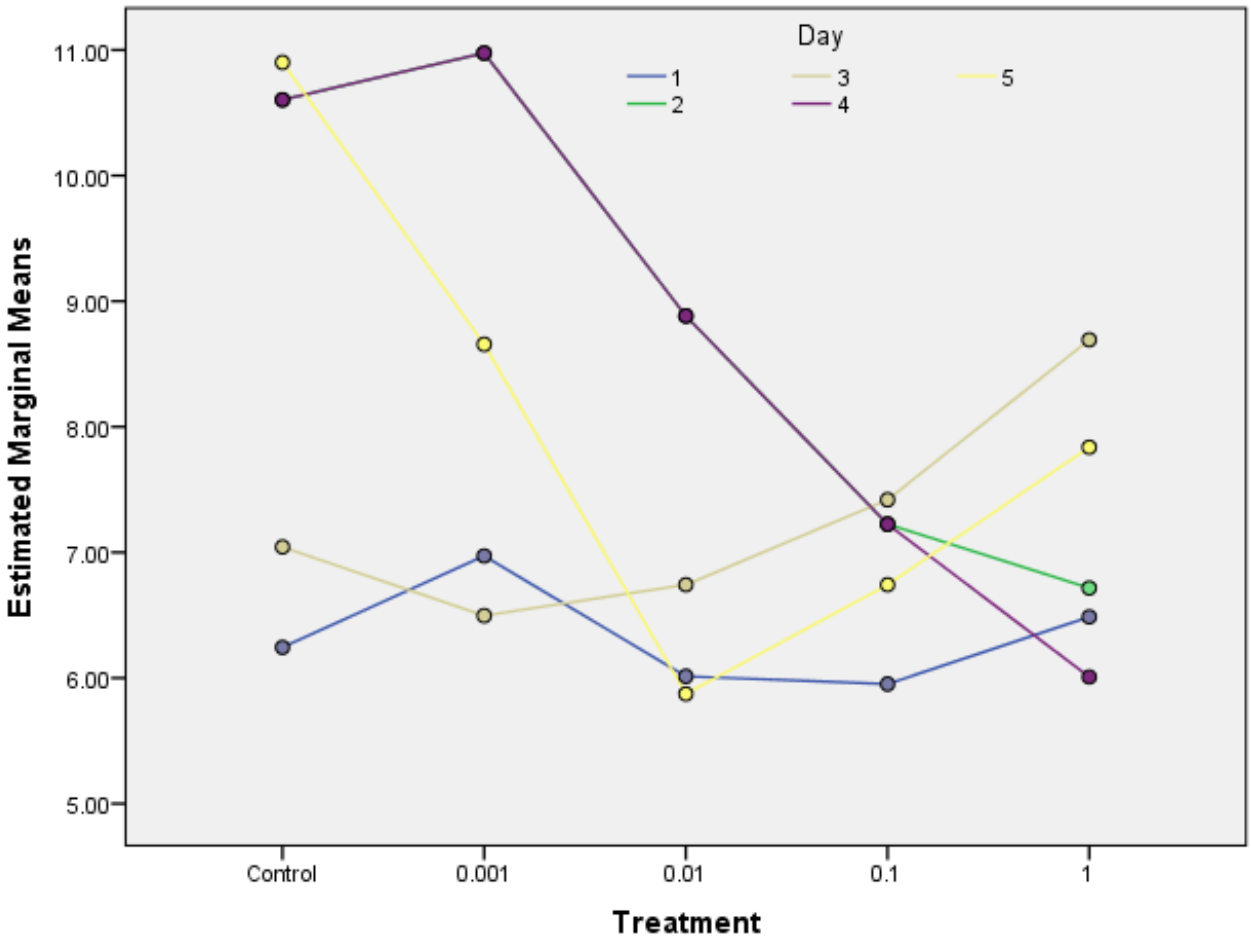
0.01
0.1
1
Estimated Marginal Means
11.00
10.00
9.00
8.00
7.00
6.00
5.00
1
2
3
4
5
Day



TITLE Aluminium.

Aluminium

```
GLM RT1 RT2 RT3 RT5 RT7 BY Treatment
  /WSFACTOR=Day 5 Polynomial
  /MEASURE=RootT
  /METHOD=SSTYPE(3)
  /PLOT=PROFILE(Day*Treatment Treatment*Day)
  /EMMEANS=TABLES(OVERALL)
  /EMMEANS=TABLES(Treatment) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Treatment) ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Day) ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE OPOWER LOF
  /CRITERIA=ALPHA(.05)
  /WSDESIGN= Day
  /DESIGN= Treatment.
```

General Linear Model

Within-Subjects Factors

Measure: RootT

Day	Dependent Variable
1	RT1
2	RT2
3	RT3
4	RT5
5	RT7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	0.001	12
	2	0.01	12
	3	0.1	12
	4	1	12

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
RT1	Control	10.9008	4.35376	12
	0.001	9.3933	2.92079	12
	0.01	7.9958	3.29420	12
	0.1	6.0725	1.83875	12
	1	6.5100	2.24761	12
	Total	8.1745	3.46447	60
RT2	Control	10.8150	4.40089	12
	0.001	9.6683	2.94729	12
	0.01	8.4317	2.67215	12

	0.1	6.3800	2.38209	12
	1	6.0967	1.54965	12
	Total	8.2783	3.38746	60
RT3	Control	4.5325	1.36607	12
	0.001	6.4950	4.97509	12
	0.01	3.9325	1.12653	12
	0.1	3.9325	1.12653	12
	1	3.9325	1.12653	12
	Total	4.5650	2.58348	60
RT5	Control	10.8150	4.40089	12
	0.001	9.6683	2.94729	12
	0.01	8.4317	2.67215	12
	0.1	6.3800	2.38209	12
	1	7.3892	3.33173	12
	Total	8.5368	3.49593	60
RT7	Control	6.4208	2.50198	12
	0.001	5.2292	1.97226	12
	0.01	6.2658	5.07795	12
	0.1	3.9325	1.12653	12
	1	4.4250	1.20572	12
	Total	5.2547	2.86142	60

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.601	19.620[b]	4.000	52.000	.000	78.479	1.000
	Wilks' Lambda	.399	19.620[b]	4.000	52.000	.000	78.479	1.000
	Hotelling's Trace	1.509	19.620[b]	4.000	52.000	.000	78.479	1.000
	Roy's Largest Root	1.509	19.620[b]	4.000	52.000	.000	78.479	1.000
Day * Treatment	Pillai's Trace	.321	1.200	16.000	220.000	.270	19.195	.767
	Wilks' Lambda	.709	1.185	16.000	159.500	.285	14.289	.590
	Hotelling's Trace	.368	1.161	16.000	202.000	.302	18.573	.747
	Roy's Largest Root	.195	2.678[c]	4.000	55.000	.041	10.710	.708

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: RootT

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.450	42.660	9	.000	.815	.936	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: RootT

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	860.615	4	215.154	30.835	.000	123.341	1.000
	Greenhouse-Geisser	860.615	3.260	263.976	30.835	.000	100.529	1.000

	Huynh-Feldt	860.615	3.742	229.986	30.835	.000	115.387	1.000
	Lower-bound	860.615	1.000	860.615	30.835	.000	30.835	1.000
Day * Treatment	Sphericity Assumed	145.884	16	9.118	1.307	.194	20.908	.812
	Greenhouse-Geisser	145.884	13.041	11.187	1.307	.212	17.041	.743
	Huynh-Feldt	145.884	14.968	9.746	1.307	.200	19.559	.790
	Lower-bound	145.884	4.000	36.471	1.307	.279	5.227	.381
Error(Day)	Sphericity Assumed	1535.055	220	6.978				
	Greenhouse-Geisser	1535.055	179.311	8.561				
	Huynh-Feldt	1535.055	205.812	7.459				
	Lower-bound	1535.055	55.000	27.910				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: RootT

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	186.897	1	186.897	28.042	.000	28.042	.999
	Quadratic	3.574	1	3.574	.434	.513	.434	.099
	Cubic	70.871	1	70.871	24.115	.000	24.115	.998
	Order 4	599.274	1	599.274	59.443	.000	59.443	1.000
Day * Treatment	Linear	38.959	4	9.740	1.461	.226	5.845	.424
	Quadratic	30.828	4	7.707	.937	.449	3.748	.278
	Cubic	9.280	4	2.320	.789	.537	3.158	.237
	Order 4	66.818	4	16.704	1.657	.173	6.628	.476
Error(Day)	Linear	366.565	55	6.665				
	Quadratic	452.373	55	8.225				
	Cubic	161.637	55	2.939				
	Order 4	554.480	55	10.081				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: RootT
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	14540.276	1	14540.276	1016.187	.000	1016.187	1.000
Treatment	515.185	4	128.796	9.001	.000	36.005	.999
Error	786.976	55	14.309				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
RT1, RT2, RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.000	.	.	.
	Hotelling's Trace	.000		.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	50.000	1.000	.000	.050
RT1, RT2, RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT2, RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT2, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT2, RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT2, RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT2, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT2, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT2, RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT2, RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT2, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1, RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT2, RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT2, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT2, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
RT1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	514.478	55	9.354				
RT2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	475.975	55	8.654				
RT3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	334.674	55	6.085				
RT5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	571.664	55	10.394				
RT7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	425.240	55	7.732				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: RootT

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
6.962	.218	6.524	7.400

2. Treatment

Estimates

Measure: RootT

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	8.697	.488	7.718	9.675
0.001	8.091	.488	7.112	9.069
0.01	7.012	.488	6.033	7.990
0.1	5.339	.488	4.361	6.318
1	5.671	.488	4.692	6.649

Pairwise Comparisons

Measure: RootT

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Control	0.001	.606	.691	1.000	-1.414	2.626
	0.01	1.685	.691	.179	-.335	3.705
	0.1	3.357*	.691	.000	1.337	5.377
	1	3.026*	.691	.001	1.006	5.046
0.001	Control	-.606	.691	1.000	-2.626	1.414
	0.01	1.079	.691	1.000	-.941	3.099
	0.1	2.751*	.691	.002	.731	4.771
	1	2.420*	.691	.009	.400	4.440
0.01	Control	-1.685	.691	.179	-3.705	.335
	0.001	-1.079	.691	1.000	-3.099	.941
	0.1	1.672	.691	.188	-.348	3.692
	1	1.341	.691	.573	-.679	3.361
0.1	Control	-3.357*	.691	.000	-5.377	-1.337
	0.001	-2.751*	.691	.002	-4.771	-.731
	0.01	-1.672	.691	.188	-3.692	.348
	1	-.331	.691	1.000	-2.351	1.689
1	Control	-3.026*	.691	.001	-5.046	-1.006
	0.001	-2.420*	.691	.009	-4.440	-.400
	0.01	-1.341	.691	.573	-3.361	.679

0.1	.331	.691	1.000	-1.689	2.351

Based on estimated marginal means
*. The mean difference is significant at the .05 level.
b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootT

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	103.037	4	25.759	9.001	.000	36.005	.999
Error	157.395	55	2.862				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: RootT

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	8.174	.395	7.383	8.966
2	8.278	.380	7.517	9.039
3	4.565	.318	3.927	5.203
4	8.537	.416	7.703	9.371
5	5.255	.359	4.535	5.974

Pairwise Comparisons

Measure: RootT

					95% Confidence Interval for Difference[b]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	Lower Bound	Upper Bound
1	2	-.104	.446	1.000	-1.409	1.202
	3	3.609*	.539	.000	2.034	5.185
	4	-.362	.491	1.000	-1.799	1.074
	5	2.920*	.511	.000	1.425	4.415
2	1	.104	.446	1.000	-1.202	1.409
	3	3.713*	.504	.000	2.240	5.186
	4	-.259	.243	1.000	-.968	.451
	5	3.024*	.483	.000	1.611	4.436
3	1	-3.609*	.539	.000	-5.185	-2.034
	2	-3.713*	.504	.000	-5.186	-2.240
	4	-3.972*	.514	.000	-5.476	-2.468
	5	-.690	.502	1.000	-2.158	.779
4	1	.362	.491	1.000	-1.074	1.799
	2	.259	.243	1.000	-.451	.968
	3	3.972*	.514	.000	2.468	5.476
	5	3.282*	.521	.000	1.758	4.807
5	1	-2.920*	.511	.000	-4.415	-1.425
	2	-3.024*	.483	.000	-4.436	-1.611
	3	.690	.502	1.000	-.779	2.158

4	-3.282*	.521	.000	-4.807	-1.758

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.601	19.620[a]	4.000	52.000	.000	78.479	1.000
Wilks' lambda	.399	19.620[a]	4.000	52.000	.000	78.479	1.000
Hotelling's trace	1.509	19.620[a]	4.000	52.000	.000	78.479	1.000
Roy's largest root	1.509	19.620[a]	4.000	52.000	.000	78.479	1.000

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: RootT

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	10.901	.883	9.131	12.670
	2	10.815	.849	9.113	12.517
	3	4.533	.712	3.105	5.960
	4	10.815	.931	8.950	12.680
	5	6.421	.803	4.812	8.029
0.001	1	9.393	.883	7.624	11.163
	2	9.668	.849	7.966	11.370
	3	6.495	.712	5.068	7.922
	4	9.668	.931	7.803	11.533
	5	5.229	.803	3.621	6.838
0.01	1	7.996	.883	6.226	9.765
	2	8.432	.849	6.730	10.134
	3	3.932	.712	2.505	5.360
	4	8.432	.931	6.567	10.297
	5	6.266	.803	4.657	7.874
0.1	1	6.072	.883	4.303	7.842
	2	6.380	.849	4.678	8.082
	3	3.932	.712	2.505	5.360
	4	6.380	.931	4.515	8.245
	5	3.933	.803	2.324	5.541
1	1	6.510	.883	4.741	8.279
	2	6.097	.849	4.395	7.799
	3	3.932	.712	2.505	5.360
	4	7.389	.931	5.524	9.254
	5	4.425	.803	2.816	6.034

Pairwise Comparisons

Measure: RootT

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	0.001	1.508	1.249	1.000	-2.144	5.159
		0.01	2.905	1.249	.237	-.747	6.557
		0.1	4.828*	1.249	.003	1.177	8.480
		1	4.391*	1.249	.009	.739	8.043
	0.001	Control	-1.508	1.249	1.000	-5.159	2.144
		0.01	1.397	1.249	1.000	-2.254	5.049
		0.1	3.321	1.249	.102	-.331	6.973
		1	2.883	1.249	.247	-.768	6.535
	0.01	Control	-2.905	1.249	.237	-6.557	.747
		0.001	-1.397	1.249	1.000	-5.049	2.254
		0.1	1.923	1.249	1.000	-1.728	5.575
		1	1.486	1.249	1.000	-2.166	5.138
	0.1	Control	-4.828*	1.249	.003	-8.480	-1.177
		0.001	-3.321	1.249	.102	-6.973	.331
		0.01	-1.923	1.249	1.000	-5.575	1.728
		1	-.438	1.249	1.000	-4.089	3.214
	1	Control	-4.391*	1.249	.009	-8.043	-.739
		0.001	-2.883	1.249	.247	-6.535	.768
		0.01	-1.486	1.249	1.000	-5.138	2.166
		0.1	.438	1.249	1.000	-3.214	4.089
2	Control	0.001	1.147	1.201	1.000	-2.366	4.659
		0.01	2.383	1.201	.522	-1.129	5.896
		0.1	4.435*	1.201	.005	.922	7.948
		1	4.718*	1.201	.002	1.206	8.231
	0.001	Control	-1.147	1.201	1.000	-4.659	2.366
		0.01	1.237	1.201	1.000	-2.276	4.749
		0.1	3.288	1.201	.083	-.224	6.801
		1	3.572*	1.201	.044	.059	7.084
	0.01	Control	-2.383	1.201	.522	-5.896	1.129
		0.001	-1.237	1.201	1.000	-4.749	2.276
		0.1	2.052	1.201	.932	-1.461	5.564
		1	2.335	1.201	.570	-1.178	5.848
	0.1	Control	-4.435*	1.201	.005	-7.948	-.922
		0.001	-3.288	1.201	.083	-6.801	.224
		0.01	-2.052	1.201	.932	-5.564	1.461
		1	.283	1.201	1.000	-3.229	3.796
	1	Control	-4.718*	1.201	.002	-8.231	-1.206
		0.001	-3.572*	1.201	.044	-7.084	-.059
		0.01	-2.335	1.201	.570	-5.848	1.178
		0.1	-.283	1.201	1.000	-3.796	3.229
3	Control	0.001	-1.962	1.007	.564	-4.908	.983
		0.01	.600	1.007	1.000	-2.345	3.545
		0.1	.600	1.007	1.000	-2.345	3.545
		1	.600	1.007	1.000	-2.345	3.545
	0.001	Control	1.962	1.007	.564	-.983	4.908
		0.01	2.562	1.007	.138	-.383	5.508
		0.1	2.562	1.007	.138	-.383	5.508
		1	2.563	1.007	.138	-.383	5.508
	0.01	Control	-.600	1.007	1.000	-3.545	2.345
		0.001	-2.562	1.007	.138	-5.508	.383
		0.1	.000	1.007	1.000	-2.945	2.945

		1	5.329E-15	1.007	1.000	-2.945	2.945
	0.1	Control	-.600	1.007	1.000	-3.545	2.345
		0.001	-2.562	1.007	.138	-5.508	.383
		0.01	.000	1.007	1.000	-2.945	2.945
		1	5.329E-15	1.007	1.000	-2.945	2.945
	1	Control	-.600	1.007	1.000	-3.545	2.345
		0.001	-2.563	1.007	.138	-5.508	.383
		0.01	-5.329E-15	1.007	1.000	-2.945	2.945
		0.1	-5.329E-15	1.007	1.000	-2.945	2.945
4	Control	0.001	1.147	1.316	1.000	-2.703	4.996
		0.01	2.383	1.316	.756	-1.466	6.233
		0.1	4.435*	1.316	.014	.586	8.284
		1	3.426	1.316	.119	-.424	7.275
	0.001	Control	-1.147	1.316	1.000	-4.996	2.703
		0.01	1.237	1.316	1.000	-2.613	5.086
		0.1	3.288	1.316	.155	-.561	7.138
		1	2.279	1.316	.889	-1.570	6.129
	0.01	Control	-2.383	1.316	.756	-6.233	1.466
		0.001	-1.237	1.316	1.000	-5.086	2.613
		0.1	2.052	1.316	1.000	-1.798	5.901
		1	1.043	1.316	1.000	-2.807	4.892
	0.1	Control	-4.435*	1.316	.014	-8.284	-.586
		0.001	-3.288	1.316	.155	-7.138	.561
		0.01	-2.052	1.316	1.000	-5.901	1.798
		1	-1.009	1.316	1.000	-4.859	2.840
	1	Control	-3.426	1.316	.119	-7.275	.424
		0.001	-2.279	1.316	.889	-6.129	1.570
		0.01	-1.043	1.316	1.000	-4.892	2.807
		0.1	1.009	1.316	1.000	-2.840	4.859
5	Control	0.001	1.192	1.135	1.000	-2.128	4.512
		0.01	.155	1.135	1.000	-3.165	3.475
		0.1	2.488	1.135	.326	-.832	5.808
		1	1.996	1.135	.843	-1.324	5.316
	0.001	Control	-1.192	1.135	1.000	-4.512	2.128
		0.01	-1.037	1.135	1.000	-4.357	2.283
		0.1	1.297	1.135	1.000	-2.023	4.617
		1	.804	1.135	1.000	-2.516	4.124
	0.01	Control	-.155	1.135	1.000	-3.475	3.165
		0.001	1.037	1.135	1.000	-2.283	4.357
		0.1	2.333	1.135	.446	-.987	5.653
		1	1.841	1.135	1.000	-1.479	5.161
	0.1	Control	-2.488	1.135	.326	-5.808	.832
		0.001	-1.297	1.135	1.000	-4.617	2.023
		0.01	-2.333	1.135	.446	-5.653	.987
		1	-.493	1.135	1.000	-3.813	2.828
	1	Control	-1.996	1.135	.843	-5.316	1.324
		0.001	-.804	1.135	1.000	-4.124	2.516
		0.01	-1.841	1.135	1.000	-5.161	1.479
		0.1	.493	1.135	1.000	-2.828	3.813

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootT

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	193.672	4	48.418	5.176	.001	20.704	.955
	Error	514.478	55	9.354				
2	Contrast	201.044	4	50.261	5.808	.001	23.231	.974
	Error	475.975	55	8.654				
3	Contrast	59.114	4	14.778	2.429	.059	9.715	.659
	Error	334.674	55	6.085				
4	Contrast	149.406	4	37.351	3.594	.011	14.374	.843
	Error	571.664	55	10.394				
5	Contrast	57.834	4	14.459	1.870	.129	7.480	.531
	Error	425.240	55	7.732				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: RootT

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	10.901	.883	9.131	12.670
	2	10.815	.849	9.113	12.517
	3	4.533	.712	3.105	5.960
	4	10.815	.931	8.950	12.680
	5	6.421	.803	4.812	8.029
0.001	1	9.393	.883	7.624	11.163
	2	9.668	.849	7.966	11.370
	3	6.495	.712	5.068	7.922
	4	9.668	.931	7.803	11.533
	5	5.229	.803	3.621	6.838
0.01	1	7.996	.883	6.226	9.765
	2	8.432	.849	6.730	10.134
	3	3.932	.712	2.505	5.360
	4	8.432	.931	6.567	10.297
	5	6.266	.803	4.657	7.874
0.1	1	6.072	.883	4.303	7.842
	2	6.380	.849	4.678	8.082
	3	3.932	.712	2.505	5.360
	4	6.380	.931	4.515	8.245
	5	3.933	.803	2.324	5.541
1	1	6.510	.883	4.741	8.279
	2	6.097	.849	4.395	7.799
	3	3.932	.712	2.505	5.360
	4	7.389	.931	5.524	9.254
	5	4.425	.803	2.816	6.034

Pairwise Comparisons

Measure: RootT

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	.086	.998	1.000	-2.834	3.005
		3	6.368*	1.204	.000	2.846	9.891
		4	.086	1.098	1.000	-3.126	3.298
		5	4.480*	1.143	.002	1.137	7.823
	2	1	-.086	.998	1.000	-3.005	2.834
		3	6.283*	1.126	.000	2.989	9.576
		4	-1.776E-15	.542	1.000	-1.587	1.587
		5	4.394*	1.080	.002	1.236	7.552
	3	1	-6.368*	1.204	.000	-9.891	-2.846
		2	-6.283*	1.126	.000	-9.576	-2.989
		4	-6.283*	1.150	.000	-9.646	-2.919
		5	-1.888	1.123	.982	-5.171	1.395
	4	1	-.086	1.098	1.000	-3.298	3.126
		2	1.776E-15	.542	1.000	-1.587	1.587
		3	6.283*	1.150	.000	2.919	9.646
		5	4.394*	1.166	.004	.985	7.803
	5	1	-4.480*	1.143	.002	-7.823	-1.137
		2	-4.394*	1.080	.002	-7.552	-1.236
		3	1.888	1.123	.982	-1.395	5.171
		4	-4.394*	1.166	.004	-7.803	-.985
0.001	1	2	-.275	.998	1.000	-3.194	2.644
		3	2.898	1.204	.195	-.624	6.421
		4	-.275	1.098	1.000	-3.487	2.937
		5	4.164*	1.143	.006	.821	7.507
	2	1	.275	.998	1.000	-2.644	3.194
		3	3.173	1.126	.067	-.120	6.467
		4	-1.776E-15	.542	1.000	-1.587	1.587
		5	4.439*	1.080	.001	1.281	7.597
	3	1	-2.898	1.204	.195	-6.421	.624
		2	-3.173	1.126	.067	-6.467	.120
		4	-3.173	1.150	.078	-6.537	.190
		5	1.266	1.123	1.000	-2.017	4.549
	4	1	.275	1.098	1.000	-2.937	3.487
		2	1.776E-15	.542	1.000	-1.587	1.587
		3	3.173	1.150	.078	-.190	6.537
		5	4.439*	1.166	.004	1.030	7.848
	5	1	-4.164*	1.143	.006	-7.507	-.821
		2	-4.439*	1.080	.001	-7.597	-1.281
		3	-1.266	1.123	1.000	-4.549	2.017
		4	-4.439*	1.166	.004	-7.848	-1.030
0.01	1	2	-.436	.998	1.000	-3.355	2.484
		3	4.063*	1.204	.014	.541	7.586
		4	-.436	1.098	1.000	-3.648	2.776
		5	1.730	1.143	1.000	-1.613	5.073
	2	1	.436	.998	1.000	-2.484	3.355

		3	4.499*	1.126	.002	1.205	7.793
		4	.000	.542	1.000	-1.587	1.587
		5	2.166	1.080	.498	-.992	5.324
	3	1	-4.063*	1.204	.014	-7.586	-.541
		2	-4.499*	1.126	.002	-7.793	-1.205
		4	-4.499*	1.150	.003	-7.863	-1.136
		5	-2.333	1.123	.423	-5.616	.950
	4	1	.436	1.098	1.000	-2.776	3.648
		2	.000	.542	1.000	-1.587	1.587
		3	4.499*	1.150	.003	1.136	7.863
		5	2.166	1.166	.685	-1.243	5.575
	5	1	-1.730	1.143	1.000	-5.073	1.613
		2	-2.166	1.080	.498	-5.324	.992
		3	2.333	1.123	.423	-.950	5.616
		4	-2.166	1.166	.685	-5.575	1.243
0.1	1	2	-.308	.998	1.000	-3.227	2.612
		3	2.140	1.204	.811	-1.383	5.663
		4	-.308	1.098	1.000	-3.519	2.904
		5	2.140	1.143	.665	-1.203	5.483
	2	1	.308	.998	1.000	-2.612	3.227
		3	2.447	1.126	.341	-.846	5.741
		4	-8.882E-16	.542	1.000	-1.587	1.587
		5	2.447	1.080	.274	-.711	5.606
	3	1	-2.140	1.204	.811	-5.663	1.383
		2	-2.447	1.126	.341	-5.741	.846
		4	-2.448	1.150	.378	-5.811	.916
		5	-4.441E-16	1.123	1.000	-3.283	3.283
	4	1	.308	1.098	1.000	-2.904	3.519
		2	8.882E-16	.542	1.000	-1.587	1.587
		3	2.448	1.150	.378	-.916	5.811
		5	2.448	1.166	.403	-.961	5.856
	5	1	-2.140	1.143	.665	-5.483	1.203
		2	-2.447	1.080	.274	-5.606	.711
		3	4.441E-16	1.123	1.000	-3.283	3.283
		4	-2.448	1.166	.403	-5.856	.961
1	1	2	.413	.998	1.000	-2.506	3.333
		3	2.578	1.204	.368	-.945	6.100
		4	-.879	1.098	1.000	-4.091	2.333
		5	2.085	1.143	.735	-1.258	5.428
	2	1	-.413	.998	1.000	-3.333	2.506
		3	2.164	1.126	.598	-1.130	5.458
		4	-1.293	.542	.207	-2.879	.294
		5	1.672	1.080	1.000	-1.486	4.830
	3	1	-2.578	1.204	.368	-6.100	.945
		2	-2.164	1.126	.598	-5.458	1.130
		4	-3.457*	1.150	.040	-6.820	-.093
		5	-.493	1.123	1.000	-3.776	2.791
	4	1	.879	1.098	1.000	-2.333	4.091
		2	1.293	.542	.207	-.294	2.879
		3	3.457*	1.150	.040	.093	6.820
		5	2.964	1.166	.138	-.445	6.373

	5	1	-2.085	1.143	.735	-5.428	1.258
		2	-1.672	1.080	1.000	-4.830	1.486
		3	.493	1.123	1.000	-2.791	3.776
		4	-2.964	1.166	.138	-6.373	.445

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

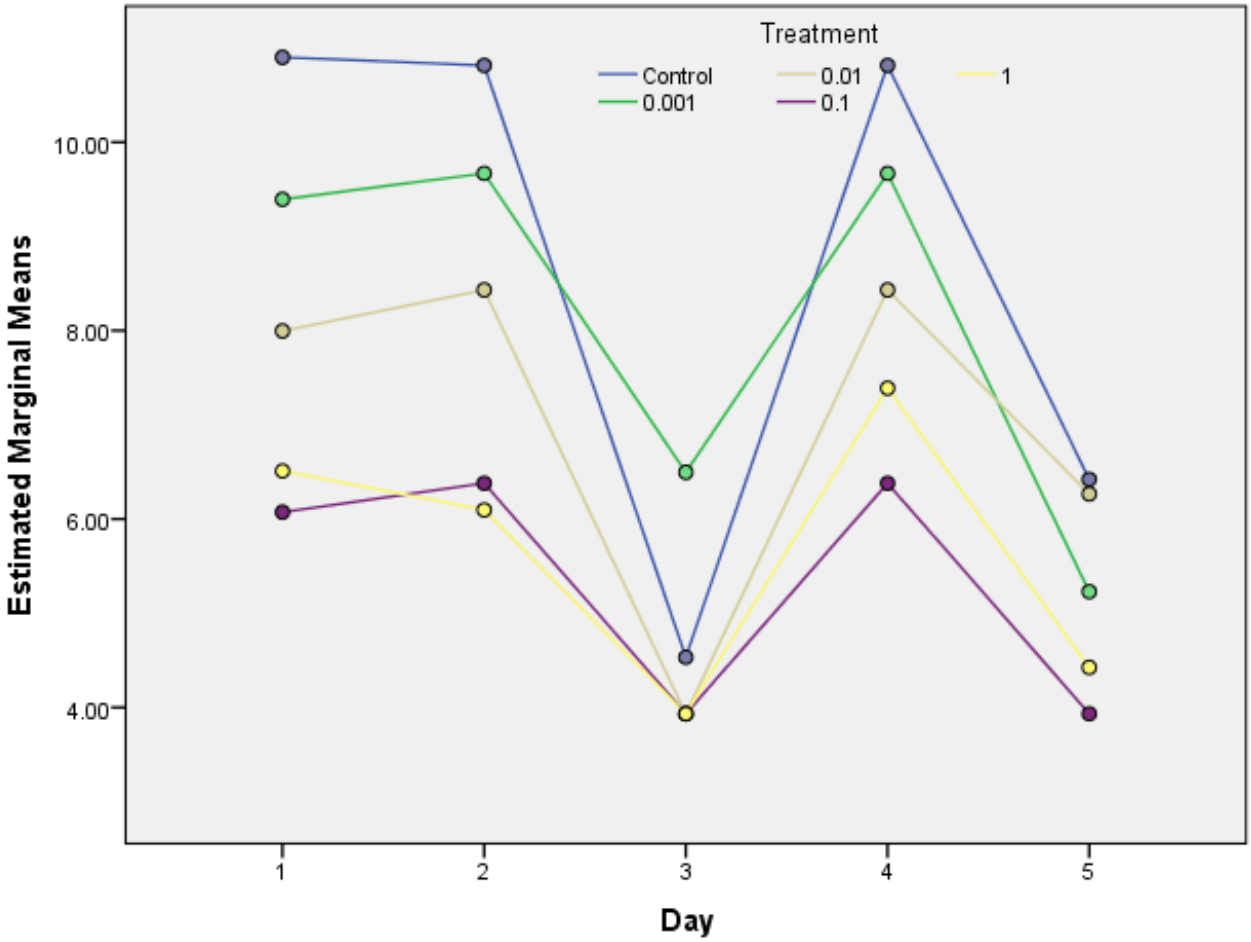
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.436	10.055[a]	4.000	52.000	.000	40.220	1.000
	Wilks' lambda	.564	10.055[a]	4.000	52.000	.000	40.220	1.000
	Hotelling's trace	.773	10.055[a]	4.000	52.000	.000	40.220	1.000
	Roy's largest root	.773	10.055[a]	4.000	52.000	.000	40.220	1.000
0.001	Pillai's trace	.277	4.971[a]	4.000	52.000	.002	19.882	.945
	Wilks' lambda	.723	4.971[a]	4.000	52.000	.002	19.882	.945
	Hotelling's trace	.382	4.971[a]	4.000	52.000	.002	19.882	.945
	Roy's largest root	.382	4.971[a]	4.000	52.000	.002	19.882	.945
0.01	Pillai's trace	.246	4.234[a]	4.000	52.000	.005	16.935	.901
	Wilks' lambda	.754	4.234[a]	4.000	52.000	.005	16.935	.901
	Hotelling's trace	.326	4.234[a]	4.000	52.000	.005	16.935	.901
	Roy's largest root	.326	4.234[a]	4.000	52.000	.005	16.935	.901
0.1	Pillai's trace	.123	1.817[a]	4.000	52.000	.140	7.266	.515
	Wilks' lambda	.877	1.817[a]	4.000	52.000	.140	7.266	.515
	Hotelling's trace	.140	1.817[a]	4.000	52.000	.140	7.266	.515
	Roy's largest root	.140	1.817[a]	4.000	52.000	.140	7.266	.515
1	Pillai's trace	.204	3.325[a]	4.000	52.000	.017	13.299	.808
	Wilks' lambda	.796	3.325[a]	4.000	52.000	.017	13.299	.808
	Hotelling's trace	.256	3.325[a]	4.000	52.000	.017	13.299	.808
	Roy's largest root	.256	3.325[a]	4.000	52.000	.017	13.299	.808

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots


Treatment
Control
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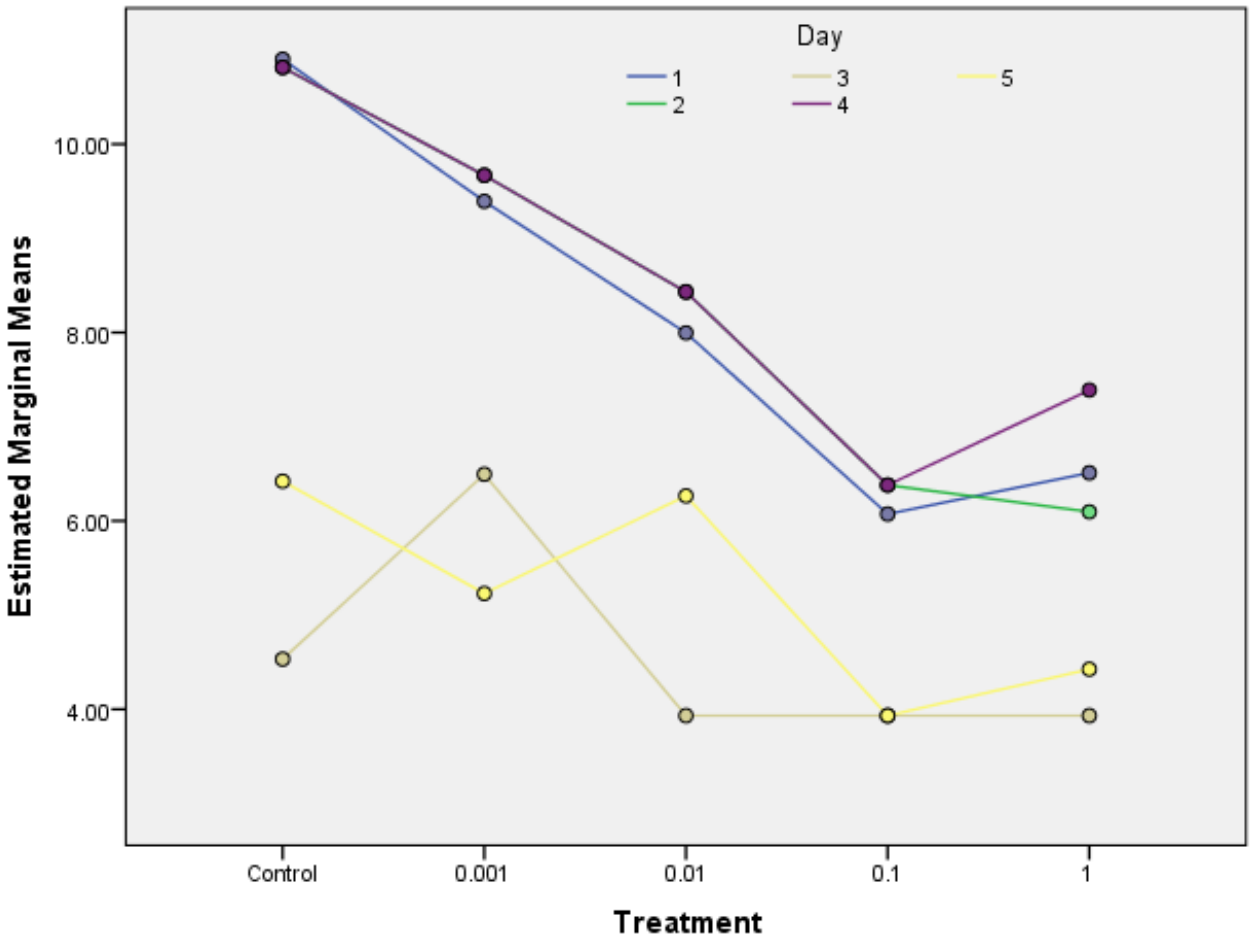
0.001
0.1
Estimated Marginal Means
10.00
8.00
6.00
4.00
1
2
3
4
5
Day



TITLE Aluminium.

Aluminium

```
GLM SA1 SA2 SA3 SA5 SA7 BY Treatment
  /WSFACTOR=Day 5 Polynomial
  /MEASURE=ShootA
  /METHOD=SSTYPE(3)
  /PLOT=PROFILE(Day*Treatment Treatment*Day)
  /EMMEANS=TABLES(OVERALL)
  /EMMEANS=TABLES(Treatment) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Treatment) ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Day) ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE OPOWER LOF
  /CRITERIA=ALPHA(.05)
  /WSDESIGN= Day
  /DESIGN= Treatment.
```

General Linear Model

Within-Subjects Factors

Measure: ShootA

Day	Dependent Variable
1	SA1
2	SA2
3	SA3
4	SA5
5	SA7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	0.001	12
	2	0.01	12
	3	0.1	12
	4	1	13

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
SA1	Control	4.1225	.89458	12
	0.001	4.5058	1.49172	12
	0.01	7.5342	4.94718	12
	0.1	4.8358	2.36273	12
	1	4.4115	1.46812	13
	Total	5.0710	2.83879	61
SA2	Control	4.1225	.89458	12
	0.001	4.5058	1.49172	12
	0.01	7.2442	4.97073	12

	0.1	5.0075	2.70010	12
	1	4.5654	1.44426	13
	Total	5.0805	2.84302	61
SA3	Control	4.1225	.89458	12
	0.001	4.5058	1.49172	12
	0.01	7.5342	4.94718	12
	0.1	5.0417	2.83749	12
	1	4.7823	2.67891	13
	Total	5.1905	3.06823	61
SA5	Control	4.1225	.89458	12
	0.001	4.8792	1.27039	12
	0.01	3.8225	1.09365	12
	0.1	4.4342	1.43852	12
	1	6.6677	4.60884	13
	Total	4.8161	2.52111	61
SA7	Control	4.1225	.89458	12
	0.001	4.8792	1.27039	12
	0.01	3.7183	.96098	12
	0.1	4.5058	1.49172	12
	1	4.9454	2.12920	13
	Total	4.4426	1.46480	61

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.089	1.301[b]	4.000	53.000	.281	5.206	.379
	Wilks' Lambda	.911	1.301[b]	4.000	53.000	.281	5.206	.379
	Hotelling's Trace	.098	1.301[b]	4.000	53.000	.281	5.206	.379
	Roy's Largest Root	.098	1.301[b]	4.000	53.000	.281	5.206	.379
Day * Treatment	Pillai's Trace	.395	1.533	16.000	224.000	.090	24.531	.884
	Wilks' Lambda	.635	1.629	16.000	162.555	.067	19.547	.766
	Hotelling's Trace	.528	1.700	16.000	206.000	.049	27.201	.920
	Roy's Largest Root	.417	5.842[c]	4.000	56.000	.001	23.369	.975

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: ShootA

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.288	67.809	9	.000	.622	.700	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: ShootA

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	23.511	4	5.878	1.306	.268	5.226	.405
	Greenhouse-Geisser	23.511	2.489	9.445	1.306	.275	3.252	.311

	Huynh-Feldt	23.511	2.800	8.396	1.306	.275	3.658	.331
	Lower-bound	23.511	1.000	23.511	1.306	.258	1.306	.202
Day * Treatment	Sphericity Assumed	221.615	16	13.851	3.079	.000	49.257	.998
	Greenhouse-Geisser	221.615	9.956	22.259	3.079	.001	30.652	.979
	Huynh-Feldt	221.615	11.200	19.786	3.079	.001	34.481	.987
	Lower-bound	221.615	4.000	55.404	3.079	.023	12.314	.776
Error(Day)	Sphericity Assumed	1007.803	224	4.499				
	Greenhouse-Geisser	1007.803	139.389	7.230				
	Huynh-Feldt	1007.803	156.805	6.427				
	Lower-bound	1007.803	56.000	17.996				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: ShootA

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	15.587	1	15.587	2.424	.125	2.424	.334
	Quadratic	6.654	1	6.654	2.743	.103	2.743	.370
	Cubic	.010	1	.010	.003	.958	.003	.050
	Order 4	1.260	1	1.260	.220	.641	.220	.075
Day * Treatment	Linear	148.891	4	37.223	5.789	.001	23.156	.973
	Quadratic	9.242	4	2.310	.953	.441	3.810	.282
	Cubic	29.423	4	7.356	2.155	.086	8.619	.600
	Order 4	34.059	4	8.515	1.487	.219	5.947	.431
Error(Day)	Linear	360.072	56	6.430				
	Quadratic	135.836	56	2.426				
	Cubic	191.170	56	3.414				
	Order 4	320.725	56	5.727				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: ShootA
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	7368.071	1	7368.071	587.418	.000	587.418	1.000
Treatment	111.595	4	27.899	2.224	.078	8.897	.616
Error	702.417	56	12.543				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
SA1, SA2, SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	51.000	1.000	.000	.050
SA1, SA2, SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA2, SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA2, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA2, SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA2, SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA2, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA2, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA2, SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA2, SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA2, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1, SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA2, SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA2, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA2, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
SA1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	389.773	56	6.960				
SA2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	410.297	56	7.327				
SA3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	477.184	56	8.521				
SA5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	317.372	56	5.667				
SA7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	115.593	56	2.064				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: ShootA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
4.918	.203	4.511	5.324

2. Treatment

Estimates

Measure: ShootA

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	4.123	.457	3.207	5.038
0.001	4.655	.457	3.739	5.571
0.01	5.971	.457	5.055	6.887
0.1	4.765	.457	3.849	5.681
1	5.074	.439	4.194	5.954

Pairwise Comparisons

Measure: ShootA

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	0.001	-.533	.647	1.000	-2.422	1.357
	0.01	-1.848	.647	.060	-3.738	.042
	0.1	-.643	.647	1.000	-2.532	1.247
	1	-.952	.634	1.000	-2.805	.901
0.001	Control	.533	.647	1.000	-1.357	2.422
	0.01	-1.316	.647	.467	-3.205	.574
	0.1	-.110	.647	1.000	-2.000	1.780
	1	-.419	.634	1.000	-2.272	1.434
0.01	Control	1.848	.647	.060	-.042	3.738
	0.001	1.316	.647	.467	-.574	3.205
	0.1	1.206	.647	.675	-.684	3.095
	1	.896	.634	1.000	-.957	2.749
0.1	Control	.643	.647	1.000	-1.247	2.532
	0.001	.110	.647	1.000	-1.780	2.000
	0.01	-1.206	.647	.675	-3.095	.684
	1	-.309	.634	1.000	-2.162	1.544
1	Control	.952	.634	1.000	-.901	2.805
	0.001	.419	.634	1.000	-1.434	2.272
	0.01	-.896	.634	1.000	-2.749	.957

0.1	.309	.634	1.000	-1.544	2.162

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootA

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	22.319	4	5.580	2.224	.078	8.897	.616
Error	140.483	56	2.509				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: ShootA

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	5.082	.338	4.405	5.759
2	5.089	.347	4.394	5.784
3	5.197	.374	4.448	5.946
4	4.785	.305	4.174	5.396
5	4.434	.184	4.066	4.803

Pairwise Comparisons

Measure: ShootA

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	-.007	.329	1.000	-.968	.954
	3	-.115	.234	1.000	-.800	.570
	4	.297	.457	1.000	-1.040	1.634
	5	.648	.359	.768	-.402	1.698
2	1	.007	.329	1.000	-.954	.968
	3	-.108	.374	1.000	-1.203	.986
	4	.304	.440	1.000	-.983	1.591
	5	.655	.379	.896	-.453	1.763
3	1	.115	.234	1.000	-.570	.800
	2	.108	.374	1.000	-.986	1.203
	4	.412	.490	1.000	-1.019	1.843
	5	.763	.430	.817	-.495	2.021
4	1	-.297	.457	1.000	-1.634	1.040
	2	-.304	.440	1.000	-1.591	.983
	3	-.412	.490	1.000	-1.843	1.019
	5	.351	.269	1.000	-.435	1.137
5	1	-.648	.359	.768	-1.698	.402
	2	-.655	.379	.896	-1.763	.453
	3	-.763	.430	.817	-2.021	.495

4	-.351	.269	1.000	-1.137	.435

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.089	1.301[a]	4.000	53.000	.281	5.206	.379
Wilks' lambda	.911	1.301[a]	4.000	53.000	.281	5.206	.379
Hotelling's trace	.098	1.301[a]	4.000	53.000	.281	5.206	.379
Roy's largest root	.098	1.301[a]	4.000	53.000	.281	5.206	.379

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: ShootA

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	4.123	.762	2.597	5.648
	2	4.123	.781	2.557	5.688
	3	4.122	.843	2.434	5.811
	4	4.123	.687	2.746	5.499
	5	4.123	.415	3.292	4.953
0.001	1	4.506	.762	2.980	6.031
	2	4.506	.781	2.941	6.071
	3	4.506	.843	2.818	6.194
	4	4.879	.687	3.502	6.256
	5	4.879	.415	4.048	5.710
0.01	1	7.534	.762	6.009	9.060
	2	7.244	.781	5.679	8.809
	3	7.534	.843	5.846	9.222
	4	3.823	.687	2.446	5.199
	5	3.718	.415	2.887	4.549
0.1	1	4.836	.762	3.310	6.361
	2	5.008	.781	3.442	6.573
	3	5.042	.843	3.354	6.730
	4	4.434	.687	3.057	5.811
	5	4.506	.415	3.675	5.337
1	1	4.412	.732	2.946	5.877
	2	4.565	.751	3.061	6.069
	3	4.782	.810	3.160	6.404
	4	6.668	.660	5.345	7.990
	5	4.945	.398	4.147	5.744

Pairwise Comparisons

Measure: ShootA

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	0.001	-.383	1.077	1.000	-3.531	2.764
		0.01	-3.412*	1.077	.025	-6.559	-.264
		0.1	-.713	1.077	1.000	-3.861	2.434
		1	-.289	1.056	1.000	-3.376	2.798
	0.001	Control	.383	1.077	1.000	-2.764	3.531
		0.01	-3.028	1.077	.068	-6.176	.119
		0.1	-.330	1.077	1.000	-3.478	2.818
		1	.094	1.056	1.000	-2.992	3.181
	0.01	Control	3.412*	1.077	.025	.264	6.559
		0.001	3.028	1.077	.068	-.119	6.176
		0.1	2.698	1.077	.152	-.449	5.846
		1	3.123*	1.056	.045	.036	6.209
	0.1	Control	.713	1.077	1.000	-2.434	3.861
		0.001	.330	1.077	1.000	-2.818	3.478
		0.01	-2.698	1.077	.152	-5.846	.449
		1	.424	1.056	1.000	-2.662	3.511
	1	Control	.289	1.056	1.000	-2.798	3.376
		0.001	-.094	1.056	1.000	-3.181	2.992
		0.01	-3.123*	1.056	.045	-6.209	-.036
		0.1	-.424	1.056	1.000	-3.511	2.662
2	Control	0.001	-.383	1.105	1.000	-3.613	2.846
		0.01	-3.122	1.105	.065	-6.351	.108
		0.1	-.885	1.105	1.000	-4.115	2.345
		1	-.443	1.084	1.000	-3.610	2.724
	0.001	Control	.383	1.105	1.000	-2.846	3.613
		0.01	-2.738	1.105	.163	-5.968	.491
		0.1	-.502	1.105	1.000	-3.731	2.728
		1	-.060	1.084	1.000	-3.226	3.107
	0.01	Control	3.122	1.105	.065	-.108	6.351
		0.001	2.738	1.105	.163	-.491	5.968
		0.1	2.237	1.105	.477	-.993	5.466
		1	2.679	1.084	.165	-.488	5.846
	0.1	Control	.885	1.105	1.000	-2.345	4.115
		0.001	.502	1.105	1.000	-2.728	3.731
		0.01	-2.237	1.105	.477	-5.466	.993
		1	.442	1.084	1.000	-2.725	3.609
	1	Control	.443	1.084	1.000	-2.724	3.610
		0.001	.060	1.084	1.000	-3.107	3.226
		0.01	-2.679	1.084	.165	-5.846	.488
		0.1	-.442	1.084	1.000	-3.609	2.725
3	Control	0.001	-.383	1.192	1.000	-3.866	3.099
		0.01	-3.412	1.192	.059	-6.894	.071
		0.1	-.919	1.192	1.000	-4.402	2.564
		1	-.660	1.169	1.000	-4.075	2.755
	0.001	Control	.383	1.192	1.000	-3.099	3.866
		0.01	-3.028	1.192	.138	-6.511	.454
		0.1	-.536	1.192	1.000	-4.019	2.947
		1	-.276	1.169	1.000	-3.692	3.139
	0.01	Control	3.412	1.192	.059	-.071	6.894
		0.001	3.028	1.192	.138	-.454	6.511
		0.1	2.493	1.192	.410	-.990	5.975
		1	2.752	1.169	.221	-.663	6.167

	0.1	Control	.919	1.192	1.000	-2.564	4.402
		0.001	.536	1.192	1.000	-2.947	4.019
		0.01	-2.493	1.192	.410	-5.975	.990
		1	.259	1.169	1.000	-3.156	3.675
	1	Control	.660	1.169	1.000	-2.755	4.075
		0.001	.276	1.169	1.000	-3.139	3.692
		0.01	-2.752	1.169	.221	-6.167	.663
		0.1	-.259	1.169	1.000	-3.675	3.156
4	Control	0.001	-.757	.972	1.000	-3.597	2.084
		0.01	.300	.972	1.000	-2.540	3.140
		0.1	-.312	.972	1.000	-3.152	2.529
		1	-2.545	.953	.099	-5.330	.240
	0.001	Control	.757	.972	1.000	-2.084	3.597
		0.01	1.057	.972	1.000	-1.784	3.897
		0.1	.445	.972	1.000	-2.395	3.285
		1	-1.789	.953	.658	-4.574	.997
	0.01	Control	-.300	.972	1.000	-3.140	2.540
		0.001	-1.057	.972	1.000	-3.897	1.784
		0.1	-.612	.972	1.000	-3.452	2.229
		1	-2.845*	.953	.042	-5.630	-.060
	0.1	Control	.312	.972	1.000	-2.529	3.152
		0.001	-.445	.972	1.000	-3.285	2.395
		0.01	.612	.972	1.000	-2.229	3.452
		1	-2.234	.953	.227	-5.019	.552
	1	Control	2.545	.953	.099	-.240	5.330
		0.001	1.789	.953	.658	-.997	4.574
		0.01	2.845*	.953	.042	.060	5.630
		0.1	2.234	.953	.227	-.552	5.019
5	Control	0.001	-.757	.587	1.000	-2.471	.958
		0.01	.404	.587	1.000	-1.310	2.118
		0.1	-.383	.587	1.000	-2.098	1.331
		1	-.823	.575	1.000	-2.504	.858
	0.001	Control	.757	.587	1.000	-.958	2.471
		0.01	1.161	.587	.527	-.553	2.875
		0.1	.373	.587	1.000	-1.341	2.088
		1	-.066	.575	1.000	-1.747	1.615
	0.01	Control	-.404	.587	1.000	-2.118	1.310
		0.001	-1.161	.587	.527	-2.875	.553
		0.1	-.788	.587	1.000	-2.502	.927
		1	-1.227	.575	.373	-2.908	.454
	0.1	Control	.383	.587	1.000	-1.331	2.098
		0.001	-.373	.587	1.000	-2.088	1.341
		0.01	.788	.587	1.000	-.927	2.502
		1	-.440	.575	1.000	-2.120	1.241
	1	Control	.823	.575	1.000	-.858	2.504
		0.001	.066	.575	1.000	-1.615	1.747
		0.01	1.227	.575	.373	-.454	2.908
		0.1	.440	.575	1.000	-1.241	2.120

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootA

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	93.752	4	23.438	3.367	.015	13.470	.816
	Error	389.773	56	6.960				
2	Contrast	74.667	4	18.667	2.548	.049	10.191	.684
	Error	410.297	56	7.327				
3	Contrast	87.658	4	21.914	2.572	.048	10.287	.688
	Error	477.184	56	8.521				
4	Contrast	63.987	4	15.997	2.823	.033	11.290	.734
	Error	317.372	56	5.667				
5	Contrast	13.146	4	3.286	1.592	.189	6.369	.460
	Error	115.593	56	2.064				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: ShootA

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	4.123	.762	2.597	5.648
	2	4.123	.781	2.557	5.688
	3	4.122	.843	2.434	5.811
	4	4.123	.687	2.746	5.499
	5	4.123	.415	3.292	4.953
0.001	1	4.506	.762	2.980	6.031
	2	4.506	.781	2.941	6.071
	3	4.506	.843	2.818	6.194
	4	4.879	.687	3.502	6.256
	5	4.879	.415	4.048	5.710
0.01	1	7.534	.762	6.009	9.060
	2	7.244	.781	5.679	8.809
	3	7.534	.843	5.846	9.222
	4	3.823	.687	2.446	5.199
	5	3.718	.415	2.887	4.549
0.1	1	4.836	.762	3.310	6.361
	2	5.008	.781	3.442	6.573
	3	5.042	.843	3.354	6.730
	4	4.434	.687	3.057	5.811
	5	4.506	.415	3.675	5.337
1	1	4.412	.732	2.946	5.877
	2	4.565	.751	3.061	6.069
	3	4.782	.810	3.160	6.404
	4	6.668	.660	5.345	7.990
	5	4.945	.398	4.147	5.744

Pairwise Comparisons

Measure: ShootA

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	8.882E-16	.741	1.000	-2.166	2.166
		3	2.665E-15	.528	1.000	-1.543	1.543
		4	8.882E-16	1.031	1.000	-3.013	3.013
		5	1.776E-15	.810	1.000	-2.367	2.367
	2	1	-8.882E-16	.741	1.000	-2.166	2.166
		3	1.776E-15	.844	1.000	-2.466	2.466
		4	.000	.992	1.000	-2.900	2.900
		5	8.882E-16	.854	1.000	-2.496	2.496
	3	1	-2.665E-15	.528	1.000	-1.543	1.543
		2	-1.776E-15	.844	1.000	-2.466	2.466
		4	-1.776E-15	1.103	1.000	-3.225	3.225
		5	-8.882E-16	.970	1.000	-2.835	2.835
	4	1	-8.882E-16	1.031	1.000	-3.013	3.013
		2	.000	.992	1.000	-2.900	2.900
		3	1.776E-15	1.103	1.000	-3.225	3.225
		5	8.882E-16	.606	1.000	-1.772	1.772
	5	1	-1.776E-15	.810	1.000	-2.367	2.367
		2	-8.882E-16	.854	1.000	-2.496	2.496
		3	8.882E-16	.970	1.000	-2.835	2.835
		4	-8.882E-16	.606	1.000	-1.772	1.772
0.001	1	2	.000	.741	1.000	-2.166	2.166
		3	1.776E-15	.528	1.000	-1.543	1.543
		4	-.373	1.031	1.000	-3.386	2.639
		5	-.373	.810	1.000	-2.740	1.993
	2	1	.000	.741	1.000	-2.166	2.166
		3	1.776E-15	.844	1.000	-2.466	2.466
		4	-.373	.992	1.000	-3.274	2.527
		5	-.373	.854	1.000	-2.870	2.123
	3	1	-1.776E-15	.528	1.000	-1.543	1.543
		2	-1.776E-15	.844	1.000	-2.466	2.466
		4	-.373	1.103	1.000	-3.598	2.851
		5	-.373	.970	1.000	-3.208	2.461
	4	1	.373	1.031	1.000	-2.639	3.386
		2	.373	.992	1.000	-2.527	3.274
		3	.373	1.103	1.000	-2.851	3.598
		5	.000	.606	1.000	-1.772	1.772
	5	1	.373	.810	1.000	-1.993	2.740
		2	.373	.854	1.000	-2.123	2.870
		3	.373	.970	1.000	-2.461	3.208
		4	.000	.606	1.000	-1.772	1.772
0.01	1	2	.290	.741	1.000	-1.876	2.456
		3	8.882E-16	.528	1.000	-1.543	1.543
		4	3.712*	1.031	.007	.699	6.724
		5	3.816*	.810	.000	1.449	6.182
	2	1	-.290	.741	1.000	-2.456	1.876
		3	-.290	.844	1.000	-2.756	2.176

		4	3.422*	.992	.011	.521	6.322
		5	3.526*	.854	.001	1.030	6.022
	3	1	-8.882E-16	.528	1.000	-1.543	1.543
		2	.290	.844	1.000	-2.176	2.756
		4	3.712*	1.103	.014	.487	6.936
		5	3.816*	.970	.002	.981	6.651
	4	1	-3.712*	1.031	.007	-6.724	-.699
		2	-3.422*	.992	.011	-6.322	-.521
		3	-3.712*	1.103	.014	-6.936	-.487
		5	.104	.606	1.000	-1.667	1.876
	5	1	-3.816*	.810	.000	-6.182	-1.449
		2	-3.526*	.854	.001	-6.022	-1.030
		3	-3.816*	.970	.002	-6.651	-.981
		4	-.104	.606	1.000	-1.876	1.667
0.1	1	2	-.172	.741	1.000	-2.338	1.994
		3	-.206	.528	1.000	-1.749	1.337
		4	.402	1.031	1.000	-2.611	3.414
		5	.330	.810	1.000	-2.037	2.697
	2	1	.172	.741	1.000	-1.994	2.338
		3	-.034	.844	1.000	-2.500	2.432
		4	.573	.992	1.000	-2.327	3.474
		5	.502	.854	1.000	-1.995	2.998
	3	1	.206	.528	1.000	-1.337	1.749
		2	.034	.844	1.000	-2.432	2.500
		4	.607	1.103	1.000	-2.617	3.832
		5	.536	.970	1.000	-2.299	3.371
	4	1	-.402	1.031	1.000	-3.414	2.611
		2	-.573	.992	1.000	-3.474	2.327
		3	-.607	1.103	1.000	-3.832	2.617
		5	-.072	.606	1.000	-1.843	1.700
	5	1	-.330	.810	1.000	-2.697	2.037
		2	-.502	.854	1.000	-2.998	1.995
		3	-.536	.970	1.000	-3.371	2.299
		4	.072	.606	1.000	-1.700	1.843
1	1	2	-.154	.712	1.000	-2.235	1.927
		3	-.371	.507	1.000	-1.853	1.112
		4	-2.256	.990	.266	-5.150	.638
		5	-.534	.778	1.000	-2.808	1.740
	2	1	.154	.712	1.000	-1.927	2.235
		3	-.217	.811	1.000	-2.586	2.152
		4	-2.102	.953	.316	-4.889	.684
		5	-.380	.821	1.000	-2.778	2.018
	3	1	.371	.507	1.000	-1.112	1.853
		2	.217	.811	1.000	-2.152	2.586
		4	-1.885	1.060	.807	-4.983	1.213
		5	-.163	.932	1.000	-2.887	2.560
	4	1	2.256	.990	.266	-.638	5.150
		2	2.102	.953	.316	-.684	4.889
		3	1.885	1.060	.807	-1.213	4.983
		5	1.722*	.582	.045	.020	3.424

	5	1	.534	.778	1.000	-1.740	2.808
		2	.380	.821	1.000	-2.018	2.778
		3	.163	.932	1.000	-2.560	2.887
		4	-1.722*	.582	.045	-3.424	-.020

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

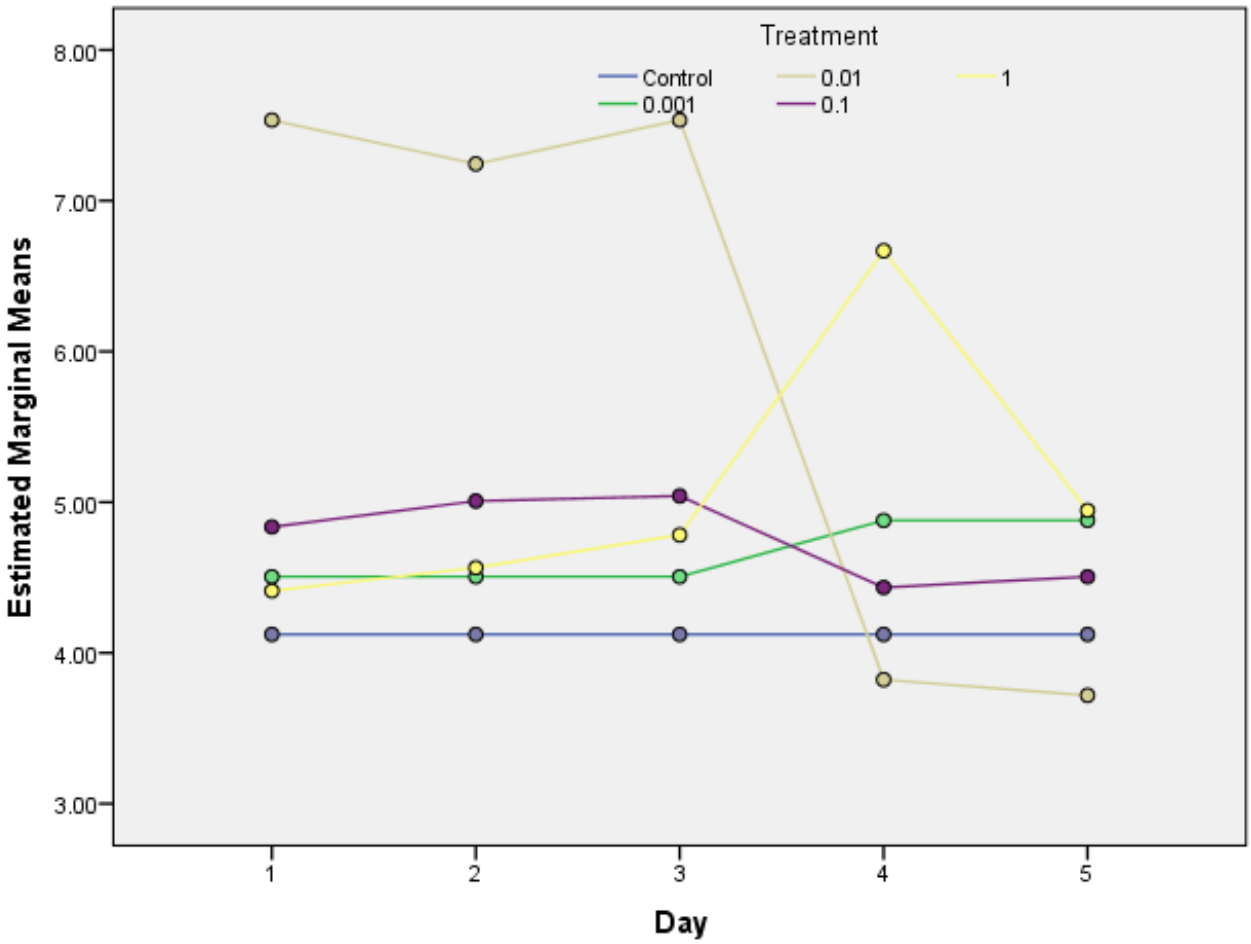
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.000	.000[a]	4.000	53.000	1.000	.000	.050
	Wilks' lambda	1.000	.000[a]	4.000	53.000	1.000	.000	.050
	Hotelling's trace	.000	.000[a]	4.000	53.000	1.000	.000	.050
	Roy's largest root	.000	.000[a]	4.000	53.000	1.000	.000	.050
0.001	Pillai's trace	.005	.060[a]	4.000	53.000	.993	.240	.061
	Wilks' lambda	.995	.060[a]	4.000	53.000	.993	.240	.061
	Hotelling's trace	.005	.060[a]	4.000	53.000	.993	.240	.061
	Roy's largest root	.005	.060[a]	4.000	53.000	.993	.240	.061
0.01	Pillai's trace	.308	5.892[a]	4.000	53.000	.001	23.570	.975
	Wilks' lambda	.692	5.892[a]	4.000	53.000	.001	23.570	.975
	Hotelling's trace	.445	5.892[a]	4.000	53.000	.001	23.570	.975
	Roy's largest root	.445	5.892[a]	4.000	53.000	.001	23.570	.975
0.1	Pillai's trace	.009	.120[a]	4.000	53.000	.975	.481	.073
	Wilks' lambda	.991	.120[a]	4.000	53.000	.975	.481	.073
	Hotelling's trace	.009	.120[a]	4.000	53.000	.975	.481	.073
	Roy's largest root	.009	.120[a]	4.000	53.000	.975	.481	.073
1	Pillai's trace	.144	2.230[a]	4.000	53.000	.078	8.920	.615
	Wilks' lambda	.856	2.230[a]	4.000	53.000	.078	8.920	.615
	Hotelling's trace	.168	2.230[a]	4.000	53.000	.078	8.920	.615
	Roy's largest root	.168	2.230[a]	4.000	53.000	.078	8.920	.615

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots


Treatment
Control
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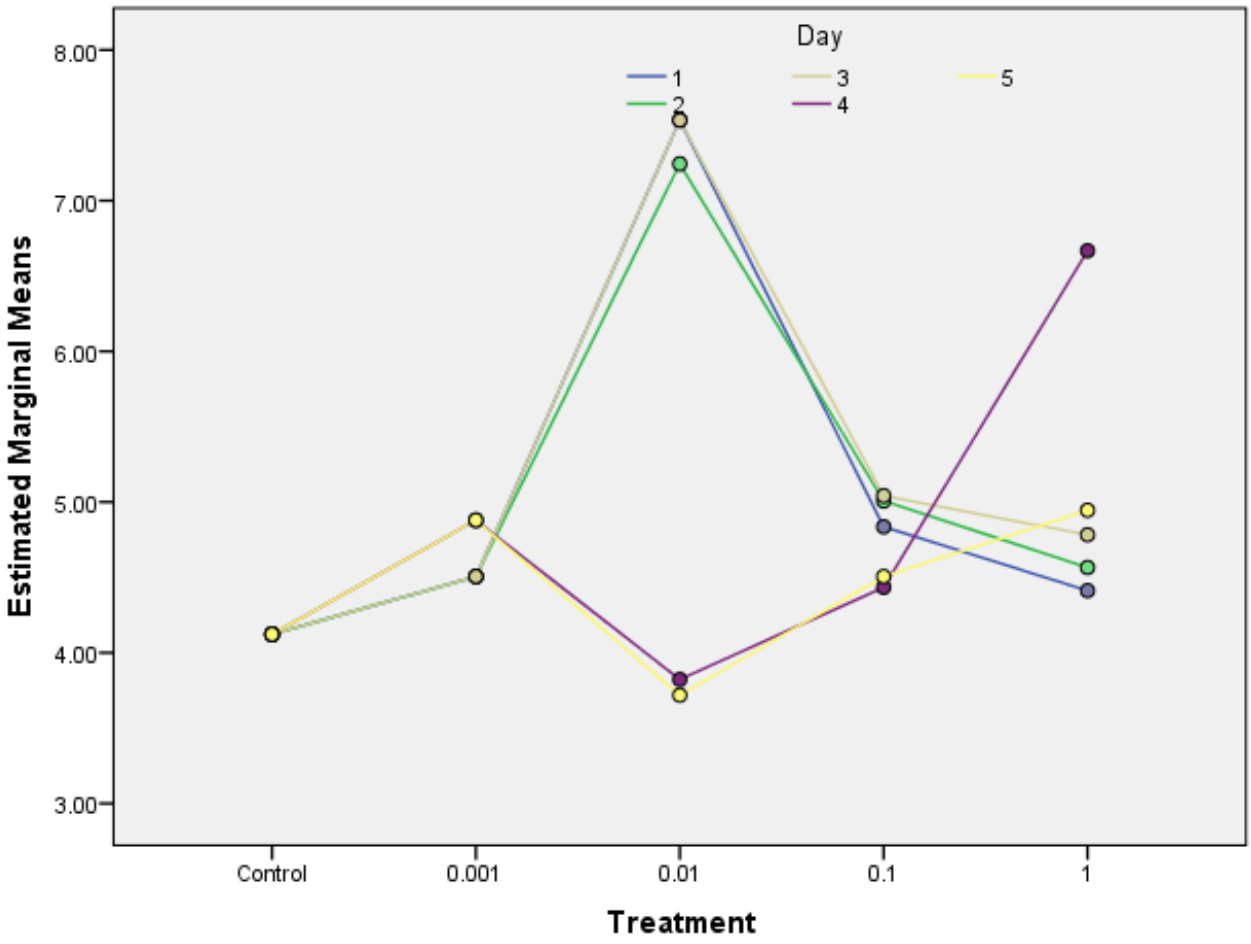
1
0.001
0.1
Estimated Marginal Means
8.00
7.00
6.00
5.00
4.00
3.00
1
2
3
4
5
Day



TITLE Aluminium.

Aluminium

```
GLM SP1 SP2 SP3 SP5 SP7 BY Treatment
  /WSFACTOR=Day 5 Polynomial
  /MEASURE=ShootP
  /METHOD=SSTYPE(3)
  /PLOT=PROFILE(Day*Treatment Treatment*Day)
  /EMMEANS=TABLES(OVERALL)
  /EMMEANS=TABLES(Treatment) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Treatment) ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Day) ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE OPOWER LOF
  /CRITERIA=ALPHA(.05)
  /WSDESIGN= Day
  /DESIGN= Treatment.
```

General Linear Model

Within-Subjects Factors

Measure: ShootP

Day	Dependent Variable
1	SP1
2	SP2
3	SP3
4	SP5
5	SP7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	0.001	12
	2	0.01	12
	3	0.1	12
	4	1	13

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
SP1	Control	3.9325	1.12653	12
	0.001	3.9325	1.12653	12
	0.01	4.1225	.89458	12
	0.1	4.8792	1.27039	12
	1	4.0177	1.12146	13
	Total	4.1743	1.13493	61
SP2	Control	3.9325	1.12653	12
	0.001	3.9325	1.12653	12
	0.01	4.1225	.89458	12

	0.1	4.8792	1.27039	12
	1	4.0177	1.12146	13
	Total	4.1743	1.13493	61
SP3	Control	3.9325	1.12653	12
	0.001	3.9325	1.12653	12
	0.01	5.3967	3.62439	12
	0.1	3.9667	1.05258	12
	1	4.5654	1.44426	13
	Total	4.3621	1.95501	61
SP5	Control	3.9325	1.12653	12
	0.001	3.8450	1.95239	12
	0.01	5.2033	2.60554	12
	0.1	3.9325	1.12653	12
	1	4.6446	1.76689	13
	Total	4.3170	1.82133	61
SP7	Control	3.9325	1.12653	12
	0.001	3.8450	1.95239	12
	0.01	5.0092	2.75040	12
	0.1	3.9325	1.12653	12
	1	4.0123	1.11630	13
	Total	4.1441	1.72901	61

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.031	.573[b]	3.000	54.000	.635	1.719	.161
	Wilks' Lambda	.969	.573[b]	3.000	54.000	.635	1.719	.161
	Hotelling's Trace	.032	.573[b]	3.000	54.000	.635	1.719	.161
	Roy's Largest Root	.032	.573[b]	3.000	54.000	.635	1.719	.161
Day * Treatment	Pillai's Trace	.225	1.136	12.000	168.000	.335	13.628	.637
	Wilks' Lambda	.782	1.163	12.000	143.162	.315	12.244	.573
	Hotelling's Trace	.270	1.187	12.000	158.000	.297	14.240	.660
	Roy's Largest Root	.233	3.259[c]	4.000	56.000	.018	13.037	.802

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: ShootP

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.000	.	9	.	.568	.636	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: ShootP

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	2.182	4	.545	.303	.875	1.214	.117
	Greenhouse-Geisser	2.182	2.272	.960	.303	.766	.690	.100

	Huynh-Feldt	2.182	2.542	.858	.303	.790	.772	.103
	Lower-bound	2.182	1.000	2.182	.303	.584	.303	.084
Day * Treatment	Sphericity Assumed	33.535	16	2.096	1.166	.297	18.660	.753
	Greenhouse-Geisser	33.535	9.088	3.690	1.166	.322	10.599	.558
	Huynh-Feldt	33.535	10.170	3.298	1.166	.318	11.860	.595
	Lower-bound	33.535	4.000	8.384	1.166	.336	4.665	.342
Error(Day)	Sphericity Assumed	402.559	224	1.797				
	Greenhouse-Geisser	402.559	127.238	3.164				
	Huynh-Feldt	402.559	142.375	2.827				
	Lower-bound	402.559	56.000	7.189				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: ShootP

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	.033	1	.033	.011	.916	.011	.051
	Quadratic	1.363	1	1.363	.995	.323	.995	.165
	Cubic	.548	1	.548	.895	.348	.895	.153
	Order 4	.237	1	.237	.106	.746	.106	.062
Day * Treatment	Linear	19.989	4	4.997	1.683	.167	6.731	.484
	Quadratic	4.949	4	1.237	.903	.469	3.611	.268
	Cubic	4.489	4	1.122	1.830	.136	7.322	.522
	Order 4	4.108	4	1.027	.459	.765	1.838	.150
Error(Day)	Linear	166.298	56	2.970				
	Quadratic	76.753	56	1.371				
	Cubic	34.331	56	.613				
	Order 4	125.177	56	2.235				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: ShootP
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	5462.250	1	5462.250	1032.641	.000	1032.641	1.000
Treatment	29.983	4	7.496	1.417	.240	5.668	.412
Error	296.217	56	5.290				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
SP1, SP2, SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000		.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP1, SP2, SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP2, SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP2, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP2, SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP1, SP2, SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP2, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP2, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP2, SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP2, SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP2, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP1, SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP2, SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP2, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP2, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
SP1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	69.567	56	1.242				
SP2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	69.567	56	1.242				
SP3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	209.636	56	3.743				
SP5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	181.990	56	3.250				
SP7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	168.015	56	3.000				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: ShootP

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
4.234	.132	3.970	4.498

2. Treatment

Estimates

Measure: ShootP

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	3.933	.297	3.338	4.527
0.001	3.898	.297	3.303	4.492
0.01	4.771	.297	4.176	5.366
0.1	4.318	.297	3.723	4.913
1	4.252	.285	3.680	4.823

Pairwise Comparisons

Measure: ShootP

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	0.001	.035	.420	1.000	-1.192	1.262
	0.01	-.838	.420	.508	-2.066	.389
	0.1	-.386	.420	1.000	-1.613	.842
	1	-.319	.412	1.000	-1.522	.884
0.001	Control	-.035	.420	1.000	-1.262	1.192
	0.01	-.873	.420	.421	-2.101	.354
	0.1	-.421	.420	1.000	-1.648	.807
	1	-.354	.412	1.000	-1.557	.849
0.01	Control	.838	.420	.508	-.389	2.066
	0.001	.873	.420	.421	-.354	2.101
	0.1	.453	.420	1.000	-.774	1.680
	1	.519	.412	1.000	-.684	1.723
0.1	Control	.386	.420	1.000	-.842	1.613
	0.001	.421	.420	1.000	-.807	1.648
	0.01	-.453	.420	1.000	-1.680	.774
	1	.066	.412	1.000	-1.137	1.270
1	Control	.319	.412	1.000	-.884	1.522
	0.001	.354	.412	1.000	-.849	1.557
	0.01	-.519	.412	1.000	-1.723	.684

0.1	-.066	.412	1.000	-1.270	1.137

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootP

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	5.997	4	1.499	1.417	.240	5.668	.412
Error	59.243	56	1.058				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: ShootP

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	4.177	.143	3.891	4.463
2	4.177	.143	3.891	4.463
3	4.359	.248	3.862	4.855
4	4.312	.231	3.849	4.774
5	4.146	.222	3.702	4.591

Pairwise Comparisons

Measure: ShootP

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	.000	.000	.	.000	.000
	3	-.182	.260	1.000	-.940	.577
	4	-.135	.241	1.000	-.840	.570
	5	.031	.244	1.000	-.682	.743
2	1	.000	.000	.	.000	.000
	3	-.182	.260	1.000	-.940	.577
	4	-.135	.241	1.000	-.840	.570
	5	.031	.244	1.000	-.682	.743
3	1	.182	.260	1.000	-.577	.940
	2	.182	.260	1.000	-.577	.940
	4	.047	.315	1.000	-.873	.968
	5	.212	.314	1.000	-.707	1.131
4	1	.135	.241	1.000	-.570	.840
	2	.135	.241	1.000	-.570	.840
	3	-.047	.315	1.000	-.968	.873
	5	.165	.148	1.000	-.266	.597
5	1	-.031	.244	1.000	-.743	.682
	2	-.031	.244	1.000	-.743	.682
	3	-.212	.314	1.000	-1.131	.707

4	-.165	.148	1.000	-.597	.266

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.031	.573[a]	3.000	54.000	.635	1.719	.161
Wilks' lambda	.969	.573[a]	3.000	54.000	.635	1.719	.161
Hotelling's trace	.032	.573[a]	3.000	54.000	.635	1.719	.161
Roy's largest root	.032	.573[a]	3.000	54.000	.635	1.719	.161

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: ShootP

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	3.933	.322	3.288	4.577
	2	3.933	.322	3.288	4.577
	3	3.933	.559	2.814	5.051
	4	3.933	.520	2.890	4.975
	5	3.932	.500	2.931	4.934
0.001	1	3.933	.322	3.288	4.577
	2	3.933	.322	3.288	4.577
	3	3.933	.559	2.814	5.051
	4	3.845	.520	2.803	4.887
	5	3.845	.500	2.843	4.847
0.01	1	4.123	.322	3.478	4.767
	2	4.123	.322	3.478	4.767
	3	5.397	.559	4.278	6.516
	4	5.203	.520	4.161	6.246
	5	5.009	.500	4.008	6.011
0.1	1	4.879	.322	4.235	5.524
	2	4.879	.322	4.235	5.524
	3	3.967	.559	2.848	5.086
	4	3.933	.520	2.890	4.975
	5	3.932	.500	2.931	4.934
1	1	4.018	.309	3.398	4.637
	2	4.018	.309	3.398	4.637
	3	4.565	.537	3.490	5.640
	4	4.645	.500	3.643	5.646
	5	4.012	.480	3.050	4.975

Pairwise Comparisons

Measure: ShootP

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
1	Control	0.001	5.551E-17	.455	1.000	-1.330	1.330
		0.01	-.190	.455	1.000	-1.520	1.140
		0.1	-.947	.455	.421	-2.276	.383
		1	-.085	.446	1.000	-1.389	1.219
	0.001	Control	-5.551E-17	.455	1.000	-1.330	1.330
		0.01	-.190	.455	1.000	-1.520	1.140
		0.1	-.947	.455	.421	-2.276	.383
		1	-.085	.446	1.000	-1.389	1.219
	0.01	Control	.190	.455	1.000	-1.140	1.520
		0.001	.190	.455	1.000	-1.140	1.520
		0.1	-.757	.455	1.000	-2.086	.573
		1	.105	.446	1.000	-1.199	1.409
	0.1	Control	.947	.455	.421	-.383	2.276
		0.001	.947	.455	.421	-.383	2.276
		0.01	.757	.455	1.000	-.573	2.086
		1	.861	.446	.586	-.443	2.165
	1	Control	.085	.446	1.000	-1.219	1.389
		0.001	.085	.446	1.000	-1.219	1.389
		0.01	-.105	.446	1.000	-1.409	1.199
		0.1	-.861	.446	.586	-2.165	.443
2	Control	0.001	5.551E-17	.455	1.000	-1.330	1.330
		0.01	-.190	.455	1.000	-1.520	1.140
		0.1	-.947	.455	.421	-2.276	.383
		1	-.085	.446	1.000	-1.389	1.219
	0.001	Control	-5.551E-17	.455	1.000	-1.330	1.330
		0.01	-.190	.455	1.000	-1.520	1.140
		0.1	-.947	.455	.421	-2.276	.383
		1	-.085	.446	1.000	-1.389	1.219
	0.01	Control	.190	.455	1.000	-1.140	1.520
		0.001	.190	.455	1.000	-1.140	1.520
		0.1	-.757	.455	1.000	-2.086	.573
		1	.105	.446	1.000	-1.199	1.409
	0.1	Control	.947	.455	.421	-.383	2.276
		0.001	.947	.455	.421	-.383	2.276
		0.01	.757	.455	1.000	-.573	2.086
		1	.861	.446	.586	-.443	2.165
	1	Control	.085	.446	1.000	-1.219	1.389
		0.001	.085	.446	1.000	-1.219	1.389
		0.01	-.105	.446	1.000	-1.409	1.199
		0.1	-.861	.446	.586	-2.165	.443
3	Control	0.001	.000	.790	1.000	-2.308	2.308
		0.01	-1.464	.790	.691	-3.773	.844
		0.1	-.034	.790	1.000	-2.343	2.274
		1	-.633	.775	1.000	-2.897	1.631
	0.001	Control	.000	.790	1.000	-2.308	2.308
		0.01	-1.464	.790	.691	-3.773	.844
		0.1	-.034	.790	1.000	-2.343	2.274
		1	-.633	.775	1.000	-2.897	1.631
	0.01	Control	1.464	.790	.691	-.844	3.773
		0.001	1.464	.790	.691	-.844	3.773
		0.1	1.430	.790	.756	-.878	3.738
		1	.831	.775	1.000	-1.432	3.095

	0.1	Control	.034	.790	1.000	-2.274	2.343
		0.001	.034	.790	1.000	-2.274	2.343
		0.01	-1.430	.790	.756	-3.738	.878
		1	-.599	.775	1.000	-2.862	1.665
	1	Control	.633	.775	1.000	-1.631	2.897
		0.001	.633	.775	1.000	-1.631	2.897
		0.01	-.831	.775	1.000	-3.095	1.432
		0.1	.599	.775	1.000	-1.665	2.862
4	Control	0.001	.087	.736	1.000	-2.063	2.238
		0.01	-1.271	.736	.897	-3.422	.880
		0.1	1.110E-16	.736	1.000	-2.151	2.151
		1	-.712	.722	1.000	-2.821	1.397
	0.001	Control	-.087	.736	1.000	-2.238	2.063
		0.01	-1.358	.736	.702	-3.509	.793
		0.1	-.087	.736	1.000	-2.238	2.063
		1	-.800	.722	1.000	-2.909	1.309
	0.01	Control	1.271	.736	.897	-.880	3.422
		0.001	1.358	.736	.702	-.793	3.509
		0.1	1.271	.736	.897	-.880	3.422
		1	.559	.722	1.000	-1.550	2.668
	0.1	Control	-1.110E-16	.736	1.000	-2.151	2.151
		0.001	.087	.736	1.000	-2.063	2.238
		0.01	-1.271	.736	.897	-3.422	.880
		1	-.712	.722	1.000	-2.821	1.397
	1	Control	.712	.722	1.000	-1.397	2.821
		0.001	.800	.722	1.000	-1.309	2.909
		0.01	-.559	.722	1.000	-2.668	1.550
		0.1	.712	.722	1.000	-1.397	2.821
5	Control	0.001	.087	.707	1.000	-1.979	2.154
		0.01	-1.077	.707	1.000	-3.143	.990
		0.1	4.163E-17	.707	1.000	-2.067	2.067
		1	-.080	.693	1.000	-2.106	1.947
	0.001	Control	-.087	.707	1.000	-2.154	1.979
		0.01	-1.164	.707	1.000	-3.231	.902
		0.1	-.087	.707	1.000	-2.154	1.979
		1	-.167	.693	1.000	-2.194	1.859
	0.01	Control	1.077	.707	1.000	-.990	3.143
		0.001	1.164	.707	1.000	-.902	3.231
		0.1	1.077	.707	1.000	-.990	3.143
		1	.997	.693	1.000	-1.030	3.023
	0.1	Control	-4.163E-17	.707	1.000	-2.067	2.067
		0.001	.087	.707	1.000	-1.979	2.154
		0.01	-1.077	.707	1.000	-3.143	.990
		1	-.080	.693	1.000	-2.106	1.947
	1	Control	.080	.693	1.000	-1.947	2.106
		0.001	.167	.693	1.000	-1.859	2.194
		0.01	-.997	.693	1.000	-3.023	1.030
		0.1	.080	.693	1.000	-1.947	2.106

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootP

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	7.716	4	1.929	1.553	.200	6.211	.449
	Error	69.567	56	1.242				
2	Contrast	7.716	4	1.929	1.553	.200	6.211	.449
	Error	69.567	56	1.242				
3	Contrast	19.687	4	4.922	1.315	.276	5.259	.384
	Error	209.636	56	3.743				
4	Contrast	17.044	4	4.261	1.311	.277	5.245	.383
	Error	181.990	56	3.250				
5	Contrast	11.354	4	2.839	.946	.444	3.784	.281
	Error	168.015	56	3.000				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: ShootP

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	3.933	.322	3.288	4.577
	2	3.933	.322	3.288	4.577
	3	3.933	.559	2.814	5.051
	4	3.933	.520	2.890	4.975
	5	3.932	.500	2.931	4.934
0.001	1	3.933	.322	3.288	4.577
	2	3.933	.322	3.288	4.577
	3	3.933	.559	2.814	5.051
	4	3.845	.520	2.803	4.887
	5	3.845	.500	2.843	4.847
0.01	1	4.123	.322	3.478	4.767
	2	4.123	.322	3.478	4.767
	3	5.397	.559	4.278	6.516
	4	5.203	.520	4.161	6.246
	5	5.009	.500	4.008	6.011
0.1	1	4.879	.322	4.235	5.524
	2	4.879	.322	4.235	5.524
	3	3.967	.559	2.848	5.086
	4	3.933	.520	2.890	4.975
	5	3.932	.500	2.931	4.934
1	1	4.018	.309	3.398	4.637
	2	4.018	.309	3.398	4.637
	3	4.565	.537	3.490	5.640
	4	4.645	.500	3.643	5.646
	5	4.012	.480	3.050	4.975

Pairwise Comparisons

Measure: ShootP

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
Control	1	2	.000	.000	.	.000	.000
		3	1.332E-15	.585	1.000	-1.710	1.710
		4	-4.441E-16	.544	1.000	-1.589	1.589
		5	1.776E-15	.549	1.000	-1.605	1.605
	2	1	.000	.000	.	.000	.000
		3	1.332E-15	.585	1.000	-1.710	1.710
		4	-4.441E-16	.544	1.000	-1.589	1.589
		5	1.776E-15	.549	1.000	-1.605	1.605
	3	1	-1.332E-15	.585	1.000	-1.710	1.710
		2	-1.332E-15	.585	1.000	-1.710	1.710
		4	-1.776E-15	.710	1.000	-2.074	2.074
		5	4.441E-16	.709	1.000	-2.071	2.071
	4	1	4.441E-16	.544	1.000	-1.589	1.589
		2	4.441E-16	.544	1.000	-1.589	1.589
		3	1.776E-15	.710	1.000	-2.074	2.074
		5	2.220E-15	.333	1.000	-.973	.973
	5	1	-1.776E-15	.549	1.000	-1.605	1.605
		2	-1.776E-15	.549	1.000	-1.605	1.605
		3	-4.441E-16	.709	1.000	-2.071	2.071
		4	-2.220E-15	.333	1.000	-.973	.973
0.001	1	2	.000	.000	.	.000	.000
		3	1.332E-15	.585	1.000	-1.710	1.710
		4	.087	.544	1.000	-1.501	1.676
		5	.088	.549	1.000	-1.518	1.693
	2	1	.000	.000	.	.000	.000
		3	1.332E-15	.585	1.000	-1.710	1.710
		4	.087	.544	1.000	-1.501	1.676
		5	.088	.549	1.000	-1.518	1.693
	3	1	-1.332E-15	.585	1.000	-1.710	1.710
		2	-1.332E-15	.585	1.000	-1.710	1.710
		4	.087	.710	1.000	-1.987	2.162
		5	.088	.709	1.000	-1.983	2.158
	4	1	-.087	.544	1.000	-1.676	1.501
		2	-.087	.544	1.000	-1.676	1.501
		3	-.087	.710	1.000	-2.162	1.987
		5	1.776E-15	.333	1.000	-.973	.973
	5	1	-.088	.549	1.000	-1.693	1.518
		2	-.088	.549	1.000	-1.693	1.518
		3	-.088	.709	1.000	-2.158	1.983
		4	-1.776E-15	.333	1.000	-.973	.973
0.01	1	2	.000	.000	.	.000	.000
		3	-1.274	.585	.336	-2.984	.435
		4	-1.081	.544	.517	-2.669	.508
		5	-.887	.549	1.000	-2.492	.718
	2	1	.000	.000	.	.000	.000
		3	-1.274	.585	.336	-2.984	.435

		4	-1.081	.544	.517	-2.669	.508
		5	-.887	.549	1.000	-2.492	.718
	3	1	1.274	.585	.336	-.435	2.984
		2	1.274	.585	.336	-.435	2.984
		4	.193	.710	1.000	-1.881	2.268
		5	.387	.709	1.000	-1.683	2.458
	4	1	1.081	.544	.517	-.508	2.669
		2	1.081	.544	.517	-.508	2.669
		3	-.193	.710	1.000	-2.268	1.881
		5	.194	.333	1.000	-.779	1.167
	5	1	.887	.549	1.000	-.718	2.492
		2	.887	.549	1.000	-.718	2.492
		3	-.387	.709	1.000	-2.458	1.683
		4	-.194	.333	1.000	-1.167	.779
0.1	1	2	.000	.000	.	.000	.000
		3	.913	.585	1.000	-.797	2.622
		4	.947	.544	.871	-.642	2.535
		5	.947	.549	.903	-.658	2.552
	2	1	.000	.000	.	.000	.000
		3	.913	.585	1.000	-.797	2.622
		4	.947	.544	.871	-.642	2.535
		5	.947	.549	.903	-.658	2.552
	3	1	-.913	.585	1.000	-2.622	.797
		2	-.913	.585	1.000	-2.622	.797
		4	.034	.710	1.000	-2.040	2.108
		5	.034	.709	1.000	-2.037	2.105
	4	1	-.947	.544	.871	-2.535	.642
		2	-.947	.544	.871	-2.535	.642
		3	-.034	.710	1.000	-2.108	2.040
		5	1.776E-15	.333	1.000	-.973	.973
	5	1	-.947	.549	.903	-2.552	.658
		2	-.947	.549	.903	-2.552	.658
		3	-.034	.709	1.000	-2.105	2.037
		4	-1.776E-15	.333	1.000	-.973	.973
1	1	2	.000	.000	.	.000	.000
		3	-.548	.562	1.000	-2.190	1.095
		4	-.627	.522	1.000	-2.153	.899
		5	.005	.528	1.000	-1.537	1.548
	2	1	.000	.000	.	.000	.000
		3	-.548	.562	1.000	-2.190	1.095
		4	-.627	.522	1.000	-2.153	.899
		5	.005	.528	1.000	-1.537	1.548
	3	1	.548	.562	1.000	-1.095	2.190
		2	.548	.562	1.000	-1.095	2.190
		4	-.079	.682	1.000	-2.072	1.914
		5	.553	.681	1.000	-1.437	2.543
	4	1	.627	.522	1.000	-.899	2.153
		2	.627	.522	1.000	-.899	2.153
		3	.079	.682	1.000	-1.914	2.072
		5	.632	.320	.530	-.302	1.567

	5	1	-.005	.528	1.000	-1.548	1.537
		2	-.005	.528	1.000	-1.548	1.537
		3	-.553	.681	1.000	-2.543	1.437
		4	-.632	.320	.530	-1.567	.302

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

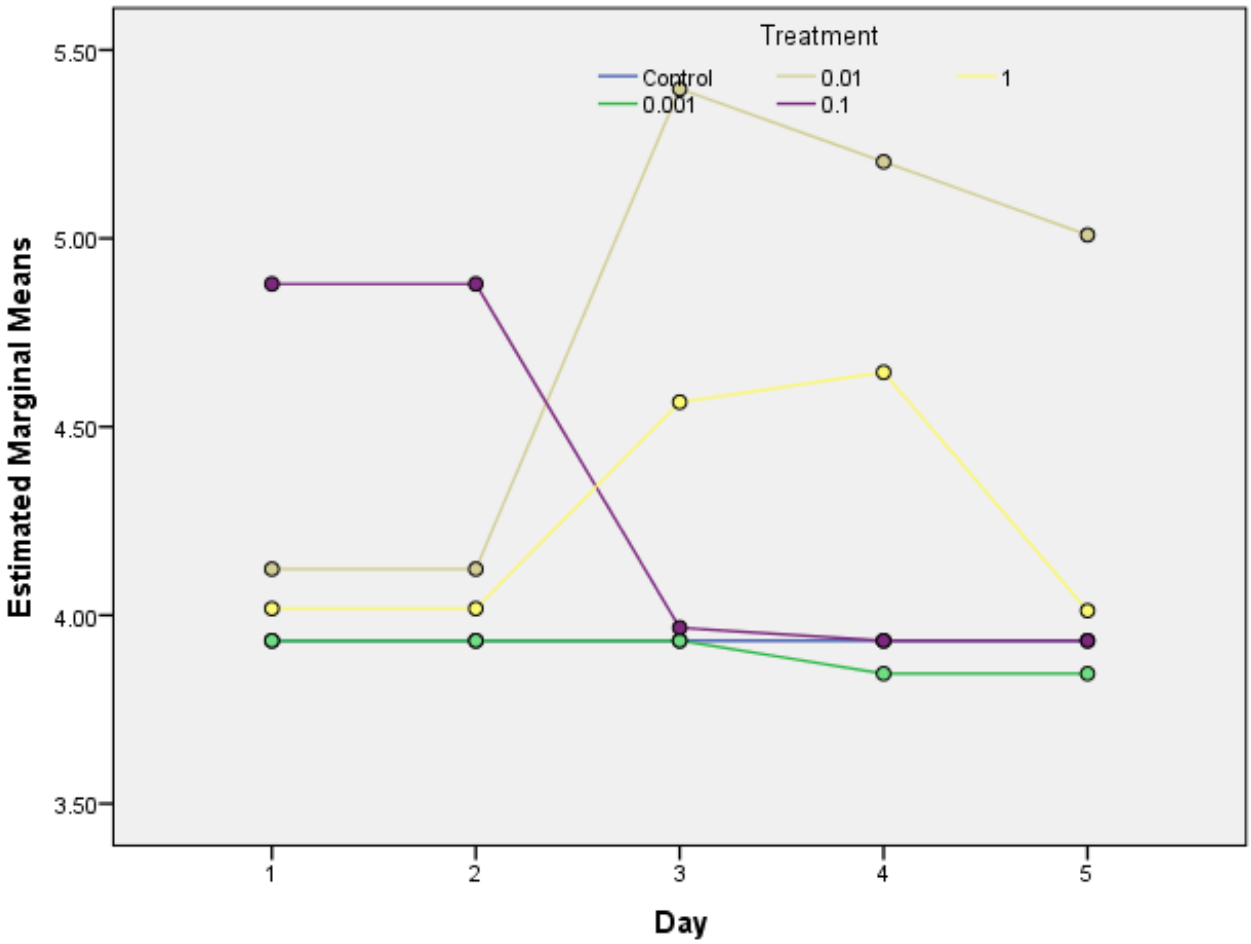
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.000	.000[a]	3.000	54.000	1.000	.000	.050
	Wilks' lambda	1.000	.000[a]	3.000	54.000	1.000	.000	.050
	Hotelling's trace	.000	.000[a]	3.000	54.000	1.000	.000	.050
	Roy's largest root	.000	.000[a]	3.000	54.000	1.000	.000	.050
0.001	Pillai's trace	.001	.010[a]	3.000	54.000	.999	.029	.052
	Wilks' lambda	.999	.010[a]	3.000	54.000	.999	.029	.052
	Hotelling's trace	.001	.010[a]	3.000	54.000	.999	.029	.052
	Roy's largest root	.001	.010[a]	3.000	54.000	.999	.029	.052
0.01	Pillai's trace	.114	2.321[a]	3.000	54.000	.085	6.963	.553
	Wilks' lambda	.886	2.321[a]	3.000	54.000	.085	6.963	.553
	Hotelling's trace	.129	2.321[a]	3.000	54.000	.085	6.963	.553
	Roy's largest root	.129	2.321[a]	3.000	54.000	.085	6.963	.553
0.1	Pillai's trace	.077	1.511[a]	3.000	54.000	.222	4.533	.377
	Wilks' lambda	.923	1.511[a]	3.000	54.000	.222	4.533	.377
	Hotelling's trace	.084	1.511[a]	3.000	54.000	.222	4.533	.377
	Roy's largest root	.084	1.511[a]	3.000	54.000	.222	4.533	.377
1	Pillai's trace	.084	1.649[a]	3.000	54.000	.189	4.948	.408
	Wilks' lambda	.916	1.649[a]	3.000	54.000	.189	4.948	.408
	Hotelling's trace	.092	1.649[a]	3.000	54.000	.189	4.948	.408
	Roy's largest root	.092	1.649[a]	3.000	54.000	.189	4.948	.408

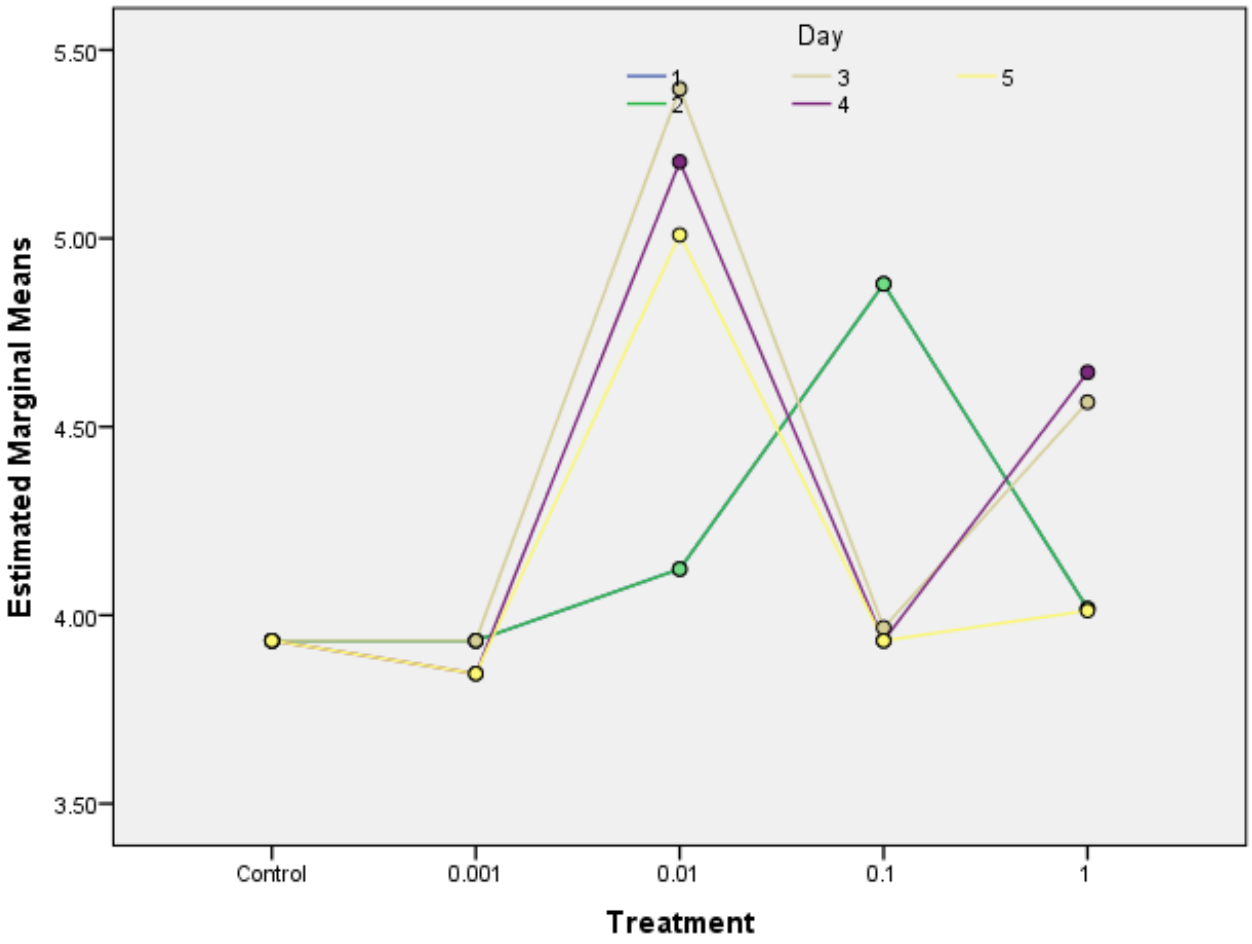
Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots


Treatment
Control
0.01
1
0.001
0.1
Estimated Marginal Means
5.50
5.00
4.50
4.00
3.50
1
2
3
4
5
Day



TITLE Aluminium.

Aluminium

```
GLM ST1 ST2 ST3 ST5 ST7 BY Treatment
  /WSFACTOR=Day 5 Polynomial
  /MEASURE=ShootT
  /METHOD=SSTYPE(3)
  /PLOT=PROFILE(Day*Treatment Treatment*Day)
  /EMMEANS=TABLES(OVERALL)
  /EMMEANS=TABLES(Treatment) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Treatment) ADJ(BONFERRONI)
  /EMMEANS=TABLES(Treatment*Day) COMPARE (Day) ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE OPOWER LOF
  /CRITERIA=ALPHA(.05)
  /WSDESIGN= Day
  /DESIGN= Treatment.
```

General Linear Model

Within-Subjects Factors

Measure: ShootT

Day	Dependent Variable
1	ST1
2	ST2
3	ST3
4	ST5
5	ST7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	0.001	12
	2	0.01	12
	3	0.1	12
	4	1	13

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
ST1	Control	7.2442	4.97073	12
	0.001	4.9358	2.73764	12
	0.01	3.9325	1.12653	12
	0.1	3.8450	1.95239	12
	1	4.7823	2.67891	13
	Total	4.9452	3.12741	61
ST2	Control	7.2442	4.97073	12
	0.001	4.9358	2.73764	12
	0.01	3.9325	1.12653	12

	0.1	3.9325	1.12653	12
	1	4.0177	1.12146	13
	Total	4.7995	2.87277	61
ST3	Control	7.2442	4.97073	12
	0.001	4.9358	2.73764	12
	0.01	5.1200	3.76559	12
	0.1	5.9167	2.62194	12
	1	4.0177	1.12146	13
	Total	5.4234	3.34979	61
ST5	Control	7.2442	4.97073	12
	0.001	5.0075	2.70010	12
	0.01	4.4308	1.47175	12
	0.1	4.9358	2.73764	12
	1	4.2585	1.01310	13
	Total	5.1603	3.00145	61
ST7	Control	3.9325	1.12653	12
	0.001	4.6317	.76575	12
	0.01	7.2442	4.97073	12
	0.1	4.9358	2.73764	12
	1	5.2392	2.95159	13
	Total	5.1974	3.03683	61

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.126	1.910[b]	4.000	53.000	.122	7.640	.540
	Wilks' Lambda	.874	1.910[b]	4.000	53.000	.122	7.640	.540
	Hotelling's Trace	.144	1.910[b]	4.000	53.000	.122	7.640	.540
	Roy's Largest Root	.144	1.910[b]	4.000	53.000	.122	7.640	.540
Day * Treatment	Pillai's Trace	.503	2.014	16.000	224.000	.013	32.228	.964
	Wilks' Lambda	.556	2.153	16.000	162.555	.008	25.705	.894
	Hotelling's Trace	.695	2.236	16.000	206.000	.005	35.781	.980
	Roy's Largest Root	.496	6.948[c]	4.000	56.000	.000	27.792	.991

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: ShootT

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.157	100.781	9	.000	.509	.567	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: ShootT

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	14.615	4	3.654	.761	.552	3.042	.243
	Greenhouse-Geisser	14.615	2.038	7.171	.761	.472	1.550	.178

	Huynh-Feldt	14.615	2.267	6.448	.761	.485	1.724	.186
	Lower-bound	14.615	1.000	14.615	.761	.387	.761	.137
Day * Treatment	Sphericity Assumed	233.538	16	14.596	3.038	.000	48.615	.998
	Greenhouse-Geisser	233.538	8.152	28.648	3.038	.004	24.769	.953
	Huynh-Feldt	233.538	9.067	25.757	3.038	.002	27.549	.967
	Lower-bound	233.538	4.000	58.385	3.038	.025	12.154	.770
Error(Day)	Sphericity Assumed	1076.067	224	4.804				
	Greenhouse-Geisser	1076.067	114.127	9.429				
	Huynh-Feldt	1076.067	126.936	8.477				
	Lower-bound	1076.067	56.000	19.215				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: ShootT

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	4.510	1	4.510	.484	.490	.484	.105
	Quadratic	1.527	1	1.527	.275	.602	.275	.081
	Cubic	1.386	1	1.386	.718	.400	.718	.133
	Order 4	7.192	1	7.192	2.973	.090	2.973	.396
Day * Treatment	Linear	123.191	4	30.798	3.305	.017	13.219	.808
	Quadratic	70.248	4	17.562	3.166	.020	12.662	.789
	Cubic	19.495	4	4.874	2.526	.051	10.105	.679
	Order 4	20.605	4	5.151	2.130	.089	8.518	.594
Error(Day)	Linear	521.886	56	9.319				
	Quadratic	310.681	56	5.548				
	Cubic	108.040	56	1.929				
	Order 4	135.459	56	2.419				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: ShootT
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	7974.357	1	7974.357	326.414	.000	326.414	1.000
Treatment	171.445	4	42.861	1.754	.151	7.018	.502
Error	1368.090	56	24.430				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
ST1, ST2, ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	51.000	1.000	.000	.050
ST1, ST2, ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST2, ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST2, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST2, ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST2, ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST2, ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST2, ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST2, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST2, ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST2, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST2, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
ST1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	496.240	56	8.861				
ST2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	397.243	56	7.094				
ST3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	600.920	56	10.731				
ST5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	470.570	56	8.403				
ST7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	479.184	56	8.557				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: ShootT

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
5.116	.283	4.549	5.683

2. Treatment

Estimates

Measure: ShootT

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	6.582	.638	5.304	7.860
0.001	4.889	.638	3.611	6.168
0.01	4.932	.638	3.654	6.210
0.1	4.713	.638	3.435	5.991
1	4.463	.613	3.235	5.691

Pairwise Comparisons

Measure: ShootT

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	0.001	1.692	.902	.659	-.945	4.330
	0.01	1.650	.902	.728	-.987	4.287
	0.1	1.869	.902	.430	-.769	4.506
	1	2.119	.885	.200	-.467	4.705
0.001	Control	-1.692	.902	.659	-4.330	.945
	0.01	-.043	.902	1.000	-2.680	2.595
	0.1	.176	.902	1.000	-2.461	2.813
	1	.426	.885	1.000	-2.160	3.012
0.01	Control	-1.650	.902	.728	-4.287	.987
	0.001	.043	.902	1.000	-2.595	2.680
	0.1	.219	.902	1.000	-2.418	2.856
	1	.469	.885	1.000	-2.117	3.055
0.1	Control	-1.869	.902	.430	-4.506	.769
	0.001	-.176	.902	1.000	-2.813	2.461
	0.01	-.219	.902	1.000	-2.856	2.418
	1	.250	.885	1.000	-2.336	2.836
1	Control	-2.119	.885	.200	-4.705	.467
	0.001	-.426	.885	1.000	-3.012	2.160
	0.01	-.469	.885	1.000	-3.055	2.117

0.1	-.250	.885	1.000	-2.836	2.336

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootT

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	34.289	4	8.572	1.754	.151	7.018	.502
Error	273.618	56	4.886				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: ShootT

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	4.948	.381	4.184	5.712
2	4.813	.341	4.129	5.496
3	5.447	.420	4.606	6.288
4	5.175	.371	4.431	5.919
5	5.197	.375	4.446	5.947

Pairwise Comparisons

Measure: ShootT

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	.135	.210	1.000	-.479	.750
	3	-.499	.332	1.000	-1.469	.471
	4	-.227	.299	1.000	-1.100	.646
	5	-.249	.555	1.000	-1.872	1.374
2	1	-.135	.210	1.000	-.750	.479
	3	-.634	.259	.175	-1.392	.123
	4	-.363	.247	1.000	-1.084	.358
	5	-.384	.503	1.000	-1.855	1.087
3	1	.499	.332	1.000	-.471	1.469
	2	.634	.259	.175	-.123	1.392
	4	.272	.347	1.000	-.743	1.286
	5	.250	.585	1.000	-1.459	1.960
4	1	.227	.299	1.000	-.646	1.100
	2	.363	.247	1.000	-.358	1.084
	3	-.272	.347	1.000	-1.286	.743
	5	-.021	.425	1.000	-1.264	1.221
5	1	.249	.555	1.000	-1.374	1.872
	2	.384	.503	1.000	-1.087	1.855
	3	-.250	.585	1.000	-1.960	1.459

4	.021	.425	1.000	-1.221	1.264

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.126	1.910[a]	4.000	53.000	.122	7.640	.540
Wilks' lambda	.874	1.910[a]	4.000	53.000	.122	7.640	.540
Hotelling's trace	.144	1.910[a]	4.000	53.000	.122	7.640	.540
Roy's largest root	.144	1.910[a]	4.000	53.000	.122	7.640	.540

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: ShootT

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	7.244	.859	5.523	8.966
	2	7.244	.769	5.704	8.784
	3	7.244	.946	5.350	9.139
	4	7.244	.837	5.568	8.921
	5	3.933	.844	2.241	5.624
0.001	1	4.936	.859	3.214	6.657
	2	4.936	.769	3.396	6.476
	3	4.936	.946	3.041	6.830
	4	5.008	.837	3.331	6.684
	5	4.632	.844	2.940	6.323
0.01	1	3.933	.859	2.211	5.654
	2	3.933	.769	2.392	5.473
	3	5.120	.946	3.226	7.014
	4	4.431	.837	2.754	6.107
	5	7.244	.844	5.553	8.936
0.1	1	3.845	.859	2.124	5.566
	2	3.933	.769	2.392	5.473
	3	5.917	.946	4.022	7.811
	4	4.936	.837	3.259	6.612
	5	4.936	.844	3.244	6.627
1	1	4.782	.826	3.128	6.436
	2	4.018	.739	2.538	5.497
	3	4.018	.909	2.198	5.838
	4	4.258	.804	2.648	5.869
	5	5.239	.811	3.614	6.864

Pairwise Comparisons

Measure: ShootT

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	0.001	2.308	1.215	.627	-1.243	5.860
		0.01	3.312	1.215	.086	-.240	6.863
		0.1	3.399	1.215	.071	-.153	6.951
		1	2.462	1.192	.435	-1.021	5.945
	0.001	Control	-2.308	1.215	.627	-5.860	1.243
		0.01	1.003	1.215	1.000	-2.548	4.555
		0.1	1.091	1.215	1.000	-2.461	4.643
		1	.154	1.192	1.000	-3.329	3.636
	0.01	Control	-3.312	1.215	.086	-6.863	.240
		0.001	-1.003	1.215	1.000	-4.555	2.548
		0.1	.087	1.215	1.000	-3.464	3.639
		1	-.850	1.192	1.000	-4.333	2.633
	0.1	Control	-3.399	1.215	.071	-6.951	.153
		0.001	-1.091	1.215	1.000	-4.643	2.461
		0.01	-.087	1.215	1.000	-3.639	3.464
		1	-.937	1.192	1.000	-4.420	2.545
	1	Control	-2.462	1.192	.435	-5.945	1.021
		0.001	-.154	1.192	1.000	-3.636	3.329
		0.01	.850	1.192	1.000	-2.633	4.333
		0.1	.937	1.192	1.000	-2.545	4.420
2	Control	0.001	2.308	1.087	.382	-.869	5.486
		0.01	3.312*	1.087	.035	.134	6.489
		0.1	3.312*	1.087	.035	.134	6.489
		1	3.226*	1.066	.037	.110	6.342
	0.001	Control	-2.308	1.087	.382	-5.486	.869
		0.01	1.003	1.087	1.000	-2.174	4.181
		0.1	1.003	1.087	1.000	-2.174	4.181
		1	.918	1.066	1.000	-2.198	4.034
	0.01	Control	-3.312*	1.087	.035	-6.489	-.134
		0.001	-1.003	1.087	1.000	-4.181	2.174
		0.1	.000	1.087	1.000	-3.178	3.178
		1	-.085	1.066	1.000	-3.201	3.031
	0.1	Control	-3.312*	1.087	.035	-6.489	-.134
		0.001	-1.003	1.087	1.000	-4.181	2.174
		0.01	.000	1.087	1.000	-3.178	3.178
		1	-.085	1.066	1.000	-3.201	3.031
	1	Control	-3.226*	1.066	.037	-6.342	-.110
		0.001	-.918	1.066	1.000	-4.034	2.198
		0.01	.085	1.066	1.000	-3.031	3.201
		0.1	.085	1.066	1.000	-3.031	3.201
3	Control	0.001	2.308	1.337	.898	-1.600	6.217
		0.01	2.124	1.337	1.000	-1.784	6.033
		0.1	1.328	1.337	1.000	-2.581	5.236
		1	3.226	1.311	.170	-.606	7.059
	0.001	Control	-2.308	1.337	.898	-6.217	1.600
		0.01	-.184	1.337	1.000	-4.093	3.724
		0.1	-.981	1.337	1.000	-4.889	2.928
		1	.918	1.311	1.000	-2.914	4.751
	0.01	Control	-2.124	1.337	1.000	-6.033	1.784
		0.001	.184	1.337	1.000	-3.724	4.093
		0.1	-.797	1.337	1.000	-4.705	3.112
		1	1.102	1.311	1.000	-2.730	4.935

	0.1	Control	-1.328	1.337	1.000	-5.236	2.581
		0.001	.981	1.337	1.000	-2.928	4.889
		0.01	.797	1.337	1.000	-3.112	4.705
		1	1.899	1.311	1.000	-1.934	5.731
	1	Control	-3.226	1.311	.170	-7.059	.606
		0.001	-.918	1.311	1.000	-4.751	2.914
		0.01	-1.102	1.311	1.000	-4.935	2.730
		0.1	-1.899	1.311	1.000	-5.731	1.934
4	Control	0.001	2.237	1.183	.639	-1.222	5.695
		0.01	2.813	1.183	.209	-.645	6.272
		0.1	2.308	1.183	.561	-1.150	5.767
		1	2.986	1.160	.128	-.406	6.377
	0.001	Control	-2.237	1.183	.639	-5.695	1.222
		0.01	.577	1.183	1.000	-2.882	4.035
		0.1	.072	1.183	1.000	-3.387	3.530
		1	.749	1.160	1.000	-2.642	4.140
	0.01	Control	-2.813	1.183	.209	-6.272	.645
		0.001	-.577	1.183	1.000	-4.035	2.882
		0.1	-.505	1.183	1.000	-3.964	2.954
		1	.172	1.160	1.000	-3.219	3.564
	0.1	Control	-2.308	1.183	.561	-5.767	1.150
		0.001	-.072	1.183	1.000	-3.530	3.387
		0.01	.505	1.183	1.000	-2.954	3.964
		1	.677	1.160	1.000	-2.714	4.069
	1	Control	-2.986	1.160	.128	-6.377	.406
		0.001	-.749	1.160	1.000	-4.140	2.642
		0.01	-.172	1.160	1.000	-3.564	3.219
		0.1	-.677	1.160	1.000	-4.069	2.714
5	Control	0.001	-.699	1.194	1.000	-4.189	2.791
		0.01	-3.312	1.194	.075	-6.802	.178
		0.1	-1.003	1.194	1.000	-4.493	2.487
		1	-1.307	1.171	1.000	-4.729	2.116
	0.001	Control	.699	1.194	1.000	-2.791	4.189
		0.01	-2.612	1.194	.329	-6.103	.878
		0.1	-.304	1.194	1.000	-3.794	3.186
		1	-.608	1.171	1.000	-4.030	2.815
	0.01	Control	3.312	1.194	.075	-.178	6.802
		0.001	2.612	1.194	.329	-.878	6.103
		0.1	2.308	1.194	.583	-1.182	5.798
		1	2.005	1.171	.924	-1.417	5.427
	0.1	Control	1.003	1.194	1.000	-2.487	4.493
		0.001	.304	1.194	1.000	-3.186	3.794
		0.01	-2.308	1.194	.583	-5.798	1.182
		1	-.303	1.171	1.000	-3.726	3.119
	1	Control	1.307	1.171	1.000	-2.116	4.729
		0.001	.608	1.171	1.000	-2.815	4.030
		0.01	-2.005	1.171	.924	-5.427	1.417
		0.1	.303	1.171	1.000	-3.119	3.726

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootT

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	90.601	4	22.650	2.556	.049	10.224	.685
	Error	496.240	56	8.861				
2	Contrast	97.926	4	24.482	3.451	.014	13.805	.827
	Error	397.243	56	7.094				
3	Contrast	72.347	4	18.087	1.686	.166	6.742	.485
	Error	600.920	56	10.731				
4	Contrast	69.953	4	17.488	2.081	.095	8.325	.583
	Error	470.570	56	8.403				
5	Contrast	74.155	4	18.539	2.167	.085	8.666	.603
	Error	479.184	56	8.557				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: ShootT

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	7.244	.859	5.523	8.966
	2	7.244	.769	5.704	8.784
	3	7.244	.946	5.350	9.139
	4	7.244	.837	5.568	8.921
	5	3.933	.844	2.241	5.624
0.001	1	4.936	.859	3.214	6.657
	2	4.936	.769	3.396	6.476
	3	4.936	.946	3.041	6.830
	4	5.008	.837	3.331	6.684
	5	4.632	.844	2.940	6.323
0.01	1	3.933	.859	2.211	5.654
	2	3.933	.769	2.392	5.473
	3	5.120	.946	3.226	7.014
	4	4.431	.837	2.754	6.107
	5	7.244	.844	5.553	8.936
0.1	1	3.845	.859	2.124	5.566
	2	3.933	.769	2.392	5.473
	3	5.917	.946	4.022	7.811
	4	4.936	.837	3.259	6.612
	5	4.936	.844	3.244	6.627
1	1	4.782	.826	3.128	6.436
	2	4.018	.739	2.538	5.497
	3	4.018	.909	2.198	5.838
	4	4.258	.804	2.648	5.869
	5	5.239	.811	3.614	6.864

Pairwise Comparisons

Measure: ShootT

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	.000	.474	1.000	-1.386	1.386
		3	.000	.748	1.000	-2.186	2.186
		4	8.882E-16	.673	1.000	-1.967	1.967
		5	3.312	1.251	.105	-.345	6.969
	2	1	.000	.474	1.000	-1.386	1.386
		3	.000	.584	1.000	-1.706	1.706
		4	8.882E-16	.556	1.000	-1.624	1.624
		5	3.312	1.134	.050	-.003	6.626
	3	1	.000	.748	1.000	-2.186	2.186
		2	.000	.584	1.000	-1.706	1.706
		4	8.882E-16	.782	1.000	-2.286	2.286
		5	3.312	1.318	.149	-.540	7.164
	4	1	-8.882E-16	.673	1.000	-1.967	1.967
		2	-8.882E-16	.556	1.000	-1.624	1.624
		3	-8.882E-16	.782	1.000	-2.286	2.286
		5	3.312*	.958	.010	.512	6.111
	5	1	-3.312	1.251	.105	-6.969	.345
		2	-3.312	1.134	.050	-6.626	.003
		3	-3.312	1.318	.149	-7.164	.540
		4	-3.312*	.958	.010	-6.111	-.512
0.001	1	2	8.882E-16	.474	1.000	-1.386	1.386
		3	8.882E-16	.748	1.000	-2.186	2.186
		4	-.072	.673	1.000	-2.039	1.895
		5	.304	1.251	1.000	-3.353	3.961
	2	1	-8.882E-16	.474	1.000	-1.386	1.386
		3	.000	.584	1.000	-1.706	1.706
		4	-.072	.556	1.000	-1.696	1.553
		5	.304	1.134	1.000	-3.010	3.619
	3	1	-8.882E-16	.748	1.000	-2.186	2.186
		2	.000	.584	1.000	-1.706	1.706
		4	-.072	.782	1.000	-2.358	2.215
		5	.304	1.318	1.000	-3.548	4.156
	4	1	.072	.673	1.000	-1.895	2.039
		2	.072	.556	1.000	-1.553	1.696
		3	.072	.782	1.000	-2.215	2.358
		5	.376	.958	1.000	-2.424	3.175
	5	1	-.304	1.251	1.000	-3.961	3.353
		2	-.304	1.134	1.000	-3.619	3.010
		3	-.304	1.318	1.000	-4.156	3.548
		4	-.376	.958	1.000	-3.175	2.424
0.01	1	2	8.882E-16	.474	1.000	-1.386	1.386
		3	-1.187	.748	1.000	-3.374	.999
		4	-.498	.673	1.000	-2.465	1.469
		5	-3.312	1.251	.105	-6.969	.345
	2	1	-8.882E-16	.474	1.000	-1.386	1.386
		3	-1.188	.584	.467	-2.894	.519

		4	-.498	.556	1.000	-2.123	1.126
		5	-3.312	1.134	.050	-6.626	.003
	3	1	1.187	.748	1.000	-.999	3.374
		2	1.188	.584	.467	-.519	2.894
		4	.689	.782	1.000	-1.597	2.976
		5	-2.124	1.318	1.000	-5.976	1.728
	4	1	.498	.673	1.000	-1.469	2.465
		2	.498	.556	1.000	-1.126	2.123
		3	-.689	.782	1.000	-2.976	1.597
		5	-2.813*	.958	.048	-5.613	-.014
	5	1	3.312	1.251	.105	-.345	6.969
		2	3.312	1.134	.050	-.003	6.626
		3	2.124	1.318	1.000	-1.728	5.976
		4	2.813*	.958	.048	.014	5.613
0.1	1	2	-.087	.474	1.000	-1.473	1.298
		3	-2.072	.748	.076	-4.258	.114
		4	-1.091	.673	1.000	-3.058	.876
		5	-1.091	1.251	1.000	-4.748	2.566
	2	1	.087	.474	1.000	-1.298	1.473
		3	-1.984*	.584	.013	-3.691	-.278
		4	-1.003	.556	.764	-2.628	.621
		5	-1.003	1.134	1.000	-4.318	2.311
	3	1	2.072	.748	.076	-.114	4.258
		2	1.984*	.584	.013	.278	3.691
		4	.981	.782	1.000	-1.306	3.267
		5	.981	1.318	1.000	-2.871	4.833
	4	1	1.091	.673	1.000	-.876	3.058
		2	1.003	.556	.764	-.621	2.628
		3	-.981	.782	1.000	-3.267	1.306
		5	.000	.958	1.000	-2.800	2.800
	5	1	1.091	1.251	1.000	-2.566	4.748
		2	1.003	1.134	1.000	-2.311	4.318
		3	-.981	1.318	1.000	-4.833	2.871
		4	.000	.958	1.000	-2.800	2.800
1	1	2	.765	.456	.988	-.567	2.096
		3	.765	.719	1.000	-1.336	2.865
		4	.524	.647	1.000	-1.366	2.414
		5	-.457	1.202	1.000	-3.971	3.057
	2	1	-.765	.456	.988	-2.096	.567
		3	-9.770E-15	.561	1.000	-1.640	1.640
		4	-.241	.534	1.000	-1.801	1.320
		5	-1.222	1.090	1.000	-4.406	1.963
	3	1	-.765	.719	1.000	-2.865	1.336
		2	9.770E-15	.561	1.000	-1.640	1.640
		4	-.241	.752	1.000	-2.438	1.956
		5	-1.222	1.266	1.000	-4.923	2.479
	4	1	-.524	.647	1.000	-2.414	1.366
		2	.241	.534	1.000	-1.320	1.801
		3	.241	.752	1.000	-1.956	2.438
		5	-.981	.920	1.000	-3.671	1.709

	5	1	.457	1.202	1.000	-3.057	3.971
		2	1.222	1.090	1.000	-1.963	4.406
		3	1.222	1.266	1.000	-2.479	4.923
		4	.981	.920	1.000	-1.709	3.671

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

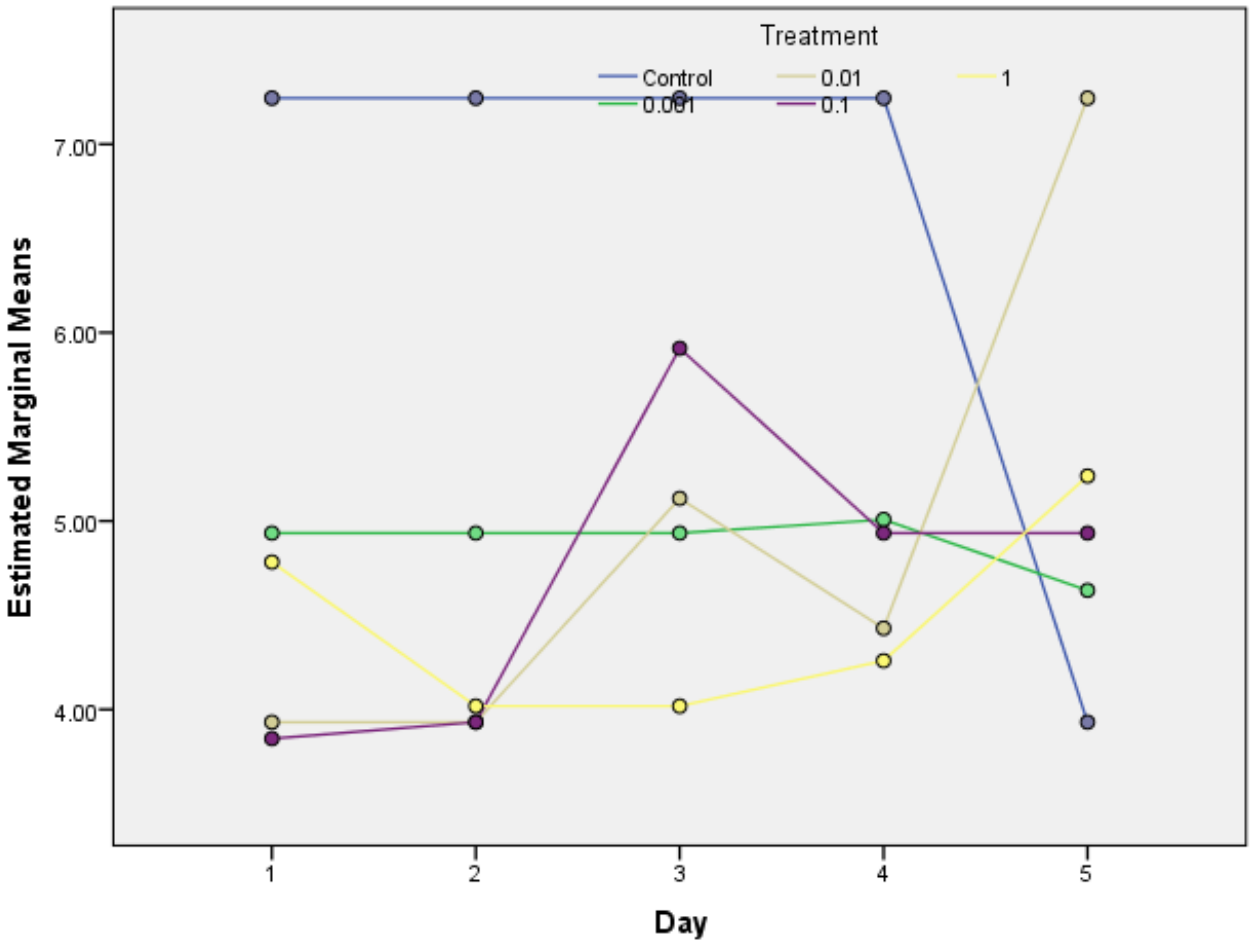
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.183	2.967[a]	4.000	53.000	.028	11.868	.756
	Wilks' lambda	.817	2.967[a]	4.000	53.000	.028	11.868	.756
	Hotelling's trace	.224	2.967[a]	4.000	53.000	.028	11.868	.756
	Roy's largest root	.224	2.967[a]	4.000	53.000	.028	11.868	.756
0.001	Pillai's trace	.003	.045[a]	4.000	53.000	.996	.178	.058
	Wilks' lambda	.997	.045[a]	4.000	53.000	.996	.178	.058
	Hotelling's trace	.003	.045[a]	4.000	53.000	.996	.178	.058
	Roy's largest root	.003	.045[a]	4.000	53.000	.996	.178	.058
0.01	Pillai's trace	.212	3.564[a]	4.000	53.000	.012	14.258	.838
	Wilks' lambda	.788	3.564[a]	4.000	53.000	.012	14.258	.838
	Hotelling's trace	.269	3.564[a]	4.000	53.000	.012	14.258	.838
	Roy's largest root	.269	3.564[a]	4.000	53.000	.012	14.258	.838
0.1	Pillai's trace	.205	3.417[a]	4.000	53.000	.015	13.668	.820
	Wilks' lambda	.795	3.417[a]	4.000	53.000	.015	13.668	.820
	Hotelling's trace	.258	3.417[a]	4.000	53.000	.015	13.668	.820
	Roy's largest root	.258	3.417[a]	4.000	53.000	.015	13.668	.820
1	Pillai's trace	.076	1.093[a]	4.000	53.000	.369	4.373	.320
	Wilks' lambda	.924	1.093[a]	4.000	53.000	.369	4.373	.320
	Hotelling's trace	.083	1.093[a]	4.000	53.000	.369	4.373	.320
	Roy's largest root	.083	1.093[a]	4.000	53.000	.369	4.373	.320

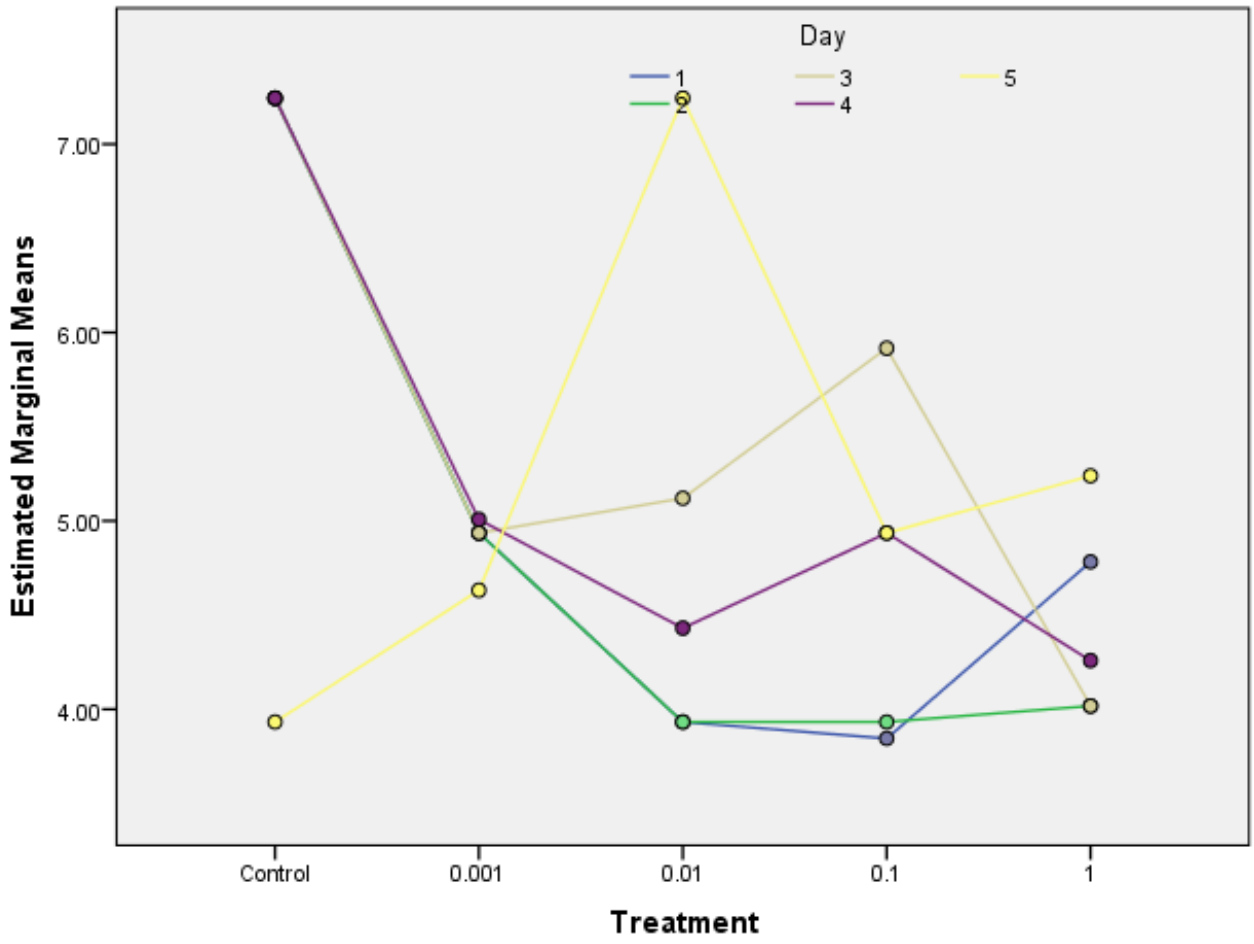
Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots


Treatment
Control
0.01
1
0.001
0.1
Estimated Marginal Means
7.00
6.00
5.00
4.00
1
2
3
4
5
Day

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DATASET CLOSE DataSet1.

GET
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DATASET NAME DataSet2 WINDOW=FRONT.

 TITLE Iron.
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Iron

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DATASET ACTIVATE DataSet2.
DESCRIPTIVES VARIABLES=RA1 RP1 RT1 RA2 RP2 RT2 RA3 RP3 RT3 RA5 RP5 RT5 RA7 RP7 RT7 SA1 SP1 ST1 SA2
    SP2 ST2 SA3 SP3 ST3 SA5 SP5 ST5 SA7 SP7 ST7
  /STATISTICS=MEAN STDDEV MIN MAX SEMEAN.
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Descriptives

[DataSet2] C:\@Data\Research\Research PostGraduate\DTech\CPUT\AyeniOlutoyosi\Data Iron new landscape.sav

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
RA1	60	2.64	40.59	14.3955	1.05038	8.13620
RP1	60	2.64	23.53	12.0731	.89345	6.92066
RT1	60	2.64	40.59	15.2733	1.05025	8.13524
RA2	60	2.64	40.59	14.2522	1.06804	8.27304
RP2	60	3.17	40.59	14.8515	1.07090	8.29516
RT2	60	2.64	40.59	15.1603	1.11281	8.61975
RA3	60	3.17	40.59	14.9571	1.02283	7.92277
RP3	60	2.64	40.59	13.7872	1.05020	8.13482
RT3	60	2.64	40.59	14.1482	1.04455	8.09108
RA5	60	2.64	40.59	13.6018	1.03543	8.02039
RP5	60	2.64	40.59	14.6701	1.07040	8.29125
RT5	60	2.64	25.37	12.8874	.92051	7.13024
RA7	60	3.17	40.59	15.6504	1.08608	8.41276
RP7	60	2.64	23.53	12.5490	.90859	7.03794
RT7	60	2.64	28.66	13.6714	.94845	7.34663
SA1	61	.84	10.01	3.8056	.30310	2.36726
SP1	61	.84	10.01	3.1865	.26545	2.07320
ST1	61	.84	10.01	3.6244	.30152	2.35495
SA2	61	.84	10.01	4.2107	.33920	2.64927
SP2	61	.84	10.01	3.7849	.30722	2.39950
ST2	61	.90	10.01	3.0128	.26002	2.03084
SA3	61	.84	10.01	3.7312	.31807	2.48419
SP3	61	.84	10.01	3.7100	.32217	2.51626
ST3	61	.90	10.01	3.1748	.26745	2.08882
SA5	61	.90	10.01	3.1528	.27822	2.17295
SP5	61	.90	10.01	3.6217	.31222	2.43849
ST5	61	.90	10.01	3.1277	.28218	2.20389
SA7	61	.90	10.01	3.4987	.30232	2.36119
SP7	61	.84	10.01	3.4021	.28989	2.26408
ST7	61	.84	10.01	3.6244	.30152	2.35495
Valid N (listwise)	60					

```
TITLE Iron.
```

Iron

```
GLM RA1 RA2 RA3 RA5 RA7 BY Treatment
 /WSFACTOR=Day 5 Polynomial
 /MEASURE=RootA
 /METHOD=SSTYPE(3)
 /PLOT=PROFILE(Day*Treatment Treatment*Day)
 /EMMEANS=TABLES(OVERALL)
 /EMMEANS=TABLES(Treatment) COMPARE ADJ(BONFERRONI)
 /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
 /EMMEANS=TABLES(Treatment*Day) COMPARE (Treatment) ADJ(BONFERRONI)
 /EMMEANS=TABLES(Treatment*Day) COMPARE (Day) ADJ(BONFERRONI)
 /CRITERIA=ALPHA(.05)
 /WSDESIGN=Day
 /DESIGN=Treatment.
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General Linear Model

Within-Subjects Factors

Measure: RootA

Day	Dependent Variable
1	RA1
2	RA2
3	RA3
4	RA5
5	RA7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	5	12
	2	10	12
	3	15	12
	4	20	12

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value
Day	Pillai's Trace	.037	.496[b]	4.000	52.000	.739
	Wilks' Lambda	.963	.496[b]	4.000	52.000	.739
	Hotelling's Trace	.038	.496[b]	4.000	52.000	.739
	Roy's Largest Root	.038	.496[b]	4.000	52.000	.739
Day * Treatment	Pillai's Trace	.619	2.518	16.000	220.000	.001
	Wilks' Lambda	.442	3.052	16.000	159.500	.000
	Hotelling's Trace	1.126	3.555	16.000	202.000	.000
	Roy's Largest Root	.998	13.717[c]	4.000	55.000	.000

a. Design: Intercept + Treatment
Within Subjects Design: Day

b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: RootA

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.858	8.174	9	.517	.934	1.000	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: RootA

Source		Type III Sum of Squares	df	Mean Square	F	p-value
Day	Sphericity Assumed	143.165	4	35.791	.688	.601
	Greenhouse-Geisser	143.165	3.738	38.300	.688	.591
	Huynh-Feldt	143.165	4.000	35.791	.688	.601
	Lower-bound	143.165	1.000	143.165	.688	.410
Day * Treatment	Sphericity Assumed	2669.619	16	166.851	3.208	.000
	Greenhouse-Geisser	2669.619	14.952	178.547	3.208	.000
	Huynh-Feldt	2669.619	16.000	166.851	3.208	.000
	Lower-bound	2669.619	4.000	667.405	3.208	.019
Error(Day)	Sphericity Assumed	11442.458	220	52.011		
	Greenhouse-Geisser	11442.458	205.589	55.657		
	Huynh-Feldt	11442.458	220.000	52.011		
	Lower-bound	11442.458	55.000	208.045		

Tests of Within-Subjects Contrasts

Measure: RootA

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value
Day	Linear	20.743	1	20.743	.456	.503
	Quadratic	23.140	1	23.140	.405	.527
	Cubic	39.193	1	39.193	.758	.388
	Order 4	60.088	1	60.088	1.119	.295
Day * Treatment	Linear	1085.168	4	271.292	5.958	.000
	Quadratic	480.632	4	120.158	2.104	.093
	Cubic	945.715	4	236.429	4.573	.003
	Order 4	158.103	4	39.526	.736	.571
Error(Day)	Linear	2504.491	55	45.536		
	Quadratic	3140.658	55	57.103		
	Cubic	2843.448	55	51.699		
	Order 4	2953.861	55	53.707		

Tests of Between-Subjects Effects

Measure: RootA
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Intercept	63697.695	1	63697.695	1084.106	.000
Treatment	2274.578	4	568.644	9.678	.000
Error	3231.578	55	58.756		

Estimated Marginal Means

1. Grand Mean

Measure: RootA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
14.571	.443	13.685	15.458

2. Treatment

Estimates

Measure: RootA

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	18.077	.990	16.094	20.060
5	17.221	.990	15.238	19.204
10	14.513	.990	12.530	16.497
15	11.580	.990	9.597	13.563
20	11.465	.990	9.482	13.448

Pairwise Comparisons

Measure: RootA

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Control	5	.856	1.399	1.000	-3.237	4.949
	10	3.564	1.399	.137	-.529	7.657
	15	6.497*	1.399	.000	2.404	10.590
	20	6.612*	1.399	.000	2.519	10.705
5	Control	-.856	1.399	1.000	-4.949	3.237
	10	2.708	1.399	.582	-1.385	6.801
	15	5.641*	1.399	.002	1.548	9.734
	20	5.756*	1.399	.001	1.663	9.849
10	Control	-3.564	1.399	.137	-7.657	.529
	5	-2.708	1.399	.582	-6.801	1.385

	15	2.933	1.399	.407	-1.160	7.026
	20	3.048	1.399	.337	-1.045	7.141
15	Control	-6.497*	1.399	.000	-10.590	-2.404
	5	-5.641*	1.399	.002	-9.734	-1.548
	10	-2.933	1.399	.407	-7.026	1.160
	20	.115	1.399	1.000	-3.978	4.208
20	Control	-6.612*	1.399	.000	-10.705	-2.519
	5	-5.756*	1.399	.001	-9.849	-1.663
	10	-3.048	1.399	.337	-7.141	1.045
	15	-.115	1.399	1.000	-4.208	3.978

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootA

	Sum of Squares	df	Mean Square	F	p-value
Contrast	454.916	4	113.729	9.678	.000
Error	646.316	55	11.751		

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

3. Day

Estimates

Measure: RootA

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	14.396	.964	12.464	16.327
2	14.252	.926	12.396	16.109
3	14.957	.935	13.084	16.830
4	13.602	.933	11.733	15.471
5	15.650	.957	13.732	17.569

Pairwise Comparisons

Measure: RootA

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	.143	1.380	1.000	-3.892	4.178
	3	-.562	1.398	1.000	-4.649	3.526
	4	.794	1.284	1.000	-2.963	4.550
	5	-1.255	1.373	1.000	-5.270	2.761
2	1	-.143	1.380	1.000	-4.178	3.892
	3	-.705	1.273	1.000	-4.428	3.018
	4	.650	1.165	1.000	-2.756	4.056
	5	-1.398	1.325	1.000	-5.272	2.476
3	1	.562	1.398	1.000	-3.526	4.649

	2	.705	1.273	1.000	-3.018	4.428
	4	1.355	1.259	1.000	-2.328	5.039
	5	-.693	1.214	1.000	-4.245	2.858
4	1	-.794	1.284	1.000	-4.550	2.963
	2	-.650	1.165	1.000	-4.056	2.756
	3	-1.355	1.259	1.000	-5.039	2.328
	5	-2.049	1.468	1.000	-6.341	2.244
5	1	1.255	1.373	1.000	-2.761	5.270
	2	1.398	1.325	1.000	-2.476	5.272
	3	.693	1.214	1.000	-2.858	4.245
	4	2.049	1.468	1.000	-2.244	6.341

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value
Pillai's trace	.037	.496[a]	4.000	52.000	.739
Wilks' lambda	.963	.496[a]	4.000	52.000	.739
Hotelling's trace	.038	.496[a]	4.000	52.000	.739
Roy's largest root	.038	.496[a]	4.000	52.000	.739

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Exact statistic

4. Treatment * Day

Estimates

Measure: RootA

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	21.445	2.155	17.126	25.764
	2	23.210	2.071	19.059	27.361
	3	15.007	2.090	10.819	19.195
	4	9.886	2.086	5.706	14.066
	5	20.837	2.140	16.548	25.126
5	1	12.281	2.155	7.962	16.600
	2	12.019	2.071	7.868	16.171
	3	20.321	2.090	16.133	24.509
	4	20.442	2.086	16.263	24.622
	5	21.043	2.140	16.753	25.332
10	1	14.360	2.155	10.041	18.679
	2	11.754	2.071	7.603	15.905
	3	17.468	2.090	13.280	21.656
	4	15.517	2.086	11.338	19.697
	5	13.467	2.140	9.178	17.756
15	1	13.332	2.155	9.013	17.651
	2	12.366	2.071	8.214	16.517
	3	10.010	2.090	5.822	14.198
	4	10.909	2.086	6.729	15.089
	5	11.283	2.140	6.994	15.572

20	1	10.559	2.155	6.240	14.878
	2	11.912	2.071	7.761	16.063
	3	11.979	2.090	7.791	16.167
	4	11.254	2.086	7.074	15.433
	5	11.623	2.140	7.334	15.912

Pairwise Comparisons

Measure: RootA

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	5	9.164*	3.048	.040	.250	18.078
		10	7.085	3.048	.238	-1.829	15.999
		15	8.113	3.048	.102	-.801	17.027
		20	10.886*	3.048	.007	1.972	19.800
	5	Control	-9.164*	3.048	.040	-18.078	-.250
		10	-2.080	3.048	1.000	-10.994	6.834
		15	-1.052	3.048	1.000	-9.966	7.862
		20	1.722	3.048	1.000	-7.192	10.636
	10	Control	-7.085	3.048	.238	-15.999	1.829
		5	2.080	3.048	1.000	-6.834	10.994
		15	1.028	3.048	1.000	-7.886	9.942
		20	3.801	3.048	1.000	-5.113	12.715
	15	Control	-8.113	3.048	.102	-17.027	.801
		5	1.052	3.048	1.000	-7.862	9.966
		10	-1.028	3.048	1.000	-9.942	7.886
		20	2.773	3.048	1.000	-6.141	11.687
	20	Control	-10.886*	3.048	.007	-19.800	-1.972
		5	-1.722	3.048	1.000	-10.636	7.192
		10	-3.801	3.048	1.000	-12.715	5.113
		15	-2.773	3.048	1.000	-11.687	6.141
2	Control	5	11.191*	2.930	.003	2.623	19.759
		10	11.456*	2.930	.003	2.888	20.024
		15	10.844*	2.930	.005	2.276	19.412
		20	11.298*	2.930	.003	2.730	19.866
	5	Control	-11.191*	2.930	.003	-19.759	-2.623
		10	.265	2.930	1.000	-8.303	8.833
		15	-.347	2.930	1.000	-8.915	8.221
		20	.107	2.930	1.000	-8.461	8.675
	10	Control	-11.456*	2.930	.003	-20.024	-2.888
		5	-.265	2.930	1.000	-8.833	8.303
		15	-.612	2.930	1.000	-9.180	7.956
		20	-.158	2.930	1.000	-8.726	8.410
	15	Control	-10.844*	2.930	.005	-19.412	-2.276
		5	.347	2.930	1.000	-8.221	8.915
		10	.612	2.930	1.000	-7.956	9.180
		20	.454	2.930	1.000	-8.114	9.022
	20	Control	-11.298*	2.930	.003	-19.866	-2.730
		5	-.107	2.930	1.000	-8.675	8.461
		10	.158	2.930	1.000	-8.410	8.726
		15	-.454	2.930	1.000	-9.022	8.114
3	Control	5	-5.314	2.955	.777	-13.957	3.330
		10	-2.461	2.955	1.000	-11.105	6.183
		15	4.997	2.955	.965	-3.647	13.641

		20	3.029	2.955	1.000	-5.615	11.672
	5	Control	5.314	2.955	.777	-3.330	13.957
		10	2.853	2.955	1.000	-5.791	11.496
		15	10.311*	2.955	.010	1.667	18.954
		20	8.342	2.955	.066	-.302	16.986
	10	Control	2.461	2.955	1.000	-6.183	11.105
		5	-2.853	2.955	1.000	-11.496	5.791
		15	7.458	2.955	.145	-1.186	16.102
		20	5.490	2.955	.686	-3.154	14.133
	15	Control	-4.997	2.955	.965	-13.641	3.647
		5	-10.311*	2.955	.010	-18.954	-1.667
		10	-7.458	2.955	.145	-16.102	1.186
		20	-1.968	2.955	1.000	-10.612	6.675
	20	Control	-3.029	2.955	1.000	-11.672	5.615
		5	-8.342	2.955	.066	-16.986	.302
		10	-5.490	2.955	.686	-14.133	3.154
		15	1.968	2.955	1.000	-6.675	10.612
4	Control	5	-10.557*	2.949	.007	-19.183	-1.930
		10	-5.632	2.949	.614	-14.258	2.995
		15	-1.023	2.949	1.000	-9.650	7.603
		20	-1.368	2.949	1.000	-9.994	7.258
	5	Control	10.557*	2.949	.007	1.930	19.183
		10	4.925	2.949	1.000	-3.701	13.551
		15	9.533*	2.949	.021	.907	18.160
		20	9.189*	2.949	.029	.562	17.815
	10	Control	5.632	2.949	.614	-2.995	14.258
		5	-4.925	2.949	1.000	-13.551	3.701
		15	4.608	2.949	1.000	-4.018	13.235
		20	4.264	2.949	1.000	-4.363	12.890
	15	Control	1.023	2.949	1.000	-7.603	9.650
		5	-9.533*	2.949	.021	-18.160	-.907
		10	-4.608	2.949	1.000	-13.235	4.018
		20	-.345	2.949	1.000	-8.971	8.282
	20	Control	1.368	2.949	1.000	-7.258	9.994
		5	-9.189*	2.949	.029	-17.815	-.562
		10	-4.264	2.949	1.000	-12.890	4.363
		15	.345	2.949	1.000	-8.282	8.971
5	Control	5	-.206	3.027	1.000	-9.058	8.647
		10	7.370	3.027	.182	-1.482	16.223
		15	9.554*	3.027	.026	.702	18.406
		20	9.214*	3.027	.036	.362	18.067
	5	Control	.206	3.027	1.000	-8.647	9.058
		10	7.576	3.027	.153	-1.277	16.428
		15	9.760*	3.027	.021	.907	18.612
		20	9.420*	3.027	.029	.567	18.272
	10	Control	-7.370	3.027	.182	-16.223	1.482
		5	-7.576	3.027	.153	-16.428	1.277
		15	2.184	3.027	1.000	-6.668	11.036
		20	1.844	3.027	1.000	-7.008	10.696
	15	Control	-9.554*	3.027	.026	-18.406	-.702
		5	-9.760*	3.027	.021	-18.612	-.907
		10	-2.184	3.027	1.000	-11.036	6.668
		20	-.340	3.027	1.000	-9.192	8.512
	20	Control	-9.214*	3.027	.036	-18.067	-.362
		5	-9.420*	3.027	.029	-18.272	-.567

	10	-1.844	3.027	1.000	-10.696	7.008
	15	.340	3.027	1.000	-8.512	9.192

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootA

Day		Sum of Squares	df	Mean Square	F	p-value
1	Contrast	840.204	4	210.051	3.769	.009
	Error	3065.465	55	55.736		
2	Contrast	1206.021	4	301.505	5.855	.001
	Error	2832.126	55	51.493		
3	Contrast	821.041	4	205.260	3.917	.007
	Error	2882.407	55	52.407		
4	Contrast	924.434	4	231.109	4.428	.004
	Error	2870.837	55	52.197		
5	Contrast	1152.497	4	288.124	5.242	.001
	Error	3023.201	55	54.967		

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

5. Treatment * Day

Estimates

Measure: RootA

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	21.445	2.155	17.126	25.764
	2	23.210	2.071	19.059	27.361
	3	15.007	2.090	10.819	19.195
	4	9.886	2.086	5.706	14.066
	5	20.837	2.140	16.548	25.126
5	1	12.281	2.155	7.962	16.600
	2	12.019	2.071	7.868	16.171
	3	20.321	2.090	16.133	24.509
	4	20.442	2.086	16.263	24.622
	5	21.043	2.140	16.753	25.332
10	1	14.360	2.155	10.041	18.679
	2	11.754	2.071	7.603	15.905
	3	17.468	2.090	13.280	21.656
	4	15.517	2.086	11.338	19.697
	5	13.467	2.140	9.178	17.756
15	1	13.332	2.155	9.013	17.651
	2	12.366	2.071	8.214	16.517
	3	10.010	2.090	5.822	14.198
	4	10.909	2.086	6.729	15.089
	5	11.283	2.140	6.994	15.572
20	1	10.559	2.155	6.240	14.878

2	11.912	2.071	7.761	16.063
3	11.979	2.090	7.791	16.167
4	11.254	2.086	7.074	15.433
5	11.623	2.140	7.334	15.912

Pairwise Comparisons

Measure: RootA

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	-1.765	3.085	1.000	-10.787	7.257
		3	6.438	3.125	.441	-2.702	15.578
		4	11.559*	2.872	.002	3.160	19.959
		5	.608	3.070	1.000	-8.371	9.587
	2	1	1.765	3.085	1.000	-7.257	10.787
		3	8.203	2.847	.056	-.123	16.528
		4	13.324*	2.604	.000	5.708	20.940
		5	2.373	2.962	1.000	-6.289	11.035
	3	1	-6.438	3.125	.441	-15.578	2.702
		2	-8.203	2.847	.056	-16.528	.123
		4	5.121	2.816	.744	-3.115	13.358
		5	-5.830	2.715	.362	-13.771	2.111
	4	1	-11.559*	2.872	.002	-19.959	-3.160
		2	-13.324*	2.604	.000	-20.940	-5.708
		3	-5.121	2.816	.744	-13.358	3.115
		5	-10.951*	3.282	.015	-20.550	-1.353
	5	1	-.608	3.070	1.000	-9.587	8.371
		2	-2.373	2.962	1.000	-11.035	6.289
		3	5.830	2.715	.362	-2.111	13.771
		4	10.951*	3.282	.015	1.353	20.550
5	1	2	.262	3.085	1.000	-8.761	9.284
		3	-8.040	3.125	.128	-17.180	1.100
		4	-8.162	2.872	.063	-16.561	.238
		5	-8.762	3.070	.061	-17.741	.217
	2	1	-.262	3.085	1.000	-9.284	8.761
		3	-8.302	2.847	.051	-16.627	.024
		4	-8.423*	2.604	.021	-16.039	-.807
		5	-9.023*	2.962	.036	-17.686	-.361
	3	1	8.040	3.125	.128	-1.100	17.180
		2	8.302	2.847	.051	-.024	16.627
		4	-.122	2.816	1.000	-8.358	8.115
		5	-.722	2.715	1.000	-8.663	7.219
	4	1	8.162	2.872	.063	-.238	16.561
		2	8.423*	2.604	.021	.807	16.039
		3	.122	2.816	1.000	-8.115	8.358
		5	-.600	3.282	1.000	-10.199	8.998
	5	1	8.762	3.070	.061	-.217	17.741
		2	9.023*	2.962	.036	.361	17.686
		3	.722	2.715	1.000	-7.219	8.663
		4	.600	3.282	1.000	-8.998	10.199

10	1	2	2.606	3.085	1.000	-6.416	11.628
		3	-3.108	3.125	1.000	-12.248	6.032
		4	-1.157	2.872	1.000	-9.557	7.242
		5	.894	3.070	1.000	-8.085	9.872
	2	1	-2.606	3.085	1.000	-11.628	6.416
		3	-5.714	2.847	.496	-14.040	2.611
		4	-3.763	2.604	1.000	-11.379	3.853
		5	-1.713	2.962	1.000	-10.375	6.950
	3	1	3.108	3.125	1.000	-6.032	12.248
		2	5.714	2.847	.496	-2.611	14.040
		4	1.951	2.816	1.000	-6.285	10.187
		5	4.002	2.715	1.000	-3.940	11.943
	4	1	1.157	2.872	1.000	-7.242	9.557
		2	3.763	2.604	1.000	-3.853	11.379
		3	-1.951	2.816	1.000	-10.187	6.285
		5	2.051	3.282	1.000	-7.548	11.649
	5	1	-.894	3.070	1.000	-9.872	8.085
		2	1.713	2.962	1.000	-6.950	10.375
		3	-4.002	2.715	1.000	-11.943	3.940
		4	-2.051	3.282	1.000	-11.649	7.548
15	1	2	.967	3.085	1.000	-8.056	9.989
		3	3.322	3.125	1.000	-5.818	12.462
		4	2.423	2.872	1.000	-5.976	10.823
		5	2.050	3.070	1.000	-6.929	11.028
	2	1	-.967	3.085	1.000	-9.989	8.056
		3	2.356	2.847	1.000	-5.970	10.681
		4	1.457	2.604	1.000	-6.159	9.073
		5	1.083	2.962	1.000	-7.579	9.745
	3	1	-3.322	3.125	1.000	-12.462	5.818
		2	-2.356	2.847	1.000	-10.681	5.970
		4	-.899	2.816	1.000	-9.135	7.337
		5	-1.273	2.715	1.000	-9.214	6.669
	4	1	-2.423	2.872	1.000	-10.823	5.976
		2	-1.457	2.604	1.000	-9.073	6.159
		3	.899	2.816	1.000	-7.337	9.135
		5	-.374	3.282	1.000	-9.972	9.225
	5	1	-2.050	3.070	1.000	-11.028	6.929
		2	-1.083	2.962	1.000	-9.745	7.579
		3	1.273	2.715	1.000	-6.669	9.214
		4	.374	3.282	1.000	-9.225	9.972
20	1	2	-1.353	3.085	1.000	-10.375	7.669
		3	-1.420	3.125	1.000	-10.560	7.720
		4	-.695	2.872	1.000	-9.094	7.705
		5	-1.064	3.070	1.000	-10.043	7.915
	2	1	1.353	3.085	1.000	-7.669	10.375
		3	-.067	2.847	1.000	-8.392	8.259
		4	.658	2.604	1.000	-6.958	8.274
		5	.289	2.962	1.000	-8.373	8.952
	3	1	1.420	3.125	1.000	-7.720	10.560
		2	.067	2.847	1.000	-8.259	8.392
		4	.725	2.816	1.000	-7.511	8.961

		5	.356	2.715	1.000	-7.585	8.297
	4	1	.695	2.872	1.000	-7.705	9.094
		2	-.658	2.604	1.000	-8.274	6.958
		3	-.725	2.816	1.000	-8.961	7.511
		5	-.369	3.282	1.000	-9.968	9.230
	5	1	1.064	3.070	1.000	-7.915	10.043
		2	-.289	2.962	1.000	-8.952	8.373
		3	-.356	2.715	1.000	-8.297	7.585
		4	.369	3.282	1.000	-9.230	9.968

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

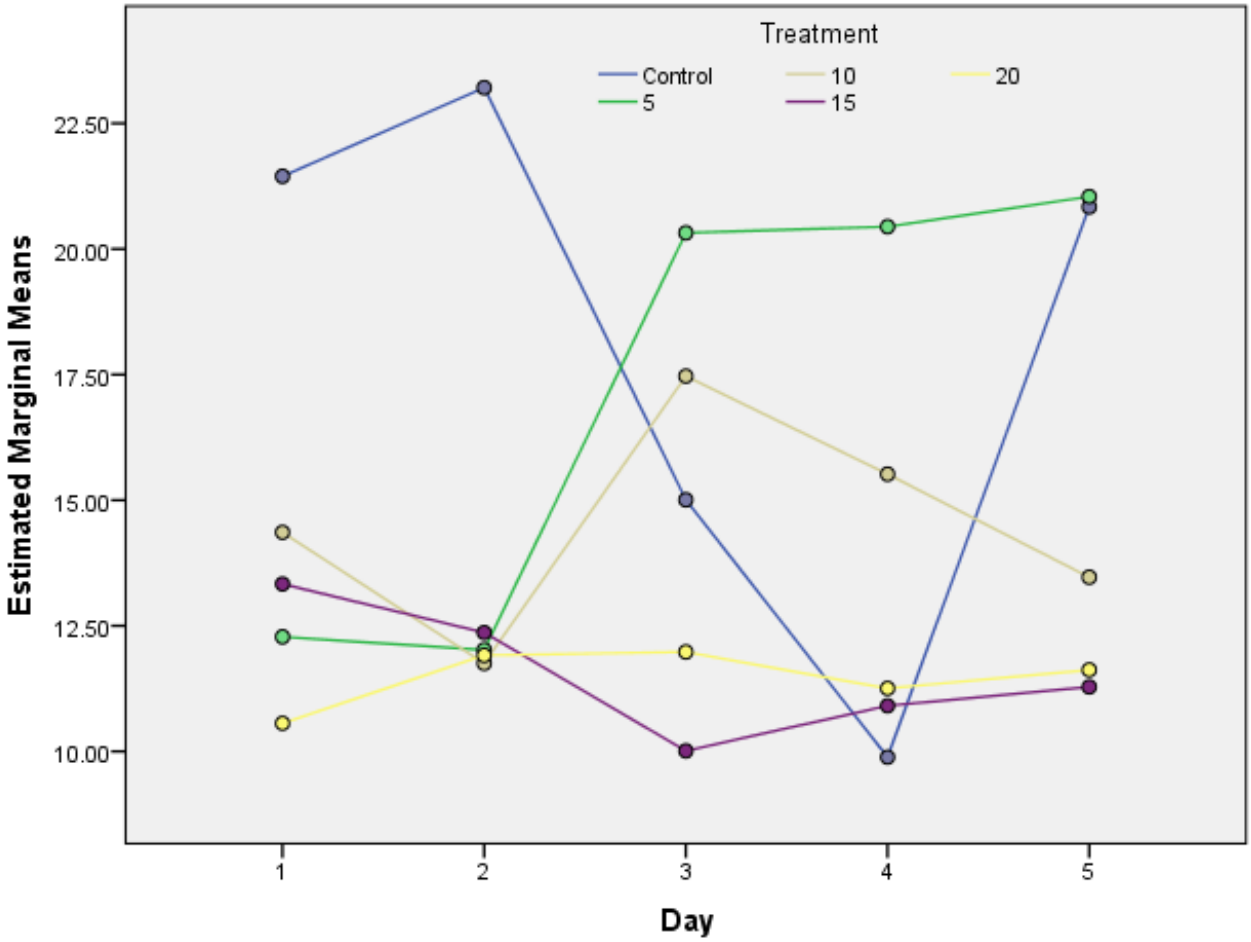
Multivariate Tests

Treatment		Value	F	Hypothesis df	Error df	p-value
Control	Pillai's trace	.377	7.854[a]	4.000	52.000	.000
	Wilks' lambda	.623	7.854[a]	4.000	52.000	.000
	Hotelling's trace	.604	7.854[a]	4.000	52.000	.000
	Roy's largest root	.604	7.854[a]	4.000	52.000	.000
5	Pillai's trace	.305	5.705[a]	4.000	52.000	.001
	Wilks' lambda	.695	5.705[a]	4.000	52.000	.001
	Hotelling's trace	.439	5.705[a]	4.000	52.000	.001
	Roy's largest root	.439	5.705[a]	4.000	52.000	.001
10	Pillai's trace	.081	1.148[a]	4.000	52.000	.344
	Wilks' lambda	.919	1.148[a]	4.000	52.000	.344
	Hotelling's trace	.088	1.148[a]	4.000	52.000	.344
	Roy's largest root	.088	1.148[a]	4.000	52.000	.344
15	Pillai's trace	.027	.367[a]	4.000	52.000	.831
	Wilks' lambda	.973	.367[a]	4.000	52.000	.831
	Hotelling's trace	.028	.367[a]	4.000	52.000	.831
	Roy's largest root	.028	.367[a]	4.000	52.000	.831
20	Pillai's trace	.005	.063[a]	4.000	52.000	.992
	Wilks' lambda	.995	.063[a]	4.000	52.000	.992
	Hotelling's trace	.005	.063[a]	4.000	52.000	.992
	Roy's largest root	.005	.063[a]	4.000	52.000	.992

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

Profile Plots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20
5
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15.00
12.50
10.00
1
2
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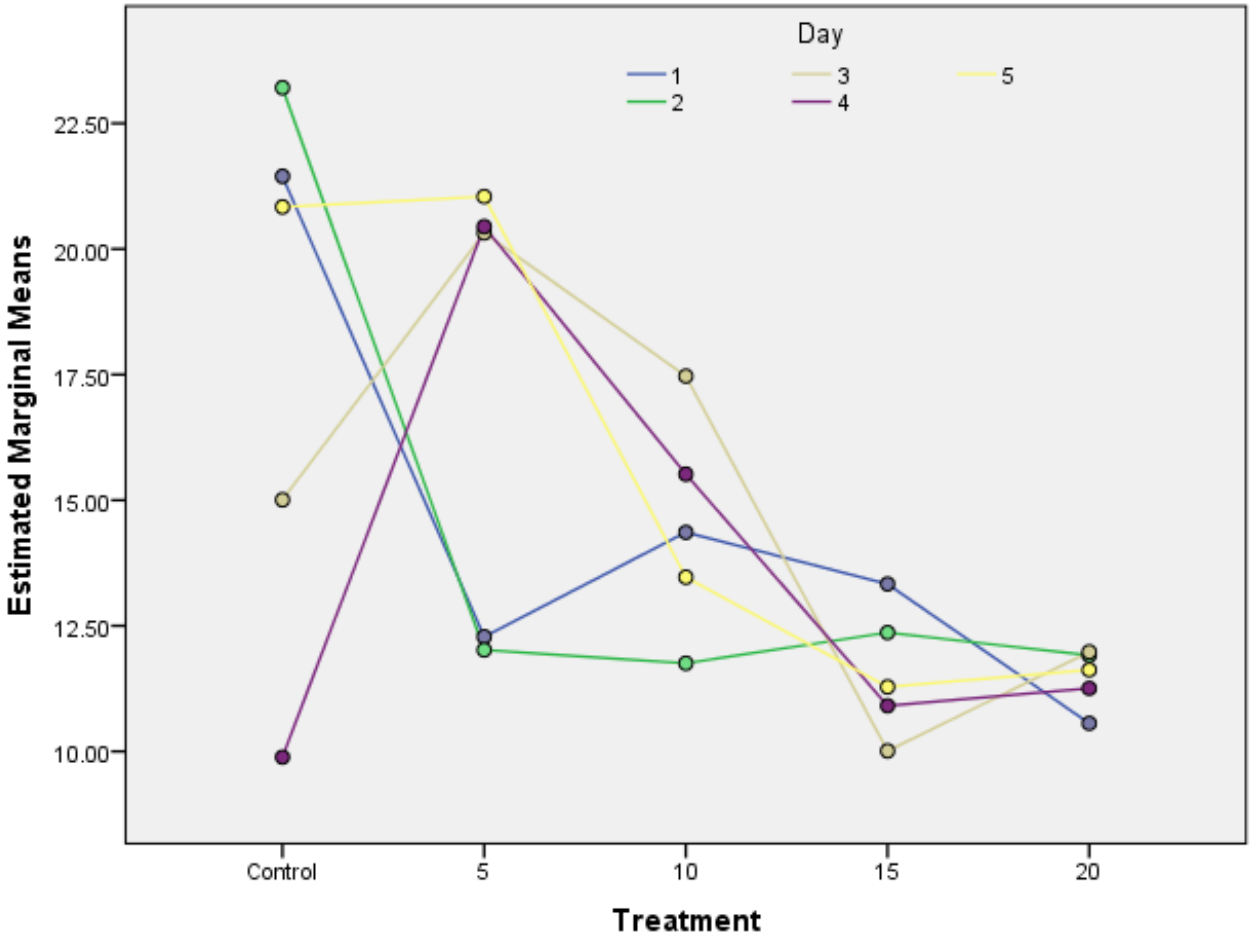
5
Day


Day
1
2
3
4
5
Estimated Marginal Means
22.50
20.00
17.50
15.00
12.50
10.00
Control
5
10
15
20
Treatment

TITLE Iron.

Iron

```
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 /EMMEANS=TABLES(OVERALL)
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 /EMMEANS=TABLES(Day) COMPARE ADJ(BONFERRONI)
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 /CRITERIA=ALPHA(.05)
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General Linear Model

Within-Subjects Factors

Measure: RootP

Day	Dependent Variable
1	RP1
2	RP2
3	RP3
4	RP5
5	RP7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	5	12
	2	10	12
	3	15	12
	4	20	12

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
RP1	Control	9.8859	6.38932	12
	5	12.9428	6.84021	12
	10	12.9097	6.90199	12
	15	13.3365	7.24537	12
	20	11.2906	7.74162	12
	Total	12.0731	6.92066	60
RP2	Control	14.8307	7.98153	12
	5	22.0210	8.19554	12
	10	14.2995	7.54164	12

	15	11.5895	6.34307	12
	20	11.5168	7.80649	12
	Total	14.8515	8.29516	60
RP3	Control	21.4450	8.35537	12
	5	12.2807	7.22519	12
	10	11.1754	6.63797	12
	15	13.8603	7.73700	12
	20	10.1747	6.39285	12
	Total	13.7872	8.13482	60
RP5	Control	23.7128	7.75517	12
	5	12.3767	7.11451	12
	10	12.9428	6.84021	12
	15	11.5698	6.37762	12
	20	12.7483	7.60189	12
	Total	14.6701	8.29125	60
RP7	Control	13.0836	7.52390	12
	5	11.4568	6.99899	12
	10	11.6838	6.33080	12
	15	14.6214	7.52952	12
	20	11.8996	7.46539	12
	Total	12.5490	7.03794	60

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.121	1.794[b]	4.000	52.000	.144	7.174	.510
	Wilks' Lambda	.879	1.794[b]	4.000	52.000	.144	7.174	.510
	Hotelling's Trace	.138	1.794[b]	4.000	52.000	.144	7.174	.510
	Roy's Largest Root	.138	1.794[b]	4.000	52.000	.144	7.174	.510
Day * Treatment	Pillai's Trace	.626	2.550	16.000	220.000	.001	40.806	.992
	Wilks' Lambda	.462	2.863	16.000	159.500	.000	33.922	.969
	Hotelling's Trace	.976	3.080	16.000	202.000	.000	49.275	.998
	Roy's Largest Root	.734	10.089[c]	4.000	55.000	.000	40.355	1.000

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: RootP

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.844	9.034	9	.434	.923	1.000	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: RootP

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	370.880	4	92.720	1.696	.152	6.784	.515
	Greenhouse-Geisser	370.880	3.693	100.440	1.696	.157	6.263	.493

	Huynh-Feldt	370.880	4.000	92.720	1.696	.152	6.784	.515
	Lower-bound	370.880	1.000	370.880	1.696	.198	1.696	.249
Day * Treatment	Sphericity Assumed	2376.213	16	148.513	2.717	.001	43.465	.995
	Greenhouse-Geisser	2376.213	14.770	160.879	2.717	.001	40.124	.992
	Huynh-Feldt	2376.213	16.000	148.513	2.717	.001	43.465	.995
	Lower-bound	2376.213	4.000	594.053	2.717	.039	10.866	.715
Error(Day)	Sphericity Assumed	12027.310	220	54.670				
	Greenhouse-Geisser	12027.310	203.090	59.222				
	Huynh-Feldt	12027.310	220.000	54.670				
	Lower-bound	12027.310	55.000	218.678				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: RootP

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	3.561	1	3.561	.086	.770	.086	.060
	Quadratic	264.213	1	264.213	4.185	.046	4.185	.520
	Cubic	4.221	1	4.221	.085	.772	.085	.059
	Order 4	98.885	1	98.885	1.531	.221	1.531	.229
Day * Treatment	Linear	499.932	4	124.983	3.031	.025	12.122	.768
	Quadratic	928.700	4	232.175	3.678	.010	14.710	.852
	Cubic	639.610	4	159.903	3.215	.019	12.861	.795
	Order 4	307.971	4	76.993	1.192	.324	4.770	.349
Error(Day)	Linear	2268.258	55	41.241				
	Quadratic	3472.364	55	63.134				
	Cubic	2735.357	55	49.734				
	Order 4	3551.330	55	64.570				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: RootP
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	55375.327	1	55375.327	1235.355	.000	1235.355	1.000
Treatment	899.401	4	224.850	5.016	.002	20.065	.948
Error	2465.399	55	44.825				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
RP1, RP2, RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	50.000	1.000	.000	.050
RP1, RP2, RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.

	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP2, RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP2, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP2, RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RP1, RP2, RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP2, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP2, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP2, RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP2, RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP2, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP3, RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RP1, RP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1, RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.

	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP2, RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP2, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP2, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP3, RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP3, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP5, RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RP1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
RP1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	2724.453	55	49.536				
RP2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	3178.161	55	57.785				
RP3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	2934.886	55	53.362				
RP5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	2816.112	55	51.202				
RP7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	2839.096	55	51.620				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: RootP

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
13.586	.387	12.812	14.361

2. Treatment

Estimates

Measure: RootP

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	16.592	.864	14.859	18.324
5	14.216	.864	12.483	15.948
10	12.602	.864	10.870	14.334
15	12.996	.864	11.263	14.728
20	11.526	.864	9.794	13.258

Pairwise Comparisons

Measure: RootP

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Control	5	2.376	1.222	.570	-1.199	5.951
	10	3.989*	1.222	.019	.414	7.564
	15	3.596*	1.222	.048	.021	7.171
	20	5.066*	1.222	.001	1.491	8.641
5	Control	-2.376	1.222	.570	-5.951	1.199
	10	1.613	1.222	1.000	-1.962	5.188
	15	1.220	1.222	1.000	-2.355	4.795
	20	2.690	1.222	.320	-.885	6.265
10	Control	-3.989*	1.222	.019	-7.564	-.414
	5	-1.613	1.222	1.000	-5.188	1.962
	15	-.393	1.222	1.000	-3.968	3.182
	20	1.076	1.222	1.000	-2.499	4.651
15	Control	-3.596*	1.222	.048	-7.171	-.021
	5	-1.220	1.222	1.000	-4.795	2.355
	10	.393	1.222	1.000	-3.182	3.968
	20	1.470	1.222	1.000	-2.106	5.045
20	Control	-5.066*	1.222	.001	-8.641	-1.491
	5	-2.690	1.222	.320	-6.265	.885

10	-1.076	1.222	1.000	-4.651	2.499
15	-1.470	1.222	1.000	-5.045	2.106

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootP

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	179.880	4	44.970	5.016	.002	20.065	.948
Error	493.080	55	8.965				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

3. Day

Estimates

Measure: RootP

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	12.073	.909	10.252	13.894
2	14.852	.981	12.885	16.818
3	13.787	.943	11.897	15.677
4	14.670	.924	12.819	16.521
5	12.549	.928	10.690	14.408

Pairwise Comparisons

Measure: RootP

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	-2.778	1.362	.462	-6.762	1.206
	3	-1.714	1.465	1.000	-5.997	2.569
	4	-2.597	1.109	.228	-5.840	.646
	5	-.476	1.234	1.000	-4.085	3.133
2	1	2.778	1.362	.462	-1.206	6.762
	3	1.064	1.461	1.000	-3.208	5.337
	4	.181	1.229	1.000	-3.412	3.775
	5	2.302	1.342	.918	-1.623	6.227
3	1	1.714	1.465	1.000	-2.569	5.997
	2	-1.064	1.461	1.000	-5.337	3.208
	4	-.883	1.459	1.000	-5.151	3.385
	5	1.238	1.536	1.000	-3.253	5.729
4	1	2.597	1.109	.228	-.646	5.840
	2	-.181	1.229	1.000	-3.775	3.412
	3	.883	1.459	1.000	-3.385	5.151
	5	2.121	1.240	.929	-1.507	5.749
5	1	.476	1.234	1.000	-3.133	4.085

2	-2.302	1.342	.918	-6.227	1.623
3	-1.238	1.536	1.000	-5.729	3.253
4	-2.121	1.240	.929	-5.749	1.507

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.121	1.794[a]	4.000	52.000	.144	7.174	.510
Wilks' lambda	.879	1.794[a]	4.000	52.000	.144	7.174	.510
Hotelling's trace	.138	1.794[a]	4.000	52.000	.144	7.174	.510
Roy's largest root	.138	1.794[a]	4.000	52.000	.144	7.174	.510

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: RootP

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	9.886	2.032	5.814	13.958
	2	14.831	2.194	10.433	19.228
	3	21.445	2.109	17.219	25.671
	4	23.713	2.066	19.573	27.852
	5	13.084	2.074	8.927	17.240
5	1	12.943	2.032	8.871	17.014
	2	22.021	2.194	17.623	26.419
	3	12.281	2.109	8.055	16.507
	4	12.377	2.066	8.237	16.516
	5	11.457	2.074	7.300	15.613
10	1	12.910	2.032	8.838	16.981
	2	14.300	2.194	9.902	18.697
	3	11.175	2.109	6.949	15.401
	4	12.943	2.066	8.803	17.082
	5	11.684	2.074	7.527	15.840
15	1	13.336	2.032	9.265	17.408
	2	11.590	2.194	7.192	15.987
	3	13.860	2.109	9.634	18.086
	4	11.570	2.066	7.430	15.709
	5	14.621	2.074	10.465	18.778
20	1	11.291	2.032	7.219	15.362
	2	11.517	2.194	7.119	15.915
	3	10.175	2.109	5.949	14.401
	4	12.748	2.066	8.609	16.888
	5	11.900	2.074	7.743	16.056

Pairwise Comparisons

Measure: RootP

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	5	-3.057	2.873	1.000	-11.460	5.347
		10	-3.024	2.873	1.000	-11.427	5.380
		15	-3.451	2.873	1.000	-11.854	4.953
		20	-1.405	2.873	1.000	-9.808	6.999
	5	Control	3.057	2.873	1.000	-5.347	11.460
		10	.033	2.873	1.000	-8.371	8.437
		15	-.394	2.873	1.000	-8.797	8.010
		20	1.652	2.873	1.000	-6.751	10.056
	10	Control	3.024	2.873	1.000	-5.380	11.427
		5	-.033	2.873	1.000	-8.437	8.371
		15	-.427	2.873	1.000	-8.830	7.977
		20	1.619	2.873	1.000	-6.784	10.023
	15	Control	3.451	2.873	1.000	-4.953	11.854
		5	.394	2.873	1.000	-8.010	8.797
		10	.427	2.873	1.000	-7.977	8.830
		20	2.046	2.873	1.000	-6.358	10.449
	20	Control	1.405	2.873	1.000	-6.999	9.808
		5	-1.652	2.873	1.000	-10.056	6.751
		10	-1.619	2.873	1.000	-10.023	6.784
		15	-2.046	2.873	1.000	-10.449	6.358
2	Control	5	-7.190	3.103	.243	-16.267	1.886
		10	.531	3.103	1.000	-8.545	9.608
		15	3.241	3.103	1.000	-5.835	12.318
		20	3.314	3.103	1.000	-5.763	12.390
	5	Control	7.190	3.103	.243	-1.886	16.267
		10	7.722	3.103	.159	-1.355	16.798
		15	10.431*	3.103	.014	1.355	19.508
		20	10.504*	3.103	.013	1.428	19.581
	10	Control	-.531	3.103	1.000	-9.608	8.545
		5	-7.722	3.103	.159	-16.798	1.355
		15	2.710	3.103	1.000	-6.366	11.786
		20	2.783	3.103	1.000	-6.294	11.859
	15	Control	-3.241	3.103	1.000	-12.318	5.835
		5	-10.431*	3.103	.014	-19.508	-1.355
		10	-2.710	3.103	1.000	-11.786	6.366
		20	.073	3.103	1.000	-9.004	9.149
	20	Control	-3.314	3.103	1.000	-12.390	5.763
		5	-10.504*	3.103	.013	-19.581	-1.428
		10	-2.783	3.103	1.000	-11.859	6.294
		15	-.073	3.103	1.000	-9.149	9.004
3	Control	5	9.164*	2.982	.033	.442	17.886
		10	10.270*	2.982	.011	1.547	18.992
		15	7.585	2.982	.138	-1.137	16.307
		20	11.270*	2.982	.004	2.548	19.992
	5	Control	-9.164*	2.982	.033	-17.886	-.442
		10	1.105	2.982	1.000	-7.617	9.827
		15	-1.580	2.982	1.000	-10.302	7.142
		20	2.106	2.982	1.000	-6.616	10.828
	10	Control	-10.270*	2.982	.011	-18.992	-1.547
		5	-1.105	2.982	1.000	-9.827	7.617

		15	-2.685	2.982	1.000	-11.407	6.037
		20	1.001	2.982	1.000	-7.721	9.723
	15	Control	-7.585	2.982	.138	-16.307	1.137
		5	1.580	2.982	1.000	-7.142	10.302
		10	2.685	2.982	1.000	-6.037	11.407
		20	3.686	2.982	1.000	-5.036	12.408
	20	Control	-11.270*	2.982	.004	-19.992	-2.548
		5	-2.106	2.982	1.000	-10.828	6.616
		10	-1.001	2.982	1.000	-9.723	7.721
		15	-3.686	2.982	1.000	-12.408	5.036
4	Control	5	11.336*	2.921	.003	2.792	19.880
		10	10.770*	2.921	.005	2.226	19.314
		15	12.143*	2.921	.001	3.599	20.687
		20	10.965*	2.921	.004	2.421	19.508
	5	Control	-11.336*	2.921	.003	-19.880	-2.792
		10	-.566	2.921	1.000	-9.110	7.978
		15	.807	2.921	1.000	-7.737	9.351
		20	-.372	2.921	1.000	-8.915	8.172
	10	Control	-10.770*	2.921	.005	-19.314	-2.226
		5	.566	2.921	1.000	-7.978	9.110
		15	1.373	2.921	1.000	-7.171	9.917
		20	.194	2.921	1.000	-8.349	8.738
	15	Control	-12.143*	2.921	.001	-20.687	-3.599
		5	-.807	2.921	1.000	-9.351	7.737
		10	-1.373	2.921	1.000	-9.917	7.171
		20	-1.178	2.921	1.000	-9.722	7.365
	20	Control	-10.965*	2.921	.004	-19.508	-2.421
		5	.372	2.921	1.000	-8.172	8.915
		10	-.194	2.921	1.000	-8.738	8.349
		15	1.178	2.921	1.000	-7.365	9.722
5	Control	5	1.627	2.933	1.000	-6.952	10.205
		10	1.400	2.933	1.000	-7.179	9.978
		15	-1.538	2.933	1.000	-10.116	7.041
		20	1.184	2.933	1.000	-7.395	9.763
	5	Control	-1.627	2.933	1.000	-10.205	6.952
		10	-.227	2.933	1.000	-8.806	8.352
		15	-3.165	2.933	1.000	-11.743	5.414
		20	-.443	2.933	1.000	-9.021	8.136
	10	Control	-1.400	2.933	1.000	-9.978	7.179
		5	.227	2.933	1.000	-8.352	8.806
		15	-2.938	2.933	1.000	-11.516	5.641
		20	-.216	2.933	1.000	-8.794	8.363
	15	Control	1.538	2.933	1.000	-7.041	10.116
		5	3.165	2.933	1.000	-5.414	11.743
		10	2.938	2.933	1.000	-5.641	11.516
		20	2.722	2.933	1.000	-5.857	11.300
	20	Control	-1.184	2.933	1.000	-9.763	7.395
		5	.443	2.933	1.000	-8.136	9.021
		10	.216	2.933	1.000	-8.363	8.794
		15	-2.722	2.933	1.000	-11.300	5.857

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootP

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	101.382	4	25.346	.512	.727	2.047	.163
	Error	2724.453	55	49.536				
2	Contrast	881.611	4	220.403	3.814	.008	15.257	.866
	Error	3178.161	55	57.785				
3	Contrast	969.460	4	242.365	4.542	.003	18.168	.924
	Error	2934.886	55	53.362				
4	Contrast	1239.834	4	309.959	6.054	.000	24.215	.979
	Error	2816.112	55	51.202				
5	Contrast	83.327	4	20.832	.404	.805	1.614	.136
	Error	2839.096	55	51.620				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: RootP

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	9.886	2.032	5.814	13.958
	2	14.831	2.194	10.433	19.228
	3	21.445	2.109	17.219	25.671
	4	23.713	2.066	19.573	27.852
	5	13.084	2.074	8.927	17.240
5	1	12.943	2.032	8.871	17.014
	2	22.021	2.194	17.623	26.419
	3	12.281	2.109	8.055	16.507
	4	12.377	2.066	8.237	16.516
	5	11.457	2.074	7.300	15.613
10	1	12.910	2.032	8.838	16.981
	2	14.300	2.194	9.902	18.697
	3	11.175	2.109	6.949	15.401
	4	12.943	2.066	8.803	17.082
	5	11.684	2.074	7.527	15.840
15	1	13.336	2.032	9.265	17.408
	2	11.590	2.194	7.192	15.987
	3	13.860	2.109	9.634	18.086
	4	11.570	2.066	7.430	15.709
	5	14.621	2.074	10.465	18.778
20	1	11.291	2.032	7.219	15.362
	2	11.517	2.194	7.119	15.915
	3	10.175	2.109	5.949	14.401
	4	12.748	2.066	8.609	16.888
	5	11.900	2.074	7.743	16.056

Pairwise Comparisons

Measure: RootP

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	-4.945	3.046	1.000	-13.853	3.964
		3	-11.559*	3.275	.008	-21.137	-1.981
		4	-13.827*	2.479	.000	-21.078	-6.576
		5	-3.198	2.759	1.000	-11.268	4.873
	2	1	4.945	3.046	1.000	-3.964	13.853
		3	-6.614	3.266	.477	-16.168	2.939
		4	-8.882*	2.747	.021	-16.918	-.847
		5	1.747	3.001	1.000	-7.029	10.524
	3	1	11.559*	3.275	.008	1.981	21.137
		2	6.614	3.266	.477	-2.939	16.168
		4	-2.268	3.263	1.000	-11.812	7.276
		5	8.361	3.434	.182	-1.681	18.404
	4	1	13.827*	2.479	.000	6.576	21.078
		2	8.882*	2.747	.021	.847	16.918
		3	2.268	3.263	1.000	-7.276	11.812
		5	10.629*	2.774	.003	2.517	18.741
	5	1	3.198	2.759	1.000	-4.873	11.268
		2	-1.747	3.001	1.000	-10.524	7.029
		3	-8.361	3.434	.182	-18.404	1.681
		4	-10.629*	2.774	.003	-18.741	-2.517
5	1	2	-9.078*	3.046	.043	-17.987	-.170
		3	.662	3.275	1.000	-8.916	10.240
		4	.566	2.479	1.000	-6.685	7.817
		5	1.486	2.759	1.000	-6.584	9.556
	2	1	9.078*	3.046	.043	.170	17.987
		3	9.740*	3.266	.043	.187	19.294
		4	9.644*	2.747	.009	1.609	17.680
		5	10.564*	3.001	.009	1.788	19.341
	3	1	-.662	3.275	1.000	-10.240	8.916
		2	-9.740*	3.266	.043	-19.294	-.187
		4	-.096	3.263	1.000	-9.640	9.448
		5	.824	3.434	1.000	-9.218	10.866
	4	1	-.566	2.479	1.000	-7.817	6.685
		2	-9.644*	2.747	.009	-17.680	-1.609
		3	.096	3.263	1.000	-9.448	9.640
		5	.920	2.774	1.000	-7.192	9.032
	5	1	-1.486	2.759	1.000	-9.556	6.584
		2	-10.564*	3.001	.009	-19.341	-1.788
		3	-.824	3.434	1.000	-10.866	9.218
		4	-.920	2.774	1.000	-9.032	7.192
10	1	2	-1.390	3.046	1.000	-10.298	7.519
		3	1.734	3.275	1.000	-7.843	11.312
		4	-.033	2.479	1.000	-7.284	7.218
		5	1.226	2.759	1.000	-6.844	9.296
	2	1	1.390	3.046	1.000	-7.519	10.298

		3	3.124	3.266	1.000	-6.429	12.678
		4	1.357	2.747	1.000	-6.679	9.392
		5	2.616	3.001	1.000	-6.161	11.392
	3	1	-1.734	3.275	1.000	-11.312	7.843
		2	-3.124	3.266	1.000	-12.678	6.429
		4	-1.767	3.263	1.000	-11.311	7.776
		5	-.508	3.434	1.000	-10.550	9.534
	4	1	.033	2.479	1.000	-7.218	7.284
		2	-1.357	2.747	1.000	-9.392	6.679
		3	1.767	3.263	1.000	-7.776	11.311
		5	1.259	2.774	1.000	-6.853	9.371
	5	1	-1.226	2.759	1.000	-9.296	6.844
		2	-2.616	3.001	1.000	-11.392	6.161
		3	.508	3.434	1.000	-9.534	10.550
		4	-1.259	2.774	1.000	-9.371	6.853
15	1	2	1.747	3.046	1.000	-7.161	10.655
		3	-.524	3.275	1.000	-10.102	9.054
		4	1.767	2.479	1.000	-5.484	9.018
		5	-1.285	2.759	1.000	-9.355	6.785
	2	1	-1.747	3.046	1.000	-10.655	7.161
		3	-2.271	3.266	1.000	-11.824	7.283
		4	.020	2.747	1.000	-8.016	8.055
		5	-3.032	3.001	1.000	-11.808	5.745
	3	1	.524	3.275	1.000	-9.054	10.102
		2	2.271	3.266	1.000	-7.283	11.824
		4	2.291	3.263	1.000	-7.253	11.834
		5	-.761	3.434	1.000	-10.803	9.281
	4	1	-1.767	2.479	1.000	-9.018	5.484
		2	-.020	2.747	1.000	-8.055	8.016
		3	-2.291	3.263	1.000	-11.834	7.253
		5	-3.052	2.774	1.000	-11.163	5.060
	5	1	1.285	2.759	1.000	-6.785	9.355
		2	3.032	3.001	1.000	-5.745	11.808
		3	.761	3.434	1.000	-9.281	10.803
		4	3.052	2.774	1.000	-5.060	11.163
20	1	2	-.226	3.046	1.000	-9.135	8.682
		3	1.116	3.275	1.000	-8.462	10.694
		4	-1.458	2.479	1.000	-8.709	5.793
		5	-.609	2.759	1.000	-8.679	7.461
	2	1	.226	3.046	1.000	-8.682	9.135
		3	1.342	3.266	1.000	-8.211	10.896
		4	-1.231	2.747	1.000	-9.267	6.804
		5	-.383	3.001	1.000	-9.159	8.394
	3	1	-1.116	3.275	1.000	-10.694	8.462
		2	-1.342	3.266	1.000	-10.896	8.211
		4	-2.574	3.263	1.000	-12.117	6.970
		5	-1.725	3.434	1.000	-11.767	8.317
	4	1	1.458	2.479	1.000	-5.793	8.709
		2	1.231	2.747	1.000	-6.804	9.267
		3	2.574	3.263	1.000	-6.970	12.117
		5	.849	2.774	1.000	-7.263	8.960

	5	1	.609	2.759	1.000	-7.461	8.679
		2	.383	3.001	1.000	-8.394	9.159
		3	1.725	3.434	1.000	-8.317	11.767
		4	-.849	2.774	1.000	-8.960	7.263

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

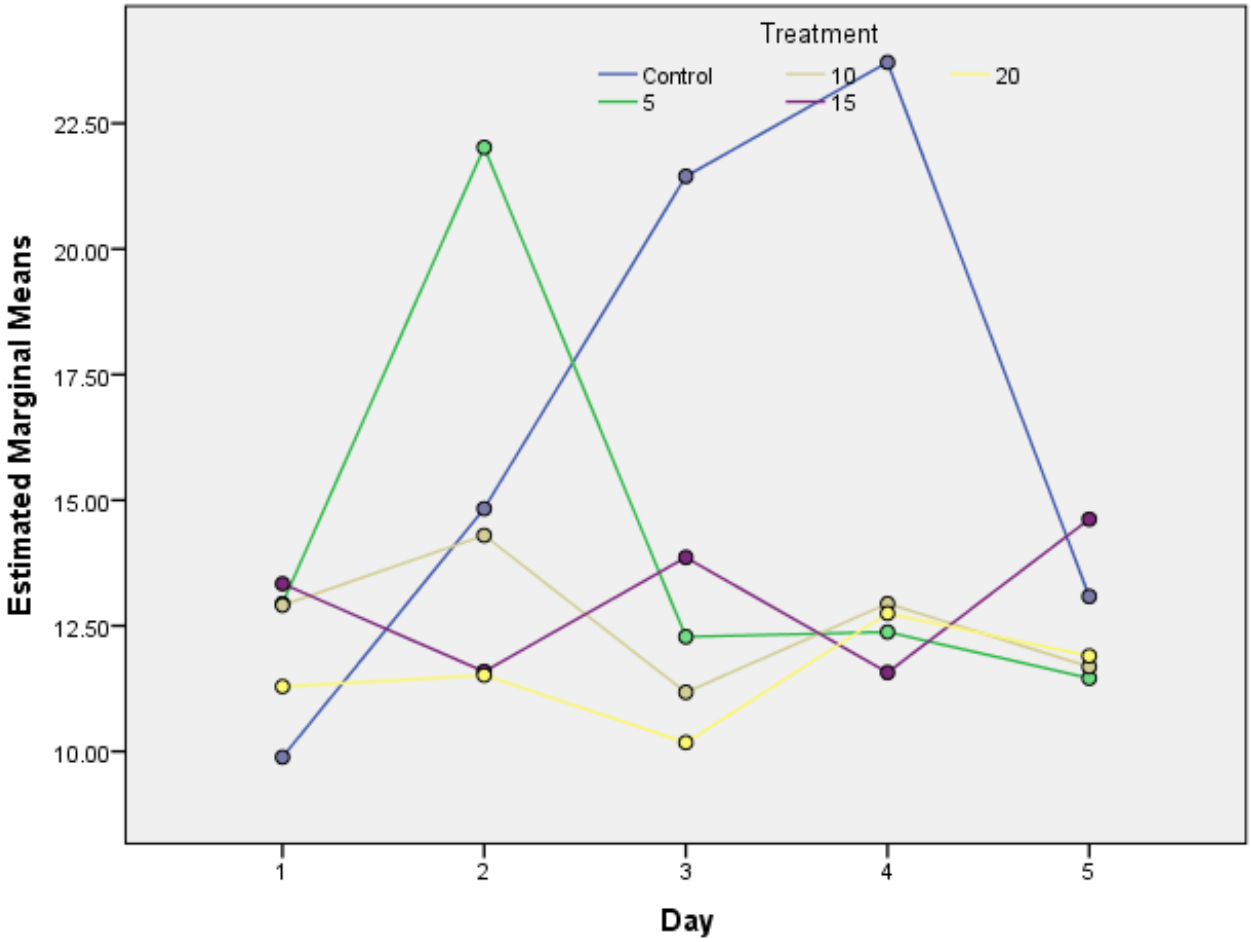
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.420	9.421[a]	4.000	52.000	.000	37.682	.999
	Wilks' lambda	.580	9.421[a]	4.000	52.000	.000	37.682	.999
	Hotelling's trace	.725	9.421[a]	4.000	52.000	.000	37.682	.999
	Roy's largest root	.725	9.421[a]	4.000	52.000	.000	37.682	.999
5	Pillai's trace	.243	4.166[a]	4.000	52.000	.005	16.663	.896
	Wilks' lambda	.757	4.166[a]	4.000	52.000	.005	16.663	.896
	Hotelling's trace	.320	4.166[a]	4.000	52.000	.005	16.663	.896
	Roy's largest root	.320	4.166[a]	4.000	52.000	.005	16.663	.896
10	Pillai's trace	.023	.302[a]	4.000	52.000	.875	1.209	.112
	Wilks' lambda	.977	.302[a]	4.000	52.000	.875	1.209	.112
	Hotelling's trace	.023	.302[a]	4.000	52.000	.875	1.209	.112
	Roy's largest root	.023	.302[a]	4.000	52.000	.875	1.209	.112
15	Pillai's trace	.031	.415[a]	4.000	52.000	.797	1.659	.139
	Wilks' lambda	.969	.415[a]	4.000	52.000	.797	1.659	.139
	Hotelling's trace	.032	.415[a]	4.000	52.000	.797	1.659	.139
	Roy's largest root	.032	.415[a]	4.000	52.000	.797	1.659	.139
20	Pillai's trace	.013	.175[a]	4.000	52.000	.950	.700	.084
	Wilks' lambda	.987	.175[a]	4.000	52.000	.950	.700	.084
	Hotelling's trace	.013	.175[a]	4.000	52.000	.950	.700	.084
	Roy's largest root	.013	.175[a]	4.000	52.000	.950	.700	.084

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots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Control
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20
5
15
Estimated Marginal Means
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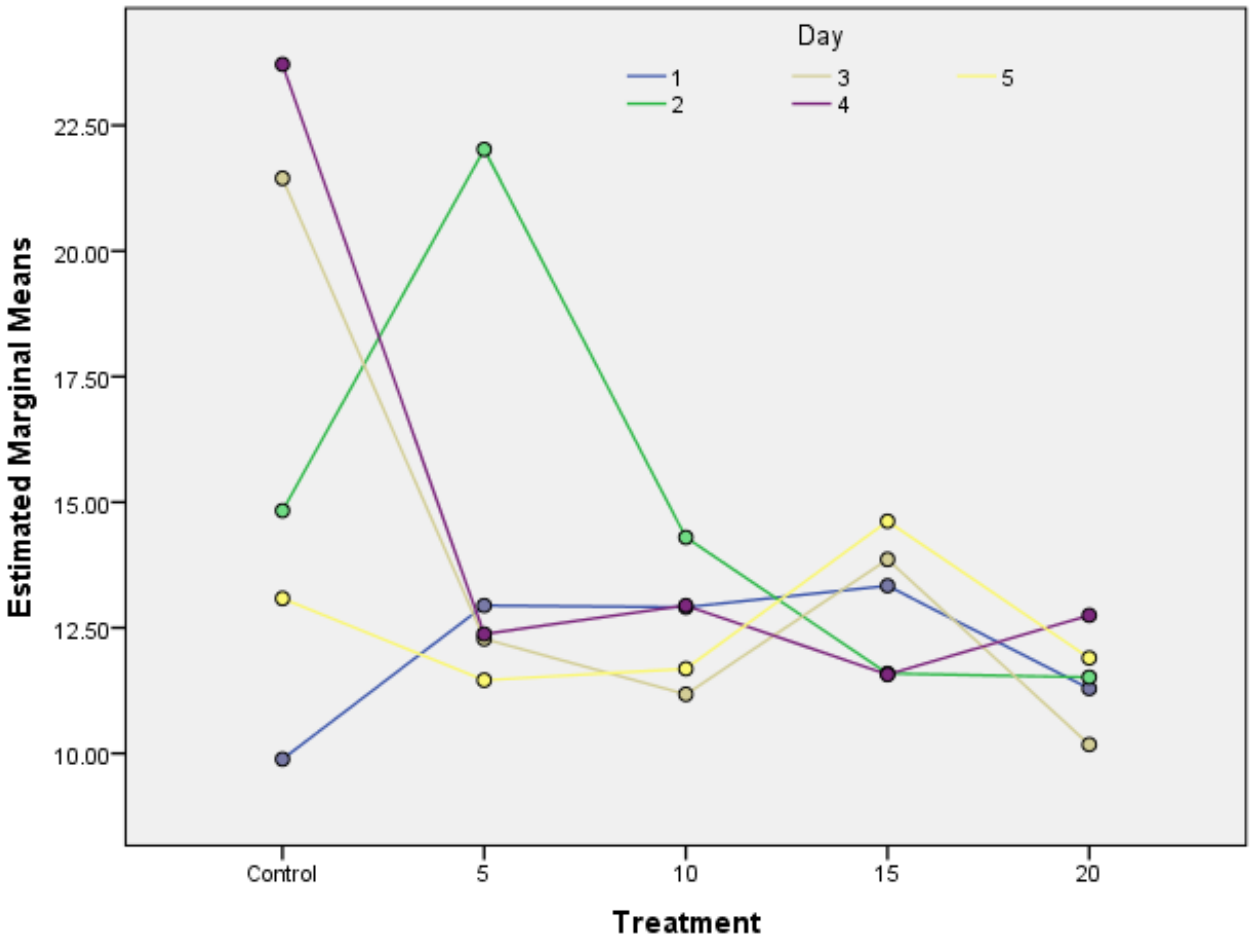
1
2
3
4
5
Day


Day
1
2
3
4
5
Estimated Marginal Means
22.50
20.00
17.50
15.00
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Control
5
10
15
20
Treatment

TITLE Iron.

Iron

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General Linear Model

Within-Subjects Factors

Measure: RootT

Day	Dependent Variable
1	RT1
2	RT2
3	RT3
4	RT5
5	RT7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	5	12
	2	10	12
	3	15	12
	4	20	12

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
RT1	Control	19.4707	6.96498	12
	5	19.6993	9.27224	12
	10	11.5444	7.15430	12
	15	13.6464	7.09818	12
	20	12.0055	6.97337	12
	Total	15.2733	8.13524	60
RT2	Control	21.1859	9.33606	12
	5	17.2613	10.06324	12
	10	12.1559	7.37110	12

	15	13.0975	6.23842	12
	20	12.1009	6.90745	12
	Total	15.1603	8.61975	60
RT3	Control	14.7112	7.76347	12
	5	20.3446	9.24451	12
	10	9.8859	6.38932	12
	15	13.9160	6.85095	12
	20	11.8833	7.06662	12
	Total	14.1482	8.09108	60
RT5	Control	9.8859	6.38932	12
	5	13.3373	6.34407	12
	10	15.5574	8.18353	12
	15	13.7730	7.41149	12
	20	11.8833	7.06662	12
	Total	12.8874	7.13024	60
RT7	Control	15.6315	8.79514	12
	5	13.4199	7.43006	12
	10	13.7666	6.86702	12
	15	11.2286	6.82032	12
	20	14.3101	7.21343	12
	Total	13.6714	7.34663	60

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.078	1.106[b]	4.000	52.000	.364	4.422	.323
	Wilks' Lambda	.922	1.106[b]	4.000	52.000	.364	4.422	.323
	Hotelling's Trace	.085	1.106[b]	4.000	52.000	.364	4.422	.323
	Roy's Largest Root	.085	1.106[b]	4.000	52.000	.364	4.422	.323
Day * Treatment	Pillai's Trace	.402	1.535	16.000	220.000	.089	24.560	.884
	Wilks' Lambda	.637	1.587	16.000	159.500	.078	19.053	.752
	Hotelling's Trace	.511	1.614	16.000	202.000	.068	25.819	.902
	Roy's Largest Root	.359	4.930[c]	4.000	55.000	.002	19.721	.944

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: RootT

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.892	6.082	9	.732	.945	1.000	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: RootT

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	244.518	4	61.129	1.123	.346	4.493	.350
	Greenhouse-Geisser	244.518	3.780	64.679	1.123	.346	4.247	.340

	Huynh-Feldt	244.518	4.000	61.129	1.123	.346	4.493	.350
	Lower-bound	244.518	1.000	244.518	1.123	.294	1.123	.181
Day * Treatment	Sphericity Assumed	1563.615	16	97.726	1.796	.033	28.734	.937
	Greenhouse-Geisser	1563.615	15.122	103.400	1.796	.036	27.157	.926
	Huynh-Feldt	1563.615	16.000	97.726	1.796	.033	28.734	.937
	Lower-bound	1563.615	4.000	390.904	1.796	.143	7.183	.512
Error(Day)	Sphericity Assumed	11971.912	220	54.418				
	Greenhouse-Geisser	11971.912	207.927	57.577				
	Huynh-Feldt	11971.912	220.000	54.418				
	Lower-bound	11971.912	55.000	217.671				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: RootT

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	179.969	1	179.969	3.262	.076	3.262	.427
	Quadratic	10.232	1	10.232	.194	.661	.194	.072
	Cubic	52.004	1	52.004	.830	.366	.830	.146
	Order 4	2.314	1	2.314	.049	.826	.049	.055
Day * Treatment	Linear	696.047	4	174.012	3.154	.021	12.615	.786
	Quadratic	142.316	4	35.579	.676	.611	2.705	.206
	Cubic	424.545	4	106.136	1.693	.165	6.772	.486
	Order 4	300.707	4	75.177	1.593	.189	6.372	.459
Error(Day)	Linear	3034.619	55	55.175				
	Quadratic	2893.832	55	52.615				
	Cubic	3448.025	55	62.691				
	Order 4	2595.436	55	47.190				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: RootT
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	60731.715	1	60731.715	892.265	.000	892.265	1.000
Treatment	1055.825	4	263.956	3.878	.008	15.512	.873
Error	3743.556	55	68.065				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
RT1, RT2, RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.000	.	.	.
	Hotelling's Trace	.000		.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	50.000	1.000	.000	.050
RT1, RT2, RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT2, RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT2, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT2, RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	53.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	51.000	1.000	.000	.050
RT1, RT2, RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT2, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT2, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT2, RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT2, RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT2, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT3, RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	52.000	1.000	.000	.050
RT1, RT2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1, RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT2, RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT2, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT2, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT3, RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT3, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT5, RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	53.000	1.000	.000	.050
RT1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050
RT7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	54.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
RT1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	3131.496	55	56.936				
RT2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	3623.340	55	65.879				
RT3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	3117.713	55	56.686				
RT5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	2781.989	55	50.582				
RT7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	3060.931	55	55.653				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: RootT

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
14.228	.476	13.274	15.183

2. Treatment

Estimates

Measure: RootT

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	16.177	1.065	14.043	18.312
5	16.812	1.065	14.678	18.947
10	12.582	1.065	10.448	14.717
15	13.132	1.065	10.998	15.267
20	12.437	1.065	10.302	14.571

Pairwise Comparisons

Measure: RootT

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	5	-.635	1.506	1.000	-5.041	3.770
	10	3.595	1.506	.205	-.810	8.000
	15	3.045	1.506	.481	-1.361	7.450
	20	3.740	1.506	.161	-.665	8.146
5	Control	.635	1.506	1.000	-3.770	5.041
	10	4.230	1.506	.069	-.175	8.636
	15	3.680	1.506	.178	-.725	8.086
	20	4.376	1.506	.053	-.030	8.781
10	Control	-3.595	1.506	.205	-8.000	.810
	5	-4.230	1.506	.069	-8.636	.175
	15	-.550	1.506	1.000	-4.956	3.855
	20	.145	1.506	1.000	-4.260	4.551
15	Control	-3.045	1.506	.481	-7.450	1.361
	5	-3.680	1.506	.178	-8.086	.725
	10	.550	1.506	1.000	-3.855	4.956
	20	.696	1.506	1.000	-3.710	5.101
20	Control	-3.740	1.506	.161	-8.146	.665
	5	-4.376	1.506	.053	-8.781	.030
	10	-.145	1.506	1.000	-4.551	4.260

15	-.696	1.506	1.000	-5.101	3.710

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootT

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	211.165	4	52.791	3.878	.008	15.512	.873
Error	748.711	55	13.613				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: RootT

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	15.273	.974	13.321	17.225
2	15.160	1.048	13.060	17.260
3	14.148	.972	12.200	16.096
4	12.887	.918	11.047	14.727
5	13.671	.963	11.741	15.601

Pairwise Comparisons

Measure: RootT

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	.113	1.356	1.000	-3.854	4.080
	3	1.125	1.208	1.000	-2.407	4.657
	4	2.386	1.392	.922	-1.686	6.458
	5	1.602	1.444	1.000	-2.621	5.825
2	1	-.113	1.356	1.000	-4.080	3.854
	3	1.012	1.284	1.000	-2.744	4.768
	4	2.273	1.358	.998	-1.699	6.245
	5	1.489	1.235	1.000	-2.123	5.101
3	1	-1.125	1.208	1.000	-4.657	2.407
	2	-1.012	1.284	1.000	-4.768	2.744
	4	1.261	1.268	1.000	-2.447	4.969
	5	.477	1.440	1.000	-3.735	4.689
4	1	-2.386	1.392	.922	-6.458	1.686
	2	-2.273	1.358	.998	-6.245	1.699
	3	-1.261	1.268	1.000	-4.969	2.447
	5	-.784	1.455	1.000	-5.040	3.472
5	1	-1.602	1.444	1.000	-5.825	2.621
	2	-1.489	1.235	1.000	-5.101	2.123
	3	-.477	1.440	1.000	-4.689	3.735

4	.784	1.455	1.000	-3.472	5.040	

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.078	1.106[a]	4.000	52.000	.364	4.422	.323
Wilks' lambda	.922	1.106[a]	4.000	52.000	.364	4.422	.323
Hotelling's trace	.085	1.106[a]	4.000	52.000	.364	4.422	.323
Roy's largest root	.085	1.106[a]	4.000	52.000	.364	4.422	.323

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: RootT

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	19.471	2.178	15.105	23.836
	2	21.186	2.343	16.490	25.882
	3	14.711	2.173	10.356	19.067
	4	9.886	2.053	5.771	14.000
	5	15.632	2.154	11.316	19.947
5	1	19.699	2.178	15.334	24.065
	2	17.261	2.343	12.566	21.957
	3	20.345	2.173	15.989	24.700
	4	13.337	2.053	9.223	17.452
	5	13.420	2.154	9.104	17.736
10	1	11.544	2.178	7.179	15.910
	2	12.156	2.343	7.460	16.852
	3	9.886	2.173	5.530	14.242
	4	15.557	2.053	11.443	19.672
	5	13.767	2.154	9.451	18.082
15	1	13.646	2.178	9.281	18.012
	2	13.098	2.343	8.402	17.793
	3	13.916	2.173	9.560	18.272
	4	13.773	2.053	9.659	17.887
	5	11.229	2.154	6.913	15.544
20	1	12.006	2.178	7.640	16.371
	2	12.101	2.343	7.405	16.796
	3	11.883	2.173	7.528	16.239
	4	11.883	2.053	7.769	15.998
	5	14.310	2.154	9.994	18.626

Pairwise Comparisons

Measure: RootT

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	5	-.229	3.080	1.000	-9.238	8.781
		10	7.926	3.080	.128	-1.083	16.936
		15	5.824	3.080	.639	-3.185	14.834
		20	7.465	3.080	.187	-1.544	16.475
	5	Control	.229	3.080	1.000	-8.781	9.238
		10	8.155	3.080	.106	-.855	17.164
		15	6.053	3.080	.545	-2.957	15.062
		20	7.694	3.080	.155	-1.316	16.703
	10	Control	-7.926	3.080	.128	-16.936	1.083
		5	-8.155	3.080	.106	-17.164	.855
		15	-2.102	3.080	1.000	-11.112	6.907
		20	-.461	3.080	1.000	-9.471	8.548
	15	Control	-5.824	3.080	.639	-14.834	3.185
		5	-6.053	3.080	.545	-15.062	2.957
		10	2.102	3.080	1.000	-6.907	11.112
		20	1.641	3.080	1.000	-7.369	10.650
	20	Control	-7.465	3.080	.187	-16.475	1.544
		5	-7.694	3.080	.155	-16.703	1.316
		10	.461	3.080	1.000	-8.548	9.471
		15	-1.641	3.080	1.000	-10.650	7.369
2	Control	5	3.925	3.314	1.000	-5.767	13.616
		10	9.030	3.314	.086	-.661	18.721
		15	8.088	3.314	.179	-1.603	17.780
		20	9.085	3.314	.082	-.606	18.776
	5	Control	-3.925	3.314	1.000	-13.616	5.767
		10	5.105	3.314	1.000	-4.586	14.797
		15	4.164	3.314	1.000	-5.527	13.855
		20	5.160	3.314	1.000	-4.531	14.852
	10	Control	-9.030	3.314	.086	-18.721	.661
		5	-5.105	3.314	1.000	-14.797	4.586
		15	-.942	3.314	1.000	-10.633	8.750
		20	.055	3.314	1.000	-9.636	9.746
	15	Control	-8.088	3.314	.179	-17.780	1.603
		5	-4.164	3.314	1.000	-13.855	5.527
		10	.942	3.314	1.000	-8.750	10.633
		20	.997	3.314	1.000	-8.695	10.688
	20	Control	-9.085	3.314	.082	-18.776	.606
		5	-5.160	3.314	1.000	-14.852	4.531
		10	-.055	3.314	1.000	-9.746	9.636
		15	-.997	3.314	1.000	-10.688	8.695
3	Control	5	-5.633	3.074	.723	-14.623	3.356
		10	4.825	3.074	1.000	-4.164	13.815
		15	.795	3.074	1.000	-8.194	9.785
		20	2.828	3.074	1.000	-6.162	11.818
	5	Control	5.633	3.074	.723	-3.356	14.623
		10	10.459*	3.074	.013	1.469	19.448
		15	6.429	3.074	.411	-2.561	15.418
		20	8.461	3.074	.080	-.528	17.451
	10	Control	-4.825	3.074	1.000	-13.815	4.164
		5	-10.459*	3.074	.013	-19.448	-1.469
		15	-4.030	3.074	1.000	-13.020	4.960
		20	-1.997	3.074	1.000	-10.987	6.992

	15	Control	-.795	3.074	1.000	-9.785	8.194
		5	-6.429	3.074	.411	-15.418	2.561
		10	4.030	3.074	1.000	-4.960	13.020
		20	2.033	3.074	1.000	-6.957	11.022
	20	Control	-2.828	3.074	1.000	-11.818	6.162
		5	-8.461	3.074	.080	-17.451	.528
		10	1.997	3.074	1.000	-6.992	10.987
		15	-2.033	3.074	1.000	-11.022	6.957
4	Control	5	-3.451	2.903	1.000	-11.943	5.040
		10	-5.671	2.903	.559	-14.163	2.820
		15	-3.887	2.903	1.000	-12.379	4.605
		20	-1.997	2.903	1.000	-10.489	6.494
	5	Control	3.451	2.903	1.000	-5.040	11.943
		10	-2.220	2.903	1.000	-10.712	6.272
		15	-.436	2.903	1.000	-8.928	8.056
		20	1.454	2.903	1.000	-7.038	9.946
	10	Control	5.671	2.903	.559	-2.820	14.163
		5	2.220	2.903	1.000	-6.272	10.712
		15	1.784	2.903	1.000	-6.707	10.276
		20	3.674	2.903	1.000	-4.818	12.166
	15	Control	3.887	2.903	1.000	-4.605	12.379
		5	.436	2.903	1.000	-8.056	8.928
		10	-1.784	2.903	1.000	-10.276	6.707
		20	1.890	2.903	1.000	-6.602	10.382
	20	Control	1.997	2.903	1.000	-6.494	10.489
		5	-1.454	2.903	1.000	-9.946	7.038
		10	-3.674	2.903	1.000	-12.166	4.818
		15	-1.890	2.903	1.000	-10.382	6.602
5	Control	5	2.212	3.046	1.000	-6.696	11.119
		10	1.865	3.046	1.000	-7.043	10.772
		15	4.403	3.046	1.000	-4.505	13.310
		20	1.321	3.046	1.000	-7.586	10.229
	5	Control	-2.212	3.046	1.000	-11.119	6.696
		10	-.347	3.046	1.000	-9.254	8.561
		15	2.191	3.046	1.000	-6.716	11.099
		20	-.890	3.046	1.000	-9.798	8.017
	10	Control	-1.865	3.046	1.000	-10.772	7.043
		5	.347	3.046	1.000	-8.561	9.254
		15	2.538	3.046	1.000	-6.369	11.445
		20	-.543	3.046	1.000	-9.451	8.364
	15	Control	-4.403	3.046	1.000	-13.310	4.505
		5	-2.191	3.046	1.000	-11.099	6.716
		10	-2.538	3.046	1.000	-11.445	6.369
		20	-3.081	3.046	1.000	-11.989	5.826
	20	Control	-1.321	3.046	1.000	-10.229	7.586
		5	.890	3.046	1.000	-8.017	9.798
		10	.543	3.046	1.000	-8.364	9.451
		15	3.081	3.046	1.000	-5.826	11.989

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: RootT

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	773.248	4	193.312	3.395	.015	13.581	.819
	Error	3131.496	55	56.936				
2	Contrast	760.367	4	190.092	2.885	.031	11.542	.744
	Error	3623.340	55	65.879				
3	Contrast	744.760	4	186.190	3.285	.017	13.138	.805
	Error	3117.713	55	56.686				
4	Contrast	217.590	4	54.398	1.075	.378	4.302	.316
	Error	2781.989	55	50.582				
5	Contrast	123.474	4	30.868	.555	.696	2.219	.174
	Error	3060.931	55	55.653				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: RootT

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	19.471	2.178	15.105	23.836
	2	21.186	2.343	16.490	25.882
	3	14.711	2.173	10.356	19.067
	4	9.886	2.053	5.771	14.000
	5	15.632	2.154	11.316	19.947
5	1	19.699	2.178	15.334	24.065
	2	17.261	2.343	12.566	21.957
	3	20.345	2.173	15.989	24.700
	4	13.337	2.053	9.223	17.452
	5	13.420	2.154	9.104	17.736
10	1	11.544	2.178	7.179	15.910
	2	12.156	2.343	7.460	16.852
	3	9.886	2.173	5.530	14.242
	4	15.557	2.053	11.443	19.672
	5	13.767	2.154	9.451	18.082
15	1	13.646	2.178	9.281	18.012
	2	13.098	2.343	8.402	17.793
	3	13.916	2.173	9.560	18.272
	4	13.773	2.053	9.659	17.887
	5	11.229	2.154	6.913	15.544
20	1	12.006	2.178	7.640	16.371
	2	12.101	2.343	7.405	16.796
	3	11.883	2.173	7.528	16.239
	4	11.883	2.053	7.769	15.998
	5	14.310	2.154	9.994	18.626

Pairwise Comparisons

Measure: RootT

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	-1.715	3.033	1.000	-10.585	7.155
		3	4.760	2.700	.835	-3.138	12.657
		4	9.585*	3.113	.032	.479	18.691
		5	3.839	3.229	1.000	-5.604	13.282
	2	1	1.715	3.033	1.000	-7.155	10.585
		3	6.475	2.872	.282	-1.924	14.874
		4	11.300*	3.036	.005	2.419	20.181
		5	5.554	2.761	.492	-2.522	13.631
	3	1	-4.760	2.700	.835	-12.657	3.138
		2	-6.475	2.872	.282	-14.874	1.924
		4	4.825	2.835	.944	-3.465	13.116
		5	-.920	3.221	1.000	-10.339	8.499
	4	1	-9.585*	3.113	.032	-18.691	-.479
		2	-11.300*	3.036	.005	-20.181	-2.419
		3	-4.825	2.835	.944	-13.116	3.465
		5	-5.746	3.254	.830	-15.263	3.772
	5	1	-3.839	3.229	1.000	-13.282	5.604
		2	-5.554	2.761	.492	-13.631	2.522
		3	.920	3.221	1.000	-8.499	10.339
		4	5.746	3.254	.830	-3.772	15.263
5	1	2	2.438	3.033	1.000	-6.432	11.308
		3	-.645	2.700	1.000	-8.543	7.252
		4	6.362	3.113	.458	-2.744	15.468
		5	6.279	3.229	.569	-3.163	15.722
	2	1	-2.438	3.033	1.000	-11.308	6.432
		3	-3.083	2.872	1.000	-11.482	5.316
		4	3.924	3.036	1.000	-4.957	12.805
		5	3.841	2.761	1.000	-4.235	11.918
	3	1	.645	2.700	1.000	-7.252	8.543
		2	3.083	2.872	1.000	-5.316	11.482
		4	7.007	2.835	.166	-1.283	15.298
		5	6.925	3.221	.360	-2.494	16.344
	4	1	-6.362	3.113	.458	-15.468	2.744
		2	-3.924	3.036	1.000	-12.805	4.957
		3	-7.007	2.835	.166	-15.298	1.283
		5	-.083	3.254	1.000	-9.600	9.435
	5	1	-6.279	3.229	.569	-15.722	3.163
		2	-3.841	2.761	1.000	-11.918	4.235
		3	-6.925	3.221	.360	-16.344	2.494
		4	.083	3.254	1.000	-9.435	9.600
10	1	2	-.612	3.033	1.000	-9.482	8.259
		3	1.658	2.700	1.000	-6.239	9.556
		4	-4.013	3.113	1.000	-13.119	5.093
		5	-2.222	3.229	1.000	-11.665	7.221
	2	1	.612	3.033	1.000	-8.259	9.482
		3	2.270	2.872	1.000	-6.129	10.669

		4	-3.401	3.036	1.000	-12.282	5.479
		5	-1.611	2.761	1.000	-9.687	6.466
	3	1	-1.658	2.700	1.000	-9.556	6.239
		2	-2.270	2.872	1.000	-10.669	6.129
		4	-5.671	2.835	.504	-13.962	2.619
		5	-3.881	3.221	1.000	-13.300	5.538
	4	1	4.013	3.113	1.000	-5.093	13.119
		2	3.401	3.036	1.000	-5.479	12.282
		3	5.671	2.835	.504	-2.619	13.962
		5	1.791	3.254	1.000	-7.727	11.308
	5	1	2.222	3.229	1.000	-7.221	11.665
		2	1.611	2.761	1.000	-6.466	9.687
		3	3.881	3.221	1.000	-5.538	13.300
		4	-1.791	3.254	1.000	-11.308	7.727
15	1	2	.549	3.033	1.000	-8.321	9.419
		3	-.270	2.700	1.000	-8.167	7.628
		4	-.127	3.113	1.000	-9.232	8.979
		5	2.418	3.229	1.000	-7.025	11.861
	2	1	-.549	3.033	1.000	-9.419	8.321
		3	-.818	2.872	1.000	-9.217	7.580
		4	-.675	3.036	1.000	-9.556	8.205
		5	1.869	2.761	1.000	-6.207	9.945
	3	1	.270	2.700	1.000	-7.628	8.167
		2	.818	2.872	1.000	-7.580	9.217
		4	.143	2.835	1.000	-8.148	8.434
		5	2.687	3.221	1.000	-6.732	12.106
	4	1	.127	3.113	1.000	-8.979	9.232
		2	.675	3.036	1.000	-8.205	9.556
		3	-.143	2.835	1.000	-8.434	8.148
		5	2.544	3.254	1.000	-6.973	12.062
	5	1	-2.418	3.229	1.000	-11.861	7.025
		2	-1.869	2.761	1.000	-9.945	6.207
		3	-2.687	3.221	1.000	-12.106	6.732
		4	-2.544	3.254	1.000	-12.062	6.973
20	1	2	-.095	3.033	1.000	-8.965	8.775
		3	.122	2.700	1.000	-7.775	8.020
		4	.122	3.113	1.000	-8.984	9.228
		5	-2.305	3.229	1.000	-11.747	7.138
	2	1	.095	3.033	1.000	-8.775	8.965
		3	.218	2.872	1.000	-8.181	8.616
		4	.218	3.036	1.000	-8.663	9.098
		5	-2.209	2.761	1.000	-10.285	5.867
	3	1	-.122	2.700	1.000	-8.020	7.775
		2	-.218	2.872	1.000	-8.616	8.181
		4	3.553E-15	2.835	1.000	-8.291	8.291
		5	-2.427	3.221	1.000	-11.846	6.992
	4	1	-.122	3.113	1.000	-9.228	8.984
		2	-.218	3.036	1.000	-9.098	8.663
		3	-3.553E-15	2.835	1.000	-8.291	8.291
		5	-2.427	3.254	1.000	-11.944	7.091

	5	1	2.305	3.229	1.000	-7.138	11.747
		2	2.209	2.761	1.000	-5.867	10.285
		3	2.427	3.221	1.000	-6.992	11.846
		4	2.427	3.254	1.000	-7.091	11.944

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

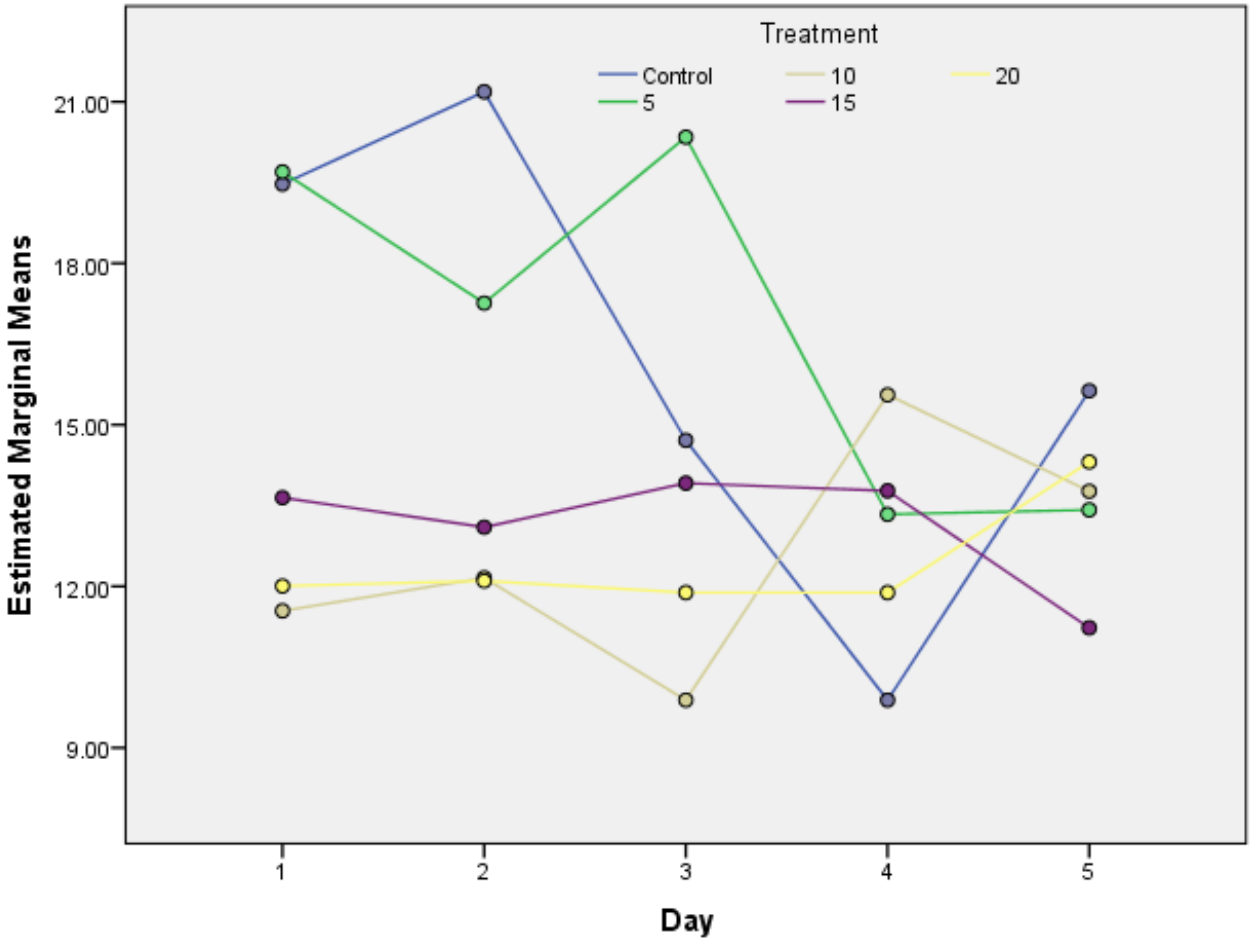
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.242	4.162[a]	4.000	52.000	.005	16.646	.895
	Wilks' lambda	.758	4.162[a]	4.000	52.000	.005	16.646	.895
	Hotelling's trace	.320	4.162[a]	4.000	52.000	.005	16.646	.895
	Roy's largest root	.320	4.162[a]	4.000	52.000	.005	16.646	.895
5	Pillai's trace	.143	2.162[a]	4.000	52.000	.086	8.649	.599
	Wilks' lambda	.857	2.162[a]	4.000	52.000	.086	8.649	.599
	Hotelling's trace	.166	2.162[a]	4.000	52.000	.086	8.649	.599
	Roy's largest root	.166	2.162[a]	4.000	52.000	.086	8.649	.599
10	Pillai's trace	.073	1.016[a]	4.000	52.000	.408	4.065	.298
	Wilks' lambda	.927	1.016[a]	4.000	52.000	.408	4.065	.298
	Hotelling's trace	.078	1.016[a]	4.000	52.000	.408	4.065	.298
	Roy's largest root	.078	1.016[a]	4.000	52.000	.408	4.065	.298
15	Pillai's trace	.016	.207[a]	4.000	52.000	.934	.827	.091
	Wilks' lambda	.984	.207[a]	4.000	52.000	.934	.827	.091
	Hotelling's trace	.016	.207[a]	4.000	52.000	.934	.827	.091
	Roy's largest root	.016	.207[a]	4.000	52.000	.934	.827	.091
20	Pillai's trace	.016	.205[a]	4.000	52.000	.934	.821	.091
	Wilks' lambda	.984	.205[a]	4.000	52.000	.934	.821	.091
	Hotelling's trace	.016	.205[a]	4.000	52.000	.934	.821	.091
	Roy's largest root	.016	.205[a]	4.000	52.000	.934	.821	.091

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots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5
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1
2
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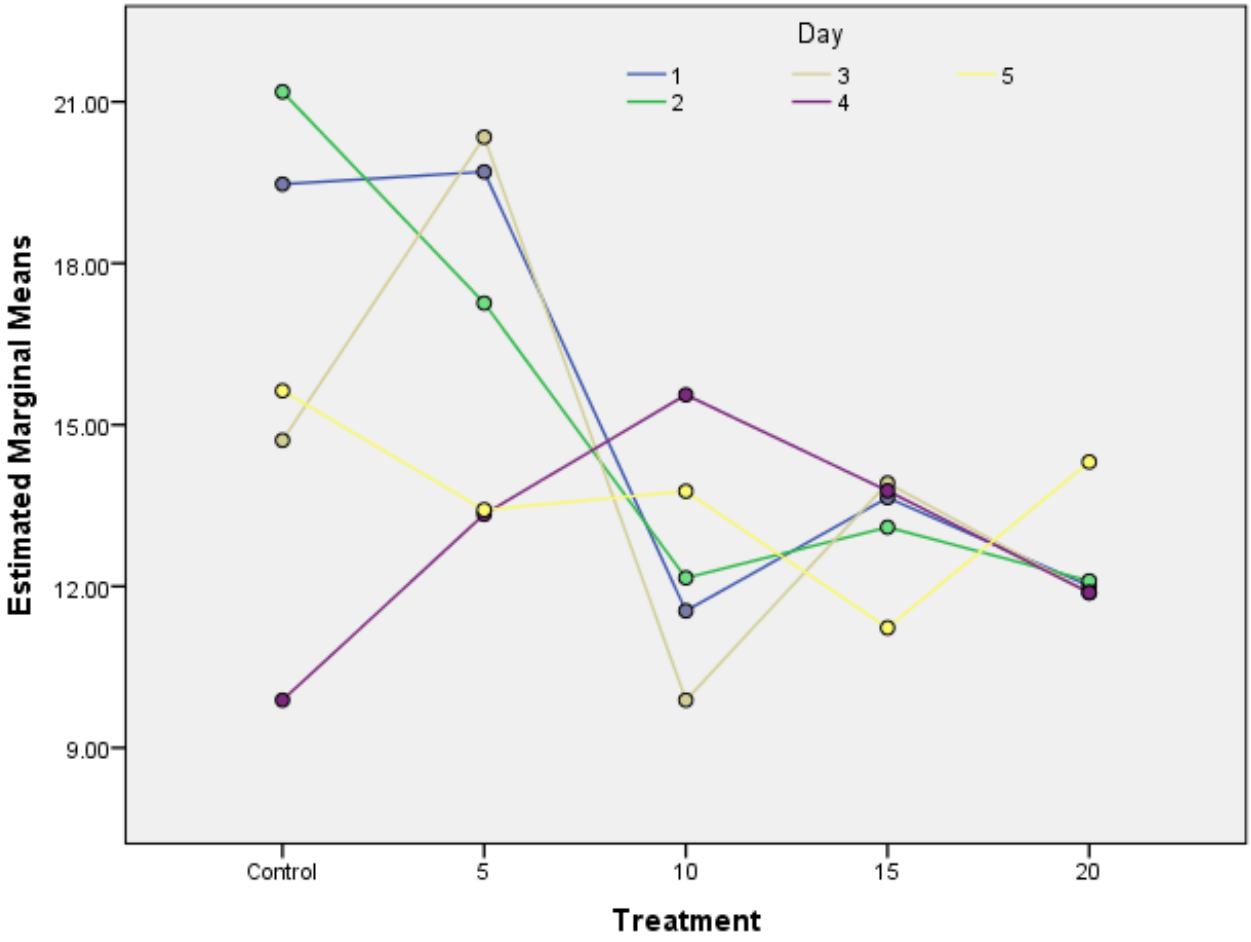
Day


Day
1
2
3
4
5
Estimated Marginal Means
21.00
18.00
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10
15
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Treatment

TITLE Iron.

Iron

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General Linear Model

Within-Subjects Factors

Measure: ShootA

Day	Dependent Variable
1	SA1
2	SA2
3	SA3
4	SA5
5	SA7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	5	12
	2	10	12
	3	15	12
	4	20	13

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
SA1	Control	3.4740	1.42628	12
	5	2.9091	1.71724	12
	10	2.9268	2.01064	12
	15	4.9116	2.90058	12
	20	4.7297	2.86348	13
	Total	3.8056	2.36726	61
SA2	Control	5.5024	3.16271	12
	5	3.8096	1.96004	12
	10	2.9444	2.34902	12

	15	4.8803	2.67827	12
	20	3.9395	2.60924	13
	Total	4.2107	2.64927	61
SA3	Control	4.9458	2.87187	12
	5	3.9602	2.24375	12
	10	2.7938	1.99786	12
	15	5.1404	2.71998	12
	20	1.9631	.67898	13
	Total	3.7312	2.48419	61
SA5	Control	3.0559	2.03139	12
	5	2.9268	2.01064	12
	10	2.9893	1.65034	12
	15	3.6185	3.29117	12
	20	3.1718	1.84117	13
	Total	3.1528	2.17295	61
SA7	Control	4.6846	1.76852	12
	5	3.1048	2.58077	12
	10	4.0866	2.50101	12
	15	2.1074	1.13769	12
	20	3.5093	2.85317	13
	Total	3.4987	2.36119	61

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.096	1.405[b]	4.000	53.000	.245	5.619	.407
	Wilks' Lambda	.904	1.405[b]	4.000	53.000	.245	5.619	.407
	Hotelling's Trace	.106	1.405[b]	4.000	53.000	.245	5.619	.407
	Roy's Largest Root	.106	1.405[b]	4.000	53.000	.245	5.619	.407
Day * Treatment	Pillai's Trace	.458	1.812	16.000	224.000	.031	28.998	.940
	Wilks' Lambda	.599	1.854	16.000	162.555	.028	22.204	.831
	Hotelling's Trace	.575	1.852	16.000	206.000	.027	29.634	.945
	Roy's Largest Root	.319	4.461[c]	4.000	56.000	.003	17.846	.919

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: ShootA

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.830	10.141	9	.339	.926	1.000	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: ShootA

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	37.556	4	9.389	1.657	.161	6.626	.505
	Greenhouse-Geisser	37.556	3.705	10.137	1.657	.166	6.138	.483

	Huynh-Feldt	37.556	4.000	9.389	1.657	.161	6.626	.505
	Lower-bound	37.556	1.000	37.556	1.657	.203	1.657	.244
Day * Treatment	Sphericity Assumed	171.981	16	10.749	1.896	.022	30.344	.952
	Greenhouse-Geisser	171.981	14.820	11.605	1.896	.026	28.106	.939
	Huynh-Feldt	171.981	16.000	10.749	1.896	.022	30.344	.952
	Lower-bound	171.981	4.000	42.995	1.896	.124	7.586	.538
Error(Day)	Sphericity Assumed	1269.590	224	5.668				
	Greenhouse-Geisser	1269.590	207.482	6.119				
	Huynh-Feldt	1269.590	224.000	5.668				
	Lower-bound	1269.590	56.000	22.671				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: ShootA

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	16.513	1	16.513	3.336	.073	3.336	.435
	Quadratic	.422	1	.422	.064	.802	.064	.057
	Cubic	20.494	1	20.494	3.110	.083	3.110	.410
	Order 4	.127	1	.127	.028	.867	.028	.053
Day * Treatment	Linear	59.972	4	14.993	3.029	.025	12.115	.768
	Quadratic	61.754	4	15.439	2.332	.067	9.329	.639
	Cubic	30.957	4	7.739	1.174	.332	4.697	.345
	Order 4	19.298	4	4.824	1.070	.380	4.278	.315
Error(Day)	Linear	277.218	56	4.950				
	Quadratic	370.689	56	6.619				
	Cubic	369.076	56	6.591				
	Order 4	252.608	56	4.511				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: ShootA
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	4133.890	1	4133.890	967.824	.000	967.824	1.000
Treatment	64.676	4	16.169	3.785	.009	15.142	.864
Error	239.194	56	4.271				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
SA1, SA2, SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	51.000	1.000	.000	.050
SA1, SA2, SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA2, SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA2, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA2, SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SA1, SA2, SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA2, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA2, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA2, SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA2, SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA2, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA3, SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SA1, SA2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1, SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA2, SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA2, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA2, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA3, SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA3, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA5, SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SA1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SA7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
SA1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	290.226	56	5.183				
SA2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	373.588	56	6.671				
SA3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	276.922	56	4.945				
SA5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	279.650	56	4.994				
SA7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	288.398	56	5.150				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: ShootA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
3.683	.118	3.446	3.921

2. Treatment

Estimates

Measure: ShootA

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	4.333	.267	3.798	4.867
5	3.342	.267	2.808	3.877
10	3.148	.267	2.614	3.683
15	4.132	.267	3.597	4.666
20	3.463	.256	2.949	3.976

Pairwise Comparisons

Measure: ShootA

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Control	5	.990	.377	.112	-.112	2.093
	10	1.184*	.377	.027	.082	2.287
	15	.201	.377	1.000	-.902	1.304
	20	.870	.370	.223	-.211	1.951
5	Control	-.990	.377	.112	-2.093	.112
	10	.194	.377	1.000	-.909	1.297
	15	-.790	.377	.409	-1.892	.313
	20	-.121	.370	1.000	-1.202	.961
10	Control	-1.184*	.377	.027	-2.287	-.082
	5	-.194	.377	1.000	-1.297	.909
	15	-.983	.377	.117	-2.086	.119
	20	-.314	.370	1.000	-1.396	.767
15	Control	-.201	.377	1.000	-1.304	.902
	5	.790	.377	.409	-.313	1.892
	10	.983	.377	.117	-.119	2.086
	20	.669	.370	.760	-.412	1.750
20	Control	-.870	.370	.223	-1.951	.211
	5	.121	.370	1.000	-.961	1.202
	10	.314	.370	1.000	-.767	1.396

15	-.669	.370	.760	-1.750	.412

Based on estimated marginal means
*. The mean difference is significant at the .05 level.
b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootA

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	12.935	4	3.234	3.785	.009	15.142	.864
Error	47.839	56	.854				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: ShootA

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	3.790	.292	3.206	4.374
2	4.215	.331	3.552	4.878
3	3.761	.285	3.190	4.331
4	3.152	.286	2.579	3.726
5	3.499	.291	2.916	4.081

Pairwise Comparisons

Measure: ShootA

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	-.425	.484	1.000	-1.839	.989
	3	.030	.451	1.000	-1.288	1.347
	4	.638	.426	1.000	-.607	1.882
	5	.292	.400	1.000	-.878	1.461
2	1	.425	.484	1.000	-.989	1.839
	3	.455	.423	1.000	-.780	1.689
	4	1.063	.468	.269	-.304	2.429
	5	.717	.409	.854	-.480	1.913
3	1	-.030	.451	1.000	-1.347	1.288
	2	-.455	.423	1.000	-1.689	.780
	4	.608	.415	1.000	-.604	1.820
	5	.262	.455	1.000	-1.066	1.591
4	1	-.638	.426	1.000	-1.882	.607
	2	-1.063	.468	.269	-2.429	.304
	3	-.608	.415	1.000	-1.820	.604
	5	-.346	.372	1.000	-1.433	.741
5	1	-.292	.400	1.000	-1.461	.878
	2	-.717	.409	.854	-1.913	.480
	3	-.262	.455	1.000	-1.591	1.066

4	.346	.372	1.000	-.741	1.433

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.096	1.405[a]	4.000	53.000	.245	5.619	.407
Wilks' lambda	.904	1.405[a]	4.000	53.000	.245	5.619	.407
Hotelling's trace	.106	1.405[a]	4.000	53.000	.245	5.619	.407
Roy's largest root	.106	1.405[a]	4.000	53.000	.245	5.619	.407

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: ShootA

				95% Confidence Interval	
Treatment	Day	Mean	Std. Error	Lower Bound	Upper Bound
Control	1	3.474	.657	2.157	4.790
	2	5.502	.746	4.009	6.996
	3	4.946	.642	3.660	6.232
	4	3.056	.645	1.764	4.348
	5	4.685	.655	3.372	5.997
5	1	2.909	.657	1.593	4.226
	2	3.810	.746	2.316	5.303
	3	3.960	.642	2.674	5.246
	4	2.927	.645	1.634	4.219
	5	3.105	.655	1.793	4.417
10	1	2.927	.657	1.610	4.243
	2	2.944	.746	1.451	4.438
	3	2.794	.642	1.508	4.080
	4	2.989	.645	1.697	4.282
	5	4.087	.655	2.774	5.399
15	1	4.912	.657	3.595	6.228
	2	4.880	.746	3.387	6.374
	3	5.140	.642	3.854	6.426
	4	3.619	.645	2.326	4.911
	5	2.107	.655	.795	3.420
20	1	4.730	.631	3.465	5.995
	2	3.939	.716	2.504	5.374
	3	1.963	.617	.728	3.199
	4	3.172	.620	1.930	4.413
	5	3.509	.629	2.248	4.770

Pairwise Comparisons

Measure: ShootA

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	5	.565	.929	1.000	-2.151	3.281
		10	.547	.929	1.000	-2.169	3.263
		15	-1.438	.929	1.000	-4.154	1.279
		20	-1.256	.911	1.000	-3.919	1.408
	5	Control	-.565	.929	1.000	-3.281	2.151
		10	-.018	.929	1.000	-2.734	2.699
		15	-2.002	.929	.355	-4.719	.714
		20	-1.821	.911	.506	-4.484	.843
	10	Control	-.547	.929	1.000	-3.263	2.169
		5	.018	.929	1.000	-2.699	2.734
		15	-1.985	.929	.371	-4.701	.731
		20	-1.803	.911	.528	-4.466	.861
	15	Control	1.438	.929	1.000	-1.279	4.154
		5	2.002	.929	.355	-.714	4.719
		10	1.985	.929	.371	-.731	4.701
		20	.182	.911	1.000	-2.482	2.845
	20	Control	1.256	.911	1.000	-1.408	3.919
		5	1.821	.911	.506	-.843	4.484
		10	1.803	.911	.528	-.861	4.466
		15	-.182	.911	1.000	-2.845	2.482
2	Control	5	1.693	1.054	1.000	-1.389	4.774
		10	2.558	1.054	.185	-.524	5.640
		15	.622	1.054	1.000	-2.460	3.704
		20	1.563	1.034	1.000	-1.459	4.585
	5	Control	-1.693	1.054	1.000	-4.774	1.389
		10	.865	1.054	1.000	-2.216	3.947
		15	-1.071	1.054	1.000	-4.152	2.011
		20	-.130	1.034	1.000	-3.152	2.892
	10	Control	-2.558	1.054	.185	-5.640	.524
		5	-.865	1.054	1.000	-3.947	2.216
		15	-1.936	1.054	.717	-5.018	1.146
		20	-.995	1.034	1.000	-4.017	2.027
	15	Control	-.622	1.054	1.000	-3.704	2.460
		5	1.071	1.054	1.000	-2.011	4.152
		10	1.936	1.054	.717	-1.146	5.018
		20	.941	1.034	1.000	-2.081	3.963
	20	Control	-1.563	1.034	1.000	-4.585	1.459
		5	.130	1.034	1.000	-2.892	3.152
		10	.995	1.034	1.000	-2.027	4.017
		15	-.941	1.034	1.000	-3.963	2.081
3	Control	5	.986	.908	1.000	-1.668	3.639
		10	2.152	.908	.212	-.501	4.805
		15	-.195	.908	1.000	-2.848	2.459
		20	2.983*	.890	.014	.381	5.584
	5	Control	-.986	.908	1.000	-3.639	1.668
		10	1.166	.908	1.000	-1.487	3.820
		15	-1.180	.908	1.000	-3.833	1.473
		20	1.997	.890	.288	-.605	4.599
	10	Control	-2.152	.908	.212	-4.805	.501
		5	-1.166	.908	1.000	-3.820	1.487
		15	-2.347	.908	.124	-5.000	.307
		20	.831	.890	1.000	-1.771	3.432

	15	Control	.195	.908	1.000	-2.459	2.848
		5	1.180	.908	1.000	-1.473	3.833
		10	2.347	.908	.124	-.307	5.000
		20	3.177*	.890	.007	.576	5.779
	20	Control	-2.983*	.890	.014	-5.584	-.381
		5	-1.997	.890	.288	-4.599	.605
		10	-.831	.890	1.000	-3.432	1.771
		15	-3.177*	.890	.007	-5.779	-.576
4	Control	5	.129	.912	1.000	-2.537	2.795
		10	.067	.912	1.000	-2.600	2.733
		15	-.563	.912	1.000	-3.229	2.104
		20	-.116	.895	1.000	-2.730	2.499
	5	Control	-.129	.912	1.000	-2.795	2.537
		10	-.063	.912	1.000	-2.729	2.604
		15	-.692	.912	1.000	-3.358	1.974
		20	-.245	.895	1.000	-2.859	2.369
	10	Control	-.067	.912	1.000	-2.733	2.600
		5	.063	.912	1.000	-2.604	2.729
		15	-.629	.912	1.000	-3.295	2.037
		20	-.182	.895	1.000	-2.797	2.432
	15	Control	.563	.912	1.000	-2.104	3.229
		5	.692	.912	1.000	-1.974	3.358
		10	.629	.912	1.000	-2.037	3.295
		20	.447	.895	1.000	-2.168	3.061
	20	Control	.116	.895	1.000	-2.499	2.730
		5	.245	.895	1.000	-2.369	2.859
		10	.182	.895	1.000	-2.432	2.797
		15	-.447	.895	1.000	-3.061	2.168
5	Control	5	1.580	.926	.937	-1.128	4.287
		10	.598	.926	1.000	-2.110	3.306
		15	2.577	.926	.074	-.130	5.285
		20	1.175	.908	1.000	-1.480	3.830
	5	Control	-1.580	.926	.937	-4.287	1.128
		10	-.982	.926	1.000	-3.689	1.726
		15	.997	.926	1.000	-1.710	3.705
		20	-.404	.908	1.000	-3.059	2.251
	10	Control	-.598	.926	1.000	-3.306	2.110
		5	.982	.926	1.000	-1.726	3.689
		15	1.979	.926	.370	-.728	4.687
		20	.577	.908	1.000	-2.078	3.232
	15	Control	-2.577	.926	.074	-5.285	.130
		5	-.997	.926	1.000	-3.705	1.710
		10	-1.979	.926	.370	-4.687	.728
		20	-1.402	.908	1.000	-4.057	1.253
	20	Control	-1.175	.908	1.000	-3.830	1.480
		5	.404	.908	1.000	-2.251	3.059
		10	-.577	.908	1.000	-3.232	2.078
		15	1.402	.908	1.000	-1.253	4.057

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootA

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	46.011	4	11.503	2.219	.078	8.878	.615
	Error	290.226	56	5.183				
2	Contrast	47.530	4	11.882	1.781	.145	7.125	.509
	Error	373.588	56	6.671				
3	Contrast	93.348	4	23.337	4.719	.002	18.877	.934
	Error	276.922	56	4.945				
4	Contrast	3.654	4	.913	.183	.946	.732	.086
	Error	279.650	56	4.994				
5	Contrast	46.115	4	11.529	2.239	.076	8.954	.619
	Error	288.398	56	5.150				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: ShootA

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	3.474	.657	2.157	4.790
	2	5.502	.746	4.009	6.996
	3	4.946	.642	3.660	6.232
	4	3.056	.645	1.764	4.348
	5	4.685	.655	3.372	5.997
5	1	2.909	.657	1.593	4.226
	2	3.810	.746	2.316	5.303
	3	3.960	.642	2.674	5.246
	4	2.927	.645	1.634	4.219
	5	3.105	.655	1.793	4.417
10	1	2.927	.657	1.610	4.243
	2	2.944	.746	1.451	4.438
	3	2.794	.642	1.508	4.080
	4	2.989	.645	1.697	4.282
	5	4.087	.655	2.774	5.399
15	1	4.912	.657	3.595	6.228
	2	4.880	.746	3.387	6.374
	3	5.140	.642	3.854	6.426
	4	3.619	.645	2.326	4.911
	5	2.107	.655	.795	3.420
20	1	4.730	.631	3.465	5.995
	2	3.939	.716	2.504	5.374
	3	1.963	.617	.728	3.199
	4	3.172	.620	1.930	4.413
	5	3.509	.629	2.248	4.770

Pairwise Comparisons

Measure: ShootA

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	-2.028	1.090	.681	-5.215	1.158
		3	-1.472	1.016	1.000	-4.440	1.496
		4	.418	.959	1.000	-2.386	3.222
		5	-1.211	.902	1.000	-3.847	1.425
	2	1	2.028	1.090	.681	-1.158	5.215
		3	.557	.952	1.000	-2.226	3.339
		4	2.446	1.054	.239	-.633	5.526
		5	.818	.922	1.000	-1.878	3.513
	3	1	1.472	1.016	1.000	-1.496	4.440
		2	-.557	.952	1.000	-3.339	2.226
		4	1.890	.935	.479	-.841	4.621
		5	.261	1.024	1.000	-2.733	3.255
	4	1	-.418	.959	1.000	-3.222	2.386
		2	-2.446	1.054	.239	-5.526	.633
		3	-1.890	.935	.479	-4.621	.841
		5	-1.629	.838	.570	-4.078	.820
	5	1	1.211	.902	1.000	-1.425	3.847
		2	-.818	.922	1.000	-3.513	1.878
		3	-.261	1.024	1.000	-3.255	2.733
		4	1.629	.838	.570	-.820	4.078
5	1	2	-.901	1.090	1.000	-4.087	2.286
		3	-1.051	1.016	1.000	-4.019	1.917
		4	-.018	.959	1.000	-2.822	2.786
		5	-.196	.902	1.000	-2.832	2.440
	2	1	.901	1.090	1.000	-2.286	4.087
		3	-.151	.952	1.000	-2.933	2.632
		4	.883	1.054	1.000	-2.196	3.962
		5	.705	.922	1.000	-1.991	3.400
	3	1	1.051	1.016	1.000	-1.917	4.019
		2	.151	.952	1.000	-2.632	2.933
		4	1.033	.935	1.000	-1.698	3.765
		5	.855	1.024	1.000	-2.138	3.849
	4	1	.018	.959	1.000	-2.786	2.822
		2	-.883	1.054	1.000	-3.962	2.196
		3	-1.033	.935	1.000	-3.765	1.698
		5	-.178	.838	1.000	-2.627	2.271
	5	1	.196	.902	1.000	-2.440	2.832
		2	-.705	.922	1.000	-3.400	1.991
		3	-.855	1.024	1.000	-3.849	2.138
		4	.178	.838	1.000	-2.271	2.627
10	1	2	-.018	1.090	1.000	-3.204	3.168
		3	.133	1.016	1.000	-2.835	3.101
		4	-.063	.959	1.000	-2.867	2.742
		5	-1.160	.902	1.000	-3.796	1.476
	2	1	.018	1.090	1.000	-3.168	3.204
		3	.151	.952	1.000	-2.632	2.933

		4	-.045	1.054	1.000	-3.124	3.034
		5	-1.142	.922	1.000	-3.838	1.553
	3	1	-.133	1.016	1.000	-3.101	2.835
		2	-.151	.952	1.000	-2.933	2.632
		4	-.196	.935	1.000	-2.927	2.536
		5	-1.293	1.024	1.000	-4.287	1.701
	4	1	.063	.959	1.000	-2.742	2.867
		2	.045	1.054	1.000	-3.034	3.124
		3	.196	.935	1.000	-2.536	2.927
		5	-1.097	.838	1.000	-3.546	1.352
	5	1	1.160	.902	1.000	-1.476	3.796
		2	1.142	.922	1.000	-1.553	3.838
		3	1.293	1.024	1.000	-1.701	4.287
		4	1.097	.838	1.000	-1.352	3.546
15	1	2	.031	1.090	1.000	-3.155	3.217
		3	-.229	1.016	1.000	-3.197	2.739
		4	1.293	.959	1.000	-1.511	4.097
		5	2.804*	.902	.029	.168	5.440
	2	1	-.031	1.090	1.000	-3.217	3.155
		3	-.260	.952	1.000	-3.043	2.523
		4	1.262	1.054	1.000	-1.817	4.341
		5	2.773*	.922	.040	.077	5.468
	3	1	.229	1.016	1.000	-2.739	3.197
		2	.260	.952	1.000	-2.523	3.043
		4	1.522	.935	1.000	-1.209	4.253
		5	3.033*	1.024	.045	.039	6.027
	4	1	-1.293	.959	1.000	-4.097	1.511
		2	-1.262	1.054	1.000	-4.341	1.817
		3	-1.522	.935	1.000	-4.253	1.209
		5	1.511	.838	.767	-.938	3.960
	5	1	-2.804*	.902	.029	-5.440	-.168
		2	-2.773*	.922	.040	-5.468	-.077
		3	-3.033*	1.024	.045	-6.027	-.039
		4	-1.511	.838	.767	-3.960	.938
20	1	2	.790	1.047	1.000	-2.271	3.851
		3	2.767	.976	.064	-.085	5.618
		4	1.558	.922	.966	-1.136	4.252
		5	1.220	.867	1.000	-1.312	3.753
	2	1	-.790	1.047	1.000	-3.851	2.271
		3	1.976	.915	.350	-.697	4.650
		4	.768	1.012	1.000	-2.191	3.726
		5	.430	.886	1.000	-2.160	3.020
	3	1	-2.767	.976	.064	-5.618	.085
		2	-1.976	.915	.350	-4.650	.697
		4	-1.209	.898	1.000	-3.833	1.416
		5	-1.546	.984	1.000	-4.423	1.330
	4	1	-1.558	.922	.966	-4.252	1.136
		2	-.768	1.012	1.000	-3.726	2.191
		3	1.209	.898	1.000	-1.416	3.833
		5	-.338	.805	1.000	-2.690	2.015

	5	1	-1.220	.867	1.000	-3.753	1.312
		2	-.430	.886	1.000	-3.020	2.160
		3	1.546	.984	1.000	-1.330	4.423
		4	.338	.805	1.000	-2.015	2.690

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

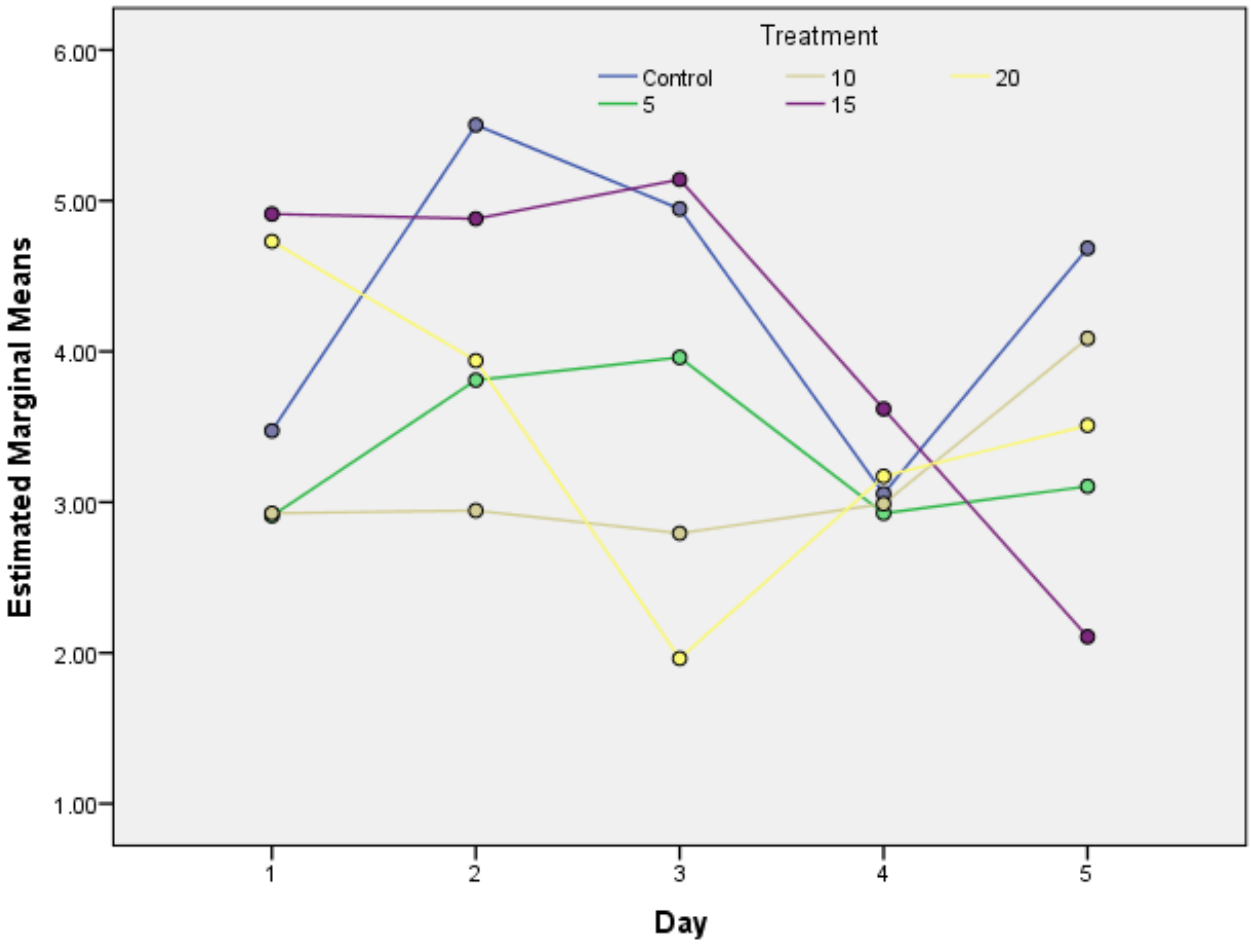
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.128	1.947[a]	4.000	53.000	.116	7.788	.549
	Wilks' lambda	.872	1.947[a]	4.000	53.000	.116	7.788	.549
	Hotelling's trace	.147	1.947[a]	4.000	53.000	.116	7.788	.549
	Roy's largest root	.147	1.947[a]	4.000	53.000	.116	7.788	.549
5	Pillai's trace	.030	.404[a]	4.000	53.000	.805	1.614	.136
	Wilks' lambda	.970	.404[a]	4.000	53.000	.805	1.614	.136
	Hotelling's trace	.030	.404[a]	4.000	53.000	.805	1.614	.136
	Roy's largest root	.030	.404[a]	4.000	53.000	.805	1.614	.136
10	Pillai's trace	.051	.710[a]	4.000	53.000	.589	2.840	.215
	Wilks' lambda	.949	.710[a]	4.000	53.000	.589	2.840	.215
	Hotelling's trace	.054	.710[a]	4.000	53.000	.589	2.840	.215
	Roy's largest root	.054	.710[a]	4.000	53.000	.589	2.840	.215
15	Pillai's trace	.214	3.603[a]	4.000	53.000	.011	14.412	.843
	Wilks' lambda	.786	3.603[a]	4.000	53.000	.011	14.412	.843
	Hotelling's trace	.272	3.603[a]	4.000	53.000	.011	14.412	.843
	Roy's largest root	.272	3.603[a]	4.000	53.000	.011	14.412	.843
20	Pillai's trace	.152	2.370[a]	4.000	53.000	.064	9.481	.645
	Wilks' lambda	.848	2.370[a]	4.000	53.000	.064	9.481	.645
	Hotelling's trace	.179	2.370[a]	4.000	53.000	.064	9.481	.645
	Roy's largest root	.179	2.370[a]	4.000	53.000	.064	9.481	.645

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots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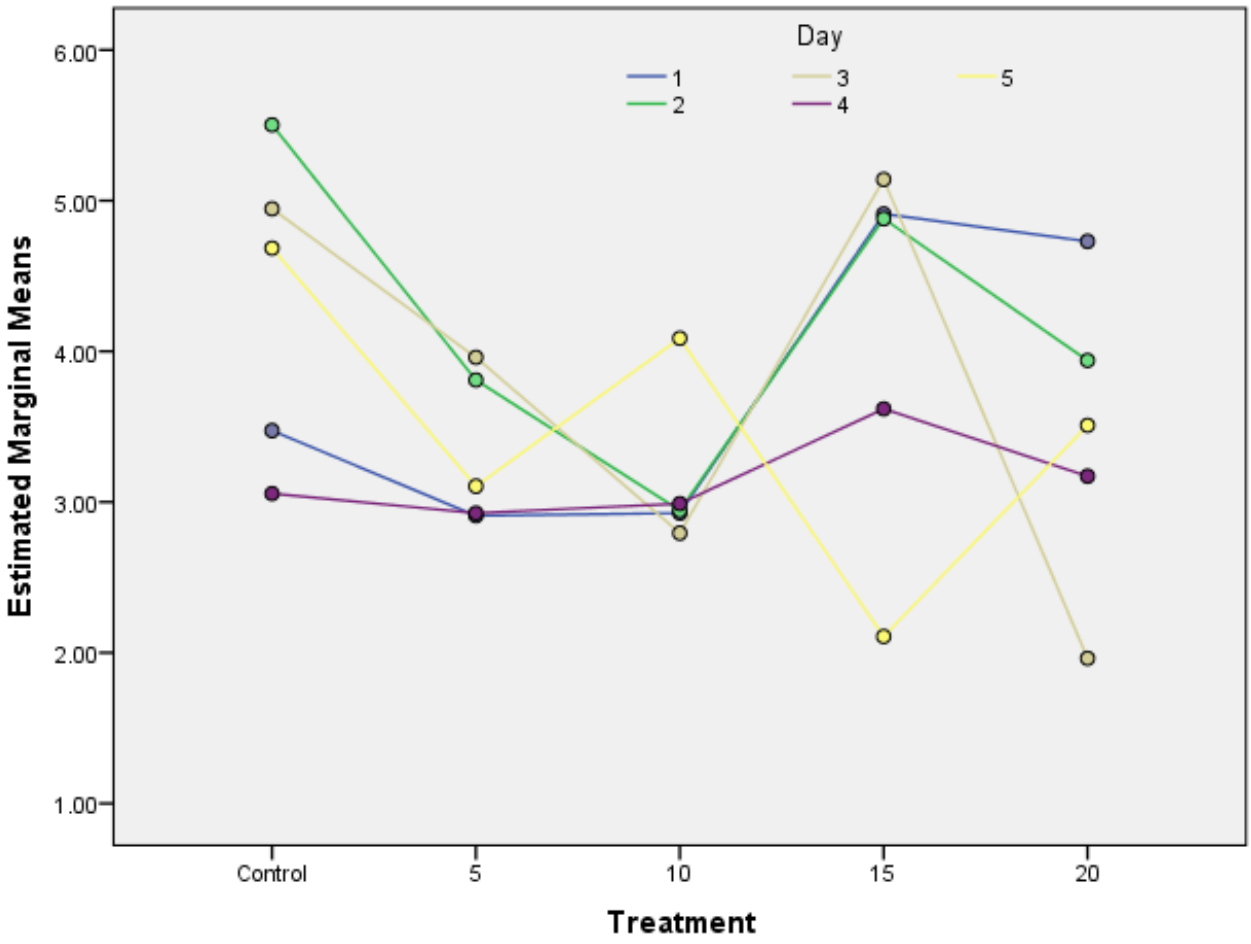
Day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Treatment

TITLE Iron.

Iron

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General Linear Model

Within-Subjects Factors

Measure: ShootP

Day	Dependent Variable
1	SP1
2	SP2
3	SP3
4	SP5
5	SP7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	5	12
	2	10	12
	3	15	12
	4	20	13

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
SP1	Control	3.1347	1.69148	12
	5	2.8868	2.34499	12
	10	4.5601	2.58465	12
	15	2.6760	1.33950	12
	20	2.7141	1.89133	13
	Total	3.1865	2.07320	61
SP2	Control	5.0199	2.74593	12
	5	3.3133	1.59339	12
	10	2.8765	1.90459	12

	15	2.2748	1.12016	12
	20	5.3128	2.77329	13
	Total	3.7849	2.39950	61
SP3	Control	4.1968	2.70409	12
	5	3.1048	2.58077	12
	10	4.4566	2.59046	12
	15	2.2286	1.07333	12
	20	4.4976	2.75958	13
	Total	3.7100	2.51626	61
SP5	Control	2.6501	1.70152	12
	5	3.3574	1.47966	12
	10	1.8329	.74146	12
	15	4.8315	2.96612	12
	20	5.2970	2.78997	13
	Total	3.6217	2.43849	61
SP7	Control	2.8737	1.67932	12
	5	3.9602	2.24375	12
	10	2.7938	1.99786	12
	15	5.1404	2.71998	12
	20	2.3317	1.66307	13
	Total	3.4021	2.26408	61

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.053	.742[b]	4.000	53.000	.567	2.970	.223
	Wilks' Lambda	.947	.742[b]	4.000	53.000	.567	2.970	.223
	Hotelling's Trace	.056	.742[b]	4.000	53.000	.567	2.970	.223
	Roy's Largest Root	.056	.742[b]	4.000	53.000	.567	2.970	.223
Day * Treatment	Pillai's Trace	.743	3.193	16.000	224.000	.000	51.086	.999
	Wilks' Lambda	.406	3.487	16.000	162.555	.000	41.094	.991
	Hotelling's Trace	1.113	3.584	16.000	206.000	.000	57.342	1.000
	Roy's Largest Root	.616	8.630[c]	4.000	56.000	.000	34.521	.998

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: ShootP

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.862	8.063	9	.528	.933	1.000	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: ShootP

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	12.755	4	3.189	.679	.607	2.717	.219
	Greenhouse-Geisser	12.755	3.731	3.419	.679	.597	2.534	.212

	Huynh-Feldt	12.755	4.000	3.189	.679	.607	2.717	.219
	Lower-bound	12.755	1.000	12.755	.679	.413	.679	.128
Day * Treatment	Sphericity Assumed	311.424	16	19.464	4.145	.000	66.327	1.000
	Greenhouse-Geisser	311.424	14.923	20.869	4.145	.000	61.863	1.000
	Huynh-Feldt	311.424	16.000	19.464	4.145	.000	66.327	1.000
	Lower-bound	311.424	4.000	77.856	4.145	.005	16.582	.896
Error(Day)	Sphericity Assumed	1051.736	224	4.695				
	Greenhouse-Geisser	1051.736	208.924	5.034				
	Huynh-Feldt	1051.736	224.000	4.695				
	Lower-bound	1051.736	56.000	18.781				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: ShootP

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	.497	1	.497	.117	.734	.117	.063
	Quadratic	10.036	1	10.036	1.900	.174	1.900	.273
	Cubic	1.891	1	1.891	.416	.521	.416	.097
	Order 4	.332	1	.332	.070	.792	.070	.058
Day * Treatment	Linear	108.520	4	27.130	6.390	.000	25.560	.984
	Quadratic	101.996	4	25.499	4.827	.002	19.307	.940
	Cubic	32.145	4	8.036	1.769	.148	7.076	.506
	Order 4	68.762	4	17.190	3.650	.010	14.600	.850
Error(Day)	Linear	237.757	56	4.246				
	Quadratic	295.835	56	5.283				
	Cubic	254.401	56	4.543				
	Order 4	263.742	56	4.710				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: ShootP
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	3802.908	1	3802.908	813.312	.000	813.312	1.000
Treatment	22.570	4	5.642	1.207	.318	4.827	.354
Error	261.847	56	4.676				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
SP1, SP2, SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.000	.	.	.
	Hotelling's Trace	.000		.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	5.000	51.000	1.000	.000	.050
SP1, SP2, SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP1, SP2, SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP1, SP2, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP1, SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP2, SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
SP1, SP2, SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP2, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP2, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP2, SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP2, SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP2, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP3, SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
SP1, SP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP2, SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP2, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP2, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP3, SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP3, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP5, SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
SP1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
SP7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
SP1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	228.108	56	4.073				
SP2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	256.868	56	4.587				
SP3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	331.568	56	5.921				
SP5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	252.162	56	4.503				
SP7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	244.877	56	4.373				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: ShootP

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
3.533	.124	3.285	3.781

2. Treatment

Estimates

Measure: ShootP

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	3.575	.279	3.016	4.134
5	3.325	.279	2.765	3.884
10	3.304	.279	2.745	3.863
15	3.430	.279	2.871	3.990
20	4.031	.268	3.493	4.568

Pairwise Comparisons

Measure: ShootP

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	5	.251	.395	1.000	-.903	1.404
	10	.271	.395	1.000	-.883	1.425
	15	.145	.395	1.000	-1.009	1.299
	20	-.456	.387	1.000	-1.587	.676
5	Control	-.251	.395	1.000	-1.404	.903
	10	.021	.395	1.000	-1.133	1.174
	15	-.106	.395	1.000	-1.260	1.048
	20	-.706	.387	.735	-1.837	.425
10	Control	-.271	.395	1.000	-1.425	.883
	5	-.021	.395	1.000	-1.174	1.133
	15	-.126	.395	1.000	-1.280	1.028
	20	-.727	.387	.657	-1.858	.405
15	Control	-.145	.395	1.000	-1.299	1.009
	5	.106	.395	1.000	-1.048	1.260
	10	.126	.395	1.000	-1.028	1.280
	20	-.600	.387	1.000	-1.732	.531
20	Control	.456	.387	1.000	-.676	1.587
	5	.706	.387	.735	-.425	1.837
	10	.727	.387	.657	-.405	1.858

15	.600	.387	1.000	-.531	1.732

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootP

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	4.514	4	1.128	1.207	.318	4.827	.354
Error	52.369	56	.935				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: ShootP

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	3.194	.259	2.676	3.712
2	3.759	.274	3.210	4.309
3	3.697	.312	3.072	4.321
4	3.594	.272	3.049	4.138
5	3.420	.268	2.883	3.957

Pairwise Comparisons

Measure: ShootP

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	-.565	.371	1.000	-1.649	.519
	3	-.503	.349	1.000	-1.522	.517
	4	-.399	.394	1.000	-1.550	.751
	5	-.226	.378	1.000	-1.331	.880
2	1	.565	.371	1.000	-.519	1.649
	3	.063	.389	1.000	-1.073	1.199
	4	.166	.381	1.000	-.949	1.280
	5	.340	.381	1.000	-.774	1.453
3	1	.503	.349	1.000	-.517	1.522
	2	-.063	.389	1.000	-1.199	1.073
	4	.103	.398	1.000	-1.060	1.267
	5	.277	.459	1.000	-1.065	1.618
4	1	.399	.394	1.000	-.751	1.550
	2	-.166	.381	1.000	-1.280	.949
	3	-.103	.398	1.000	-1.267	1.060
	5	.174	.416	1.000	-1.042	1.389
5	1	.226	.378	1.000	-.880	1.331
	2	-.340	.381	1.000	-1.453	.774
	3	-.277	.459	1.000	-1.618	1.065

4	-.174	.416	1.000	-1.389	1.042

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.053	.742[a]	4.000	53.000	.567	2.970	.223
Wilks' lambda	.947	.742[a]	4.000	53.000	.567	2.970	.223
Hotelling's trace	.056	.742[a]	4.000	53.000	.567	2.970	.223
Roy's largest root	.056	.742[a]	4.000	53.000	.567	2.970	.223

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: ShootP

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	3.135	.583	1.968	4.302
	2	5.020	.618	3.781	6.258
	3	4.197	.702	2.790	5.604
	4	2.650	.613	1.423	3.877
	5	2.874	.604	1.664	4.083
5	1	2.887	.583	1.720	4.054
	2	3.313	.618	2.075	4.552
	3	3.105	.702	1.698	4.512
	4	3.357	.613	2.130	4.585
	5	3.960	.604	2.751	5.170
10	1	4.560	.583	3.393	5.727
	2	2.877	.618	1.638	4.115
	3	4.457	.702	3.050	5.864
	4	1.833	.613	.606	3.060
	5	2.794	.604	1.585	4.003
15	1	2.676	.583	1.509	3.843
	2	2.275	.618	1.036	3.513
	3	2.229	.702	.821	3.636
	4	4.831	.613	3.604	6.059
	5	5.140	.604	3.931	6.350
20	1	2.714	.560	1.593	3.835
	2	5.313	.594	4.123	6.503
	3	4.498	.675	3.146	5.850
	4	5.297	.589	4.118	6.476
	5	2.332	.580	1.170	3.493

Pairwise Comparisons

Measure: ShootP

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	5	.248	.824	1.000	-2.160	2.656
		10	-1.425	.824	.891	-3.833	.983
		15	.459	.824	1.000	-1.949	2.867
		20	.421	.808	1.000	-1.941	2.782
	5	Control	-.248	.824	1.000	-2.656	2.160
		10	-1.673	.824	.470	-4.081	.735
		15	.211	.824	1.000	-2.197	2.619
		20	.173	.808	1.000	-2.189	2.534
	10	Control	1.425	.824	.891	-.983	3.833
		5	1.673	.824	.470	-.735	4.081
		15	1.884	.824	.260	-.524	4.292
		20	1.846	.808	.261	-.515	4.207
	15	Control	-.459	.824	1.000	-2.867	1.949
		5	-.211	.824	1.000	-2.619	2.197
		10	-1.884	.824	.260	-4.292	.524
		20	-.038	.808	1.000	-2.399	2.323
	20	Control	-.421	.808	1.000	-2.782	1.941
		5	-.173	.808	1.000	-2.534	2.189
		10	-1.846	.808	.261	-4.207	.515
		15	.038	.808	1.000	-2.323	2.399
2	Control	5	1.707	.874	.560	-.849	4.262
		10	2.143	.874	.174	-.412	4.699
		15	2.745*	.874	.027	.190	5.300
		20	-.293	.857	1.000	-2.799	2.213
	5	Control	-1.707	.874	.560	-4.262	.849
		10	.437	.874	1.000	-2.119	2.992
		15	1.038	.874	1.000	-1.517	3.594
		20	-2.000	.857	.233	-4.505	.506
	10	Control	-2.143	.874	.174	-4.699	.412
		5	-.437	.874	1.000	-2.992	2.119
		15	.602	.874	1.000	-1.954	3.157
		20	-2.436	.857	.063	-4.942	.069
	15	Control	-2.745*	.874	.027	-5.300	-.190
		5	-1.038	.874	1.000	-3.594	1.517
		10	-.602	.874	1.000	-3.157	1.954
		20	-3.038*	.857	.008	-5.544	-.532
	20	Control	.293	.857	1.000	-2.213	2.799
		5	2.000	.857	.233	-.506	4.505
		10	2.436	.857	.063	-.069	4.942
		15	3.038*	.857	.008	.532	5.544
3	Control	5	1.092	.993	1.000	-1.811	3.995
		10	-.260	.993	1.000	-3.163	2.643
		15	1.968	.993	.525	-.935	4.871
		20	-.301	.974	1.000	-3.148	2.546
	5	Control	-1.092	.993	1.000	-3.995	1.811
		10	-1.352	.993	1.000	-4.255	1.551
		15	.876	.993	1.000	-2.027	3.779
		20	-1.393	.974	1.000	-4.240	1.454
	10	Control	.260	.993	1.000	-2.643	3.163
		5	1.352	.993	1.000	-1.551	4.255
		15	2.228	.993	.289	-.675	5.131
		20	-.041	.974	1.000	-2.888	2.806

	15	Control	-1.968	.993	.525	-4.871	.935
		5	-.876	.993	1.000	-3.779	2.027
		10	-2.228	.993	.289	-5.131	.675
		20	-2.269	.974	.235	-5.116	.578
	20	Control	.301	.974	1.000	-2.546	3.148
		5	1.393	.974	1.000	-1.454	4.240
		10	.041	.974	1.000	-2.806	2.888
		15	2.269	.974	.235	-.578	5.116
4	Control	5	-.707	.866	1.000	-3.239	1.824
		10	.817	.866	1.000	-1.715	3.349
		15	-2.181	.866	.147	-4.713	.350
		20	-2.647*	.849	.029	-5.129	-.164
	5	Control	.707	.866	1.000	-1.824	3.239
		10	1.525	.866	.839	-1.007	4.056
		15	-1.474	.866	.944	-4.006	1.058
		20	-1.940	.849	.262	-4.422	.543
	10	Control	-.817	.866	1.000	-3.349	1.715
		5	-1.525	.866	.839	-4.056	1.007
		15	-2.999*	.866	.010	-5.530	-.467
		20	-3.464*	.849	.001	-5.947	-.981
	15	Control	2.181	.866	.147	-.350	4.713
		5	1.474	.866	.944	-1.058	4.006
		10	2.999*	.866	.010	.467	5.530
		20	-.465	.849	1.000	-2.948	2.017
	20	Control	2.647*	.849	.029	.164	5.129
		5	1.940	.849	.262	-.543	4.422
		10	3.464*	.849	.001	.981	5.947
		15	.465	.849	1.000	-2.017	2.948
5	Control	5	-1.087	.854	1.000	-3.582	1.408
		10	.080	.854	1.000	-2.415	2.575
		15	-2.267	.854	.103	-4.762	.228
		20	.542	.837	1.000	-1.904	2.989
	5	Control	1.087	.854	1.000	-1.408	3.582
		10	1.166	.854	1.000	-1.329	3.661
		15	-1.180	.854	1.000	-3.675	1.315
		20	1.629	.837	.567	-.818	4.075
	10	Control	-.080	.854	1.000	-2.575	2.415
		5	-1.166	.854	1.000	-3.661	1.329
		15	-2.347	.854	.080	-4.842	.148
		20	.462	.837	1.000	-1.984	2.909
	15	Control	2.267	.854	.103	-.228	4.762
		5	1.180	.854	1.000	-1.315	3.675
		10	2.347	.854	.080	-.148	4.842
		20	2.809*	.837	.014	.362	5.255
	20	Control	-.542	.837	1.000	-2.989	1.904
		5	-1.629	.837	.567	-4.075	.818
		10	-.462	.837	1.000	-2.909	1.984
		15	-2.809*	.837	.014	-5.255	-.362

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootP

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	29.781	4	7.445	1.828	.136	7.311	.521
	Error	228.108	56	4.073				
2	Contrast	88.587	4	22.147	4.828	.002	19.313	.940
	Error	256.868	56	4.587				
3	Contrast	48.326	4	12.082	2.041	.101	8.162	.573
	Error	331.568	56	5.921				
4	Contrast	104.612	4	26.153	5.808	.001	23.232	.974
	Error	252.162	56	4.503				
5	Contrast	62.687	4	15.672	3.584	.011	14.336	.842
	Error	244.877	56	4.373				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: ShootP

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	3.135	.583	1.968	4.302
	2	5.020	.618	3.781	6.258
	3	4.197	.702	2.790	5.604
	4	2.650	.613	1.423	3.877
	5	2.874	.604	1.664	4.083
5	1	2.887	.583	1.720	4.054
	2	3.313	.618	2.075	4.552
	3	3.105	.702	1.698	4.512
	4	3.357	.613	2.130	4.585
	5	3.960	.604	2.751	5.170
10	1	4.560	.583	3.393	5.727
	2	2.877	.618	1.638	4.115
	3	4.457	.702	3.050	5.864
	4	1.833	.613	.606	3.060
	5	2.794	.604	1.585	4.003
15	1	2.676	.583	1.509	3.843
	2	2.275	.618	1.036	3.513
	3	2.229	.702	.821	3.636
	4	4.831	.613	3.604	6.059
	5	5.140	.604	3.931	6.350
20	1	2.714	.560	1.593	3.835
	2	5.313	.594	4.123	6.503
	3	4.498	.675	3.146	5.850
	4	5.297	.589	4.118	6.476
	5	2.332	.580	1.170	3.493

Pairwise Comparisons

Measure: ShootP

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	-1.885	.836	.280	-4.328	.558
		3	-1.062	.786	1.000	-3.359	1.235
		4	.485	.887	1.000	-2.109	3.078
		5	.261	.852	1.000	-2.230	2.752
	2	1	1.885	.836	.280	-.558	4.328
		3	.823	.876	1.000	-1.737	3.383
		4	2.370	.859	.078	-.141	4.881
		5	2.146	.858	.153	-.362	4.655
	3	1	1.062	.786	1.000	-1.235	3.359
		2	-.823	.876	1.000	-3.383	1.737
		4	1.547	.897	.902	-1.075	4.169
		5	1.323	1.034	1.000	-1.700	4.346
	4	1	-.485	.887	1.000	-3.078	2.109
		2	-2.370	.859	.078	-4.881	.141
		3	-1.547	.897	.902	-4.169	1.075
		5	-.224	.937	1.000	-2.963	2.516
	5	1	-.261	.852	1.000	-2.752	2.230
		2	-2.146	.858	.153	-4.655	.362
		3	-1.323	1.034	1.000	-4.346	1.700
		4	.224	.937	1.000	-2.516	2.963
5	1	2	-.426	.836	1.000	-2.869	2.016
		3	-.218	.786	1.000	-2.515	2.079
		4	-.471	.887	1.000	-3.064	2.123
		5	-1.073	.852	1.000	-3.565	1.418
	2	1	.426	.836	1.000	-2.016	2.869
		3	.208	.876	1.000	-2.351	2.768
		4	-.044	.859	1.000	-2.555	2.467
		5	-.647	.858	1.000	-3.155	1.861
	3	1	.218	.786	1.000	-2.079	2.515
		2	-.208	.876	1.000	-2.768	2.351
		4	-.253	.897	1.000	-2.875	2.370
		5	-.855	1.034	1.000	-3.878	2.168
	4	1	.471	.887	1.000	-2.123	3.064
		2	.044	.859	1.000	-2.467	2.555
		3	.253	.897	1.000	-2.370	2.875
		5	-.603	.937	1.000	-3.342	2.136
	5	1	1.073	.852	1.000	-1.418	3.565
		2	.647	.858	1.000	-1.861	3.155
		3	.855	1.034	1.000	-2.168	3.878
		4	.603	.937	1.000	-2.136	3.342
10	1	2	1.684	.836	.488	-.759	4.126
		3	.103	.786	1.000	-2.193	2.400
		4	2.727*	.887	.033	.134	5.320
		5	1.766	.852	.429	-.725	4.257
	2	1	-1.684	.836	.488	-4.126	.759
		3	-1.580	.876	.766	-4.140	.980

		4	1.044	.859	1.000	-1.467	3.555
		5	.083	.858	1.000	-2.426	2.591
	3	1	-.103	.786	1.000	-2.400	2.193
		2	1.580	.876	.766	-.980	4.140
		4	2.624*	.897	.050	.002	5.246
		5	1.663	1.034	1.000	-1.360	4.686
	4	1	-2.727*	.887	.033	-5.320	-.134
		2	-1.044	.859	1.000	-3.555	1.467
		3	-2.624*	.897	.050	-5.246	-.002
		5	-.961	.937	1.000	-3.700	1.778
	5	1	-1.766	.852	.429	-4.257	.725
		2	-.083	.858	1.000	-2.591	2.426
		3	-1.663	1.034	1.000	-4.686	1.360
		4	.961	.937	1.000	-1.778	3.700
15	1	2	.401	.836	1.000	-2.042	2.844
		3	.447	.786	1.000	-1.849	2.744
		4	-2.155	.887	.184	-4.749	.438
		5	-2.464	.852	.055	-4.956	.027
	2	1	-.401	.836	1.000	-2.844	2.042
		3	.046	.876	1.000	-2.514	2.606
		4	-2.557*	.859	.043	-5.068	-.046
		5	-2.866*	.858	.015	-5.374	-.357
	3	1	-.447	.786	1.000	-2.744	1.849
		2	-.046	.876	1.000	-2.606	2.514
		4	-2.603	.897	.053	-5.225	.019
		5	-2.912	1.034	.067	-5.935	.111
	4	1	2.155	.887	.184	-.438	4.749
		2	2.557*	.859	.043	.046	5.068
		3	2.603	.897	.053	-.019	5.225
		5	-.309	.937	1.000	-3.048	2.430
	5	1	2.464	.852	.055	-.027	4.956
		2	2.866*	.858	.015	.357	5.374
		3	2.912	1.034	.067	-.111	5.935
		4	.309	.937	1.000	-2.430	3.048
20	1	2	-2.599*	.803	.020	-4.946	-.252
		3	-1.784	.755	.217	-3.990	.423
		4	-2.583*	.852	.037	-5.074	-.091
		5	.382	.819	1.000	-2.011	2.776
	2	1	2.599*	.803	.020	.252	4.946
		3	.815	.842	1.000	-1.644	3.275
		4	.016	.825	1.000	-2.397	2.428
		5	2.981*	.825	.006	.571	5.391
	3	1	1.784	.755	.217	-.423	3.990
		2	-.815	.842	1.000	-3.275	1.644
		4	-.799	.862	1.000	-3.319	1.720
		5	2.166	.994	.335	-.738	5.070
	4	1	2.583*	.852	.037	.091	5.074
		2	-.016	.825	1.000	-2.428	2.397
		3	.799	.862	1.000	-1.720	3.319
		5	2.965*	.900	.017	.334	5.597

	5	1	-.382	.819	1.000	-2.776	2.011
		2	-2.981*	.825	.006	-5.391	-.571
		3	-2.166	.994	.335	-5.070	.738
		4	-2.965*	.900	.017	-5.597	-.334

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

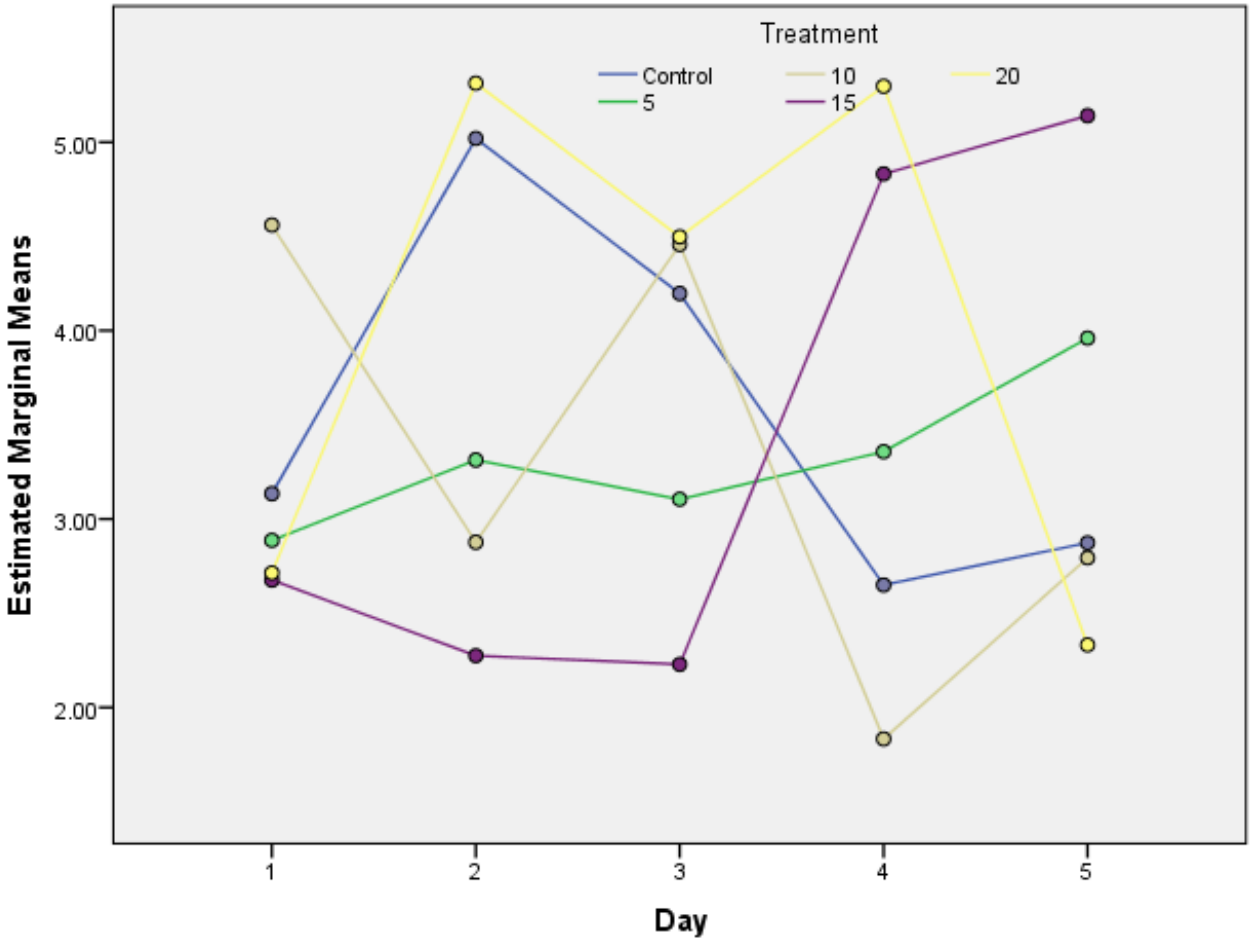
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.166	2.646[a]	4.000	53.000	.043	10.584	.700
	Wilks' lambda	.834	2.646[a]	4.000	53.000	.043	10.584	.700
	Hotelling's trace	.200	2.646[a]	4.000	53.000	.043	10.584	.700
	Roy's largest root	.200	2.646[a]	4.000	53.000	.043	10.584	.700
5	Pillai's trace	.028	.379[a]	4.000	53.000	.822	1.518	.130
	Wilks' lambda	.972	.379[a]	4.000	53.000	.822	1.518	.130
	Hotelling's trace	.029	.379[a]	4.000	53.000	.822	1.518	.130
	Roy's largest root	.029	.379[a]	4.000	53.000	.822	1.518	.130
10	Pillai's trace	.181	2.933[a]	4.000	53.000	.029	11.733	.751
	Wilks' lambda	.819	2.933[a]	4.000	53.000	.029	11.733	.751
	Hotelling's trace	.221	2.933[a]	4.000	53.000	.029	11.733	.751
	Roy's largest root	.221	2.933[a]	4.000	53.000	.029	11.733	.751
15	Pillai's trace	.260	4.648[a]	4.000	53.000	.003	18.591	.929
	Wilks' lambda	.740	4.648[a]	4.000	53.000	.003	18.591	.929
	Hotelling's trace	.351	4.648[a]	4.000	53.000	.003	18.591	.929
	Roy's largest root	.351	4.648[a]	4.000	53.000	.003	18.591	.929
20	Pillai's trace	.273	4.979[a]	4.000	53.000	.002	19.916	.946
	Wilks' lambda	.727	4.979[a]	4.000	53.000	.002	19.916	.946
	Hotelling's trace	.376	4.979[a]	4.000	53.000	.002	19.916	.946
	Roy's largest root	.376	4.979[a]	4.000	53.000	.002	19.916	.946

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots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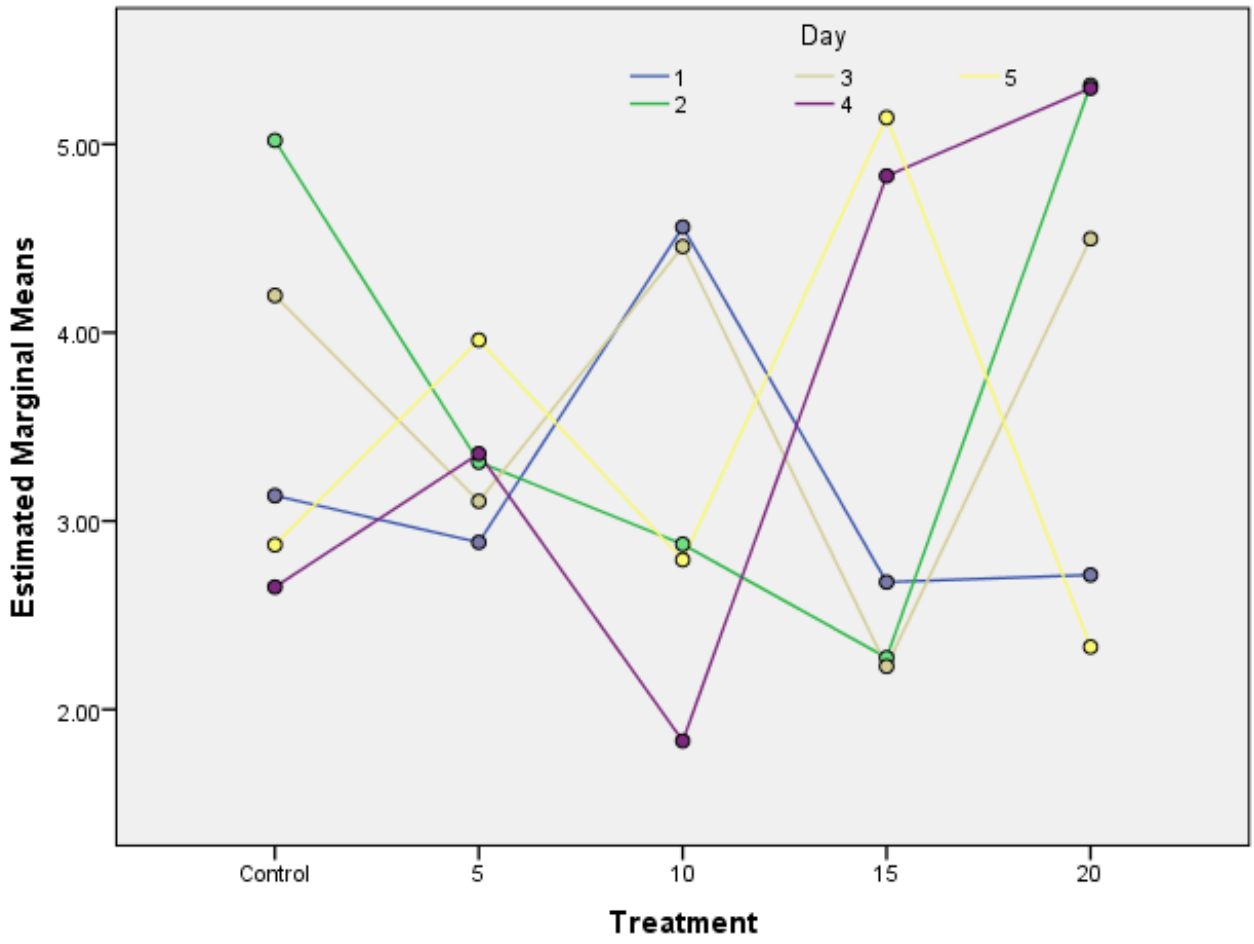
Day
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2
3
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Estimated Marginal Means
5.00
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Control
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10
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20
Treatment

TITLE Iron..

Iron.

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General Linear Model

Within-Subjects Factors

Measure: ShootT

Day	Dependent Variable
1	ST1
2	ST2
3	ST3
4	ST5
5	ST7

Between-Subjects Factors

		Value Label	N
Treatment	0	Control	12
	1	5	12
	2	10	12
	3	15	12
	4	20	13

Descriptive Statistics

	Treatment	Mean	Std. Deviation	N
ST1	Control	4.9458	2.87187	12
	5	3.2639	1.57908	12
	10	3.0745	1.74173	12
	15	2.2958	1.07534	12
	20	4.4712	3.05314	13
	Total	3.6244	2.35495	61
ST2	Control	2.8033	1.70807	12
	5	2.7221	1.62890	12
	10	2.6357	1.34560	12

	15	4.5974	3.17130	12
	20	2.3598	1.18020	13
	Total	3.0128	2.03084	61
ST3	Control	2.6357	1.34560	12
	5	3.6191	2.13089	12
	10	2.2766	1.07169	12
	15	4.5139	3.25069	12
	20	2.8555	1.43390	13
	Total	3.1748	2.08882	61
ST5	Control	2.6652	1.34281	12
	5	2.4536	2.01720	12
	10	5.1851	2.83142	12
	15	2.5228	1.30322	12
	20	2.8360	2.15358	13
	Total	3.1277	2.20389	61
ST7	Control	4.9458	2.87187	12
	5	3.2639	1.57908	12
	10	3.0745	1.74173	12
	15	2.2958	1.07534	12
	20	4.4712	3.05314	13
	Total	3.6244	2.35495	61

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[d]
Day	Pillai's Trace	.040	.756[b]	3.000	54.000	.524	2.269	.201
	Wilks' Lambda	.960	.756[b]	3.000	54.000	.524	2.269	.201
	Hotelling's Trace	.042	.756[b]	3.000	54.000	.524	2.269	.201
	Roy's Largest Root	.042	.756[b]	3.000	54.000	.524	2.269	.201
Day * Treatment	Pillai's Trace	.542	3.085	12.000	168.000	.001	37.025	.991
	Wilks' Lambda	.536	3.172	12.000	143.162	.000	33.087	.980
	Hotelling's Trace	.725	3.180	12.000	158.000	.000	38.159	.993
	Roy's Largest Root	.408	5.709[c]	4.000	56.000	.001	22.834	.971

a. Design: Intercept + Treatment
Within Subjects Design: Day
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: ShootT

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p-value	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	.000	.	9	.	.622	.699	.250

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Treatment
Within Subjects Design: Day
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: ShootT

Source		Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Sphericity Assumed	18.930	4	4.732	1.197	.313	4.788	.373
	Greenhouse-Geisser	18.930	2.487	7.612	1.197	.310	2.977	.287

	Huynh-Feldt	18.930	2.797	6.767	1.197	.312	3.348	.306
	Lower-bound	18.930	1.000	18.930	1.197	.279	1.197	.189
Day * Treatment	Sphericity Assumed	245.286	16	15.330	3.877	.000	62.039	1.000
	Greenhouse-Geisser	245.286	9.947	24.658	3.877	.000	38.570	.996
	Huynh-Feldt	245.286	11.190	21.921	3.877	.000	43.387	.998
	Lower-bound	245.286	4.000	61.322	3.877	.008	15.510	.873
Error(Day)	Sphericity Assumed	885.632	224	3.954				
	Greenhouse-Geisser	885.632	139.263	6.359				
	Huynh-Feldt	885.632	156.654	5.653				
	Lower-bound	885.632	56.000	15.815				

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: ShootT

Source	Day	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Day	Linear	.072	1	.072	.082	.775	.082	.059
	Quadratic	16.121	1	16.121	1.975	.165	1.975	.282
	Cubic	.289	1	.289	.082	.775	.082	.059
	Order 4	2.447	1	2.447	.753	.389	.753	.137
Day * Treatment	Linear	13.287	4	3.322	3.775	.009	15.098	.863
	Quadratic	139.691	4	34.923	4.278	.004	17.110	.906
	Cubic	53.149	4	13.287	3.775	.009	15.098	.863
	Order 4	39.160	4	9.790	3.012	.025	12.048	.765
Error(Day)	Linear	49.282	56	.880				
	Quadratic	457.198	56	8.164				
	Cubic	197.128	56	3.520				
	Order 4	182.024	56	3.250				

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: ShootT
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Intercept	3340.980	1	3340.980	574.549	.000	574.549	1.000
Treatment	9.616	4	2.404	.413	.798	1.654	.139
Error	325.638	56	5.815				

a. Computed using alpha = .05

Lack of Fit

Multivariate Tests

Dependent Variables		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
ST1, ST2, ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST2, ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST2, ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST2, ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	54.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	4.000	52.000	1.000	.000	.050
ST1, ST2, ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST2, ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST2, ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST2, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST3, ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	3.000	53.000	1.000	.000	.050
ST1, ST2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.

	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST2, ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST2, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST2, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST3, ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST3, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST5, ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	55.500	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	2.000	54.000	1.000	.000	.050
ST1	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST2	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST3	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST5	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050
ST7	Pillai's Trace	.000	.	.000	.000	.	.	.
	Wilks' Lambda	1.000	.	.000	56.000	.	.	.
	Hotelling's Trace	.000	.	.000	2.000	.	.	.
	Roy's Largest Root	.000	.000[a]	1.000	55.000	1.000	.000	.050

a. Exact statistic
b. Computed using alpha = .05

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
ST1	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	276.102	56	4.930				
ST2	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	208.539	56	3.724				
ST3	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	223.408	56	3.989				
ST5	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	227.118	56	4.056				
ST7	Lack of Fit	.000	0	.	.	.	.000	.
	Pure Error	276.102	56	4.930				

a. Computed using alpha = .05

Estimated Marginal Means

1. Grand Mean

Measure: ShootT

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
3.311	.138	3.035	3.588

2. Treatment

Estimates

Measure: ShootT

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	3.599	.311	2.976	4.223
5	3.065	.311	2.441	3.688
10	3.249	.311	2.626	3.873
15	3.245	.311	2.622	3.869
20	3.399	.299	2.800	3.998

Pairwise Comparisons

Measure: ShootT

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Control	5	.535	.440	1.000	-.752	1.821
	10	.350	.440	1.000	-.937	1.637
	15	.354	.440	1.000	-.933	1.641
	20	.200	.432	1.000	-1.061	1.462
5	Control	-.535	.440	1.000	-1.821	.752
	10	-.185	.440	1.000	-1.471	1.102
	15	-.181	.440	1.000	-1.467	1.106
	20	-.334	.432	1.000	-1.596	.927
10	Control	-.350	.440	1.000	-1.637	.937
	5	.185	.440	1.000	-1.102	1.471
	15	.004	.440	1.000	-1.283	1.291
	20	-.149	.432	1.000	-1.411	1.112
15	Control	-.354	.440	1.000	-1.641	.933
	5	.181	.440	1.000	-1.106	1.467
	10	-.004	.440	1.000	-1.291	1.283
	20	-.154	.432	1.000	-1.415	1.108
20	Control	-.200	.432	1.000	-1.462	1.061
	5	.334	.432	1.000	-.927	1.596
	10	.149	.432	1.000	-1.112	1.411

15	.154	.432	1.000	-1.108	1.415

Based on estimated marginal means
a. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootT

	Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
Contrast	1.923	4	.481	.413	.798	1.654	.139
Error	65.128	56	1.163				

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.
a. Computed using alpha = .05

3. Day

Estimates

Measure: ShootT

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	3.610	.284	3.040	4.180
2	3.024	.247	2.528	3.519
3	3.180	.256	2.668	3.693
4	3.133	.258	2.616	3.649
5	3.610	.284	3.040	4.180

Pairwise Comparisons

Measure: ShootT

					95% Confidence Interval for Difference[a]	
(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[a]	Lower Bound	Upper Bound
1	2	.587	.410	1.000	-.612	1.785
	3	.430	.387	1.000	-.701	1.561
	4	.478	.385	1.000	-.646	1.602
	5	.000	.000	.	.000	.000
2	1	-.587	.410	1.000	-1.785	.612
	3	-.157	.307	1.000	-1.055	.742
	4	-.109	.380	1.000	-1.220	1.002
	5	-.587	.410	1.000	-1.785	.612
3	1	-.430	.387	1.000	-1.561	.701
	2	.157	.307	1.000	-.742	1.055
	4	.048	.356	1.000	-.994	1.089
	5	-.430	.387	1.000	-1.561	.701
4	1	-.478	.385	1.000	-1.602	.646
	2	.109	.380	1.000	-1.002	1.220
	3	-.048	.356	1.000	-1.089	.994
	5	-.478	.385	1.000	-1.602	.646
5	1	.000	.000	.	.000	.000
	2	.587	.410	1.000	-.612	1.785
	3	.430	.387	1.000	-.701	1.561

4	.478	.385	1.000	-.646	1.602

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Pillai's trace	.040	.756[a]	3.000	54.000	.524	2.269	.201
Wilks' lambda	.960	.756[a]	3.000	54.000	.524	2.269	.201
Hotelling's trace	.042	.756[a]	3.000	54.000	.524	2.269	.201
Roy's largest root	.042	.756[a]	3.000	54.000	.524	2.269	.201

Each F tests the multivariate effect of Day. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

4. Treatment * Day

Estimates

Measure: ShootT

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	4.946	.641	3.662	6.230
	2	2.803	.557	1.687	3.919
	3	2.636	.577	1.481	3.791
	4	2.665	.581	1.501	3.830
	5	4.946	.641	3.662	6.230
5	1	3.264	.641	1.980	4.548
	2	2.722	.557	1.606	3.838
	3	3.619	.577	2.464	4.774
	4	2.454	.581	1.289	3.618
	5	3.264	.641	1.980	4.548
10	1	3.075	.641	1.790	4.359
	2	2.636	.557	1.520	3.752
	3	2.277	.577	1.122	3.432
	4	5.185	.581	4.020	6.350
	5	3.075	.641	1.790	4.359
15	1	2.296	.641	1.012	3.580
	2	4.597	.557	3.481	5.713
	3	4.514	.577	3.359	5.669
	4	2.523	.581	1.358	3.687
	5	2.296	.641	1.012	3.580
20	1	4.471	.616	3.238	5.705
	2	2.360	.535	1.288	3.432
	3	2.856	.554	1.746	3.965
	4	2.836	.559	1.717	3.955
	5	4.471	.616	3.238	5.705

Pairwise Comparisons

Measure: ShootT

Day	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	Control	5	1.682	.906	.688	-.967	4.331
		10	1.871	.906	.436	-.778	4.521
		15	2.650*	.906	.050	.001	5.299
		20	.475	.889	1.000	-2.123	3.072
	5	Control	-1.682	.906	.688	-4.331	.967
		10	.189	.906	1.000	-2.460	2.839
		15	.968	.906	1.000	-1.681	3.617
		20	-1.207	.889	1.000	-3.805	1.390
	10	Control	-1.871	.906	.436	-4.521	.778
		5	-.189	.906	1.000	-2.839	2.460
		15	.779	.906	1.000	-1.871	3.428
		20	-1.397	.889	1.000	-3.995	1.201
	15	Control	-2.650*	.906	.050	-5.299	-.001
		5	-.968	.906	1.000	-3.617	1.681
		10	-.779	.906	1.000	-3.428	1.871
		20	-2.175	.889	.176	-4.773	.422
	20	Control	-.475	.889	1.000	-3.072	2.123
		5	1.207	.889	1.000	-1.390	3.805
		10	1.397	.889	1.000	-1.201	3.995
		15	2.175	.889	.176	-.422	4.773
2	Control	5	.081	.788	1.000	-2.221	2.384
		10	.168	.788	1.000	-2.135	2.470
		15	-1.794	.788	.266	-4.096	.508
		20	.443	.773	1.000	-1.814	2.701
	5	Control	-.081	.788	1.000	-2.384	2.221
		10	.086	.788	1.000	-2.216	2.389
		15	-1.875	.788	.207	-4.178	.427
		20	.362	.773	1.000	-1.895	2.620
	10	Control	-.168	.788	1.000	-2.470	2.135
		5	-.086	.788	1.000	-2.389	2.216
		15	-1.962	.788	.158	-4.264	.341
		20	.276	.773	1.000	-1.982	2.534
	15	Control	1.794	.788	.266	-.508	4.096
		5	1.875	.788	.207	-.427	4.178
		10	1.962	.788	.158	-.341	4.264
		20	2.238	.773	.054	-.020	4.495
	20	Control	-.443	.773	1.000	-2.701	1.814
		5	-.362	.773	1.000	-2.620	1.895
		10	-.276	.773	1.000	-2.534	1.982
		15	-2.238	.773	.054	-4.495	.020
3	Control	5	-.983	.815	1.000	-3.366	1.400
		10	.359	.815	1.000	-2.024	2.742
		15	-1.878	.815	.250	-4.261	.505
		20	-.220	.800	1.000	-2.557	2.117
	5	Control	.983	.815	1.000	-1.400	3.366
		10	1.343	.815	1.000	-1.041	3.726
		15	-.895	.815	1.000	-3.278	1.488
		20	.764	.800	1.000	-1.573	3.100
	10	Control	-.359	.815	1.000	-2.742	2.024
		5	-1.343	.815	1.000	-3.726	1.041
		15	-2.237	.815	.081	-4.620	.146
		20	-.579	.800	1.000	-2.916	1.758

	15	Control	1.878	.815	.250	-.505	4.261
		5	.895	.815	1.000	-1.488	3.278
		10	2.237	.815	.081	-.146	4.620
		20	1.658	.800	.427	-.678	3.995
	20	Control	.220	.800	1.000	-2.117	2.557
		5	-.764	.800	1.000	-3.100	1.573
		10	.579	.800	1.000	-1.758	2.916
		15	-1.658	.800	.427	-3.995	.678
4	Control	5	.212	.822	1.000	-2.191	2.614
		10	-2.520*	.822	.033	-4.923	-.117
		15	.142	.822	1.000	-2.260	2.545
		20	-.171	.806	1.000	-2.527	2.185
	5	Control	-.212	.822	1.000	-2.614	2.191
		10	-2.731*	.822	.016	-5.134	-.329
		15	-.069	.822	1.000	-2.472	2.334
		20	-.382	.806	1.000	-2.739	1.974
	10	Control	2.520*	.822	.033	.117	4.923
		5	2.731*	.822	.016	.329	5.134
		15	2.662*	.822	.020	.260	5.065
		20	2.349	.806	.051	-.007	4.705
	15	Control	-.142	.822	1.000	-2.545	2.260
		5	.069	.822	1.000	-2.334	2.472
		10	-2.662*	.822	.020	-5.065	-.260
		20	-.313	.806	1.000	-2.669	2.043
	20	Control	.171	.806	1.000	-2.185	2.527
		5	.382	.806	1.000	-1.974	2.739
		10	-2.349	.806	.051	-4.705	.007
		15	.313	.806	1.000	-2.043	2.669
5	Control	5	1.682	.906	.688	-.967	4.331
		10	1.871	.906	.436	-.778	4.521
		15	2.650*	.906	.050	.001	5.299
		20	.475	.889	1.000	-2.123	3.072
	5	Control	-1.682	.906	.688	-4.331	.967
		10	.189	.906	1.000	-2.460	2.839
		15	.968	.906	1.000	-1.681	3.617
		20	-1.207	.889	1.000	-3.805	1.390
	10	Control	-1.871	.906	.436	-4.521	.778
		5	-.189	.906	1.000	-2.839	2.460
		15	.779	.906	1.000	-1.871	3.428
		20	-1.397	.889	1.000	-3.995	1.201
	15	Control	-2.650*	.906	.050	-5.299	-.001
		5	-.968	.906	1.000	-3.617	1.681
		10	-.779	.906	1.000	-3.428	1.871
		20	-2.175	.889	.176	-4.773	.422
	20	Control	-.475	.889	1.000	-3.072	2.123
		5	1.207	.889	1.000	-1.390	3.805
		10	1.397	.889	1.000	-1.201	3.995
		15	2.175	.889	.176	-.422	4.773

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Measure: ShootT

Day		Sum of Squares	df	Mean Square	F	p-value	Noncent. Parameter	Observed Power[a]
1	Contrast	56.645	4	14.161	2.872	.031	11.489	.743
	Error	276.102	56	4.930				
2	Contrast	38.920	4	9.730	2.613	.045	10.451	.696
	Error	208.539	56	3.724				
3	Contrast	38.382	4	9.595	2.405	.060	9.621	.655
	Error	223.408	56	3.989				
4	Contrast	64.310	4	16.077	3.964	.007	15.857	.881
	Error	227.118	56	4.056				
5	Contrast	56.645	4	14.161	2.872	.031	11.489	.743
	Error	276.102	56	4.930				

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

5. Treatment * Day

Estimates

Measure: ShootT

Treatment	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	4.946	.641	3.662	6.230
	2	2.803	.557	1.687	3.919
	3	2.636	.577	1.481	3.791
	4	2.665	.581	1.501	3.830
	5	4.946	.641	3.662	6.230
5	1	3.264	.641	1.980	4.548
	2	2.722	.557	1.606	3.838
	3	3.619	.577	2.464	4.774
	4	2.454	.581	1.289	3.618
	5	3.264	.641	1.980	4.548
10	1	3.075	.641	1.790	4.359
	2	2.636	.557	1.520	3.752
	3	2.277	.577	1.122	3.432
	4	5.185	.581	4.020	6.350
	5	3.075	.641	1.790	4.359
15	1	2.296	.641	1.012	3.580
	2	4.597	.557	3.481	5.713
	3	4.514	.577	3.359	5.669
	4	2.523	.581	1.358	3.687
	5	2.296	.641	1.012	3.580
20	1	4.471	.616	3.238	5.705
	2	2.360	.535	1.288	3.432
	3	2.856	.554	1.746	3.965
	4	2.836	.559	1.717	3.955
	5	4.471	.616	3.238	5.705

Pairwise Comparisons

Measure: ShootT

Treatment	(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	p-value[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Control	1	2	2.143	.924	.241	-.558	4.843
		3	2.310	.872	.105	-.239	4.860
		4	2.281	.867	.110	-.252	4.813
		5	.000	.000	.	.000	.000
	2	1	-2.143	.924	.241	-4.843	.558
		3	.168	.693	1.000	-1.857	2.192
		4	.138	.856	1.000	-2.365	2.641
		5	-2.143	.924	.241	-4.843	.558
	3	1	-2.310	.872	.105	-4.860	.239
		2	-.168	.693	1.000	-2.192	1.857
		4	-.030	.803	1.000	-2.376	2.317
		5	-2.310	.872	.105	-4.860	.239
	4	1	-2.281	.867	.110	-4.813	.252
		2	-.138	.856	1.000	-2.641	2.365
		3	.030	.803	1.000	-2.317	2.376
		5	-2.281	.867	.110	-4.813	.252
	5	1	.000	.000	.	.000	.000
		2	2.143	.924	.241	-.558	4.843
		3	2.310	.872	.105	-.239	4.860
		4	2.281	.867	.110	-.252	4.813
5	1	2	.542	.924	1.000	-2.159	3.242
		3	-.355	.872	1.000	-2.905	2.194
		4	.810	.867	1.000	-1.722	3.343
		5	.000	.000	.	.000	.000
	2	1	-.542	.924	1.000	-3.242	2.159
		3	-.897	.693	1.000	-2.922	1.128
		4	.268	.856	1.000	-2.234	2.771
		5	-.542	.924	1.000	-3.242	2.159
	3	1	.355	.872	1.000	-2.194	2.905
		2	.897	.693	1.000	-1.128	2.922
		4	1.165	.803	1.000	-1.181	3.512
		5	.355	.872	1.000	-2.194	2.905
	4	1	-.810	.867	1.000	-3.343	1.722
		2	-.268	.856	1.000	-2.771	2.234
		3	-1.165	.803	1.000	-3.512	1.181
		5	-.810	.867	1.000	-3.343	1.722
	5	1	.000	.000	.	.000	.000
		2	.542	.924	1.000	-2.159	3.242
		3	-.355	.872	1.000	-2.905	2.194
		4	.810	.867	1.000	-1.722	3.343
10	1	2	.439	.924	1.000	-2.262	3.139
		3	.798	.872	1.000	-1.751	3.347
		4	-2.111	.867	.181	-4.643	.422
		5	.000	.000	.	.000	.000
	2	1	-.439	.924	1.000	-3.139	2.262
		3	.359	.693	1.000	-1.666	2.384

		4	-2.549*	.856	.043	-5.052	-.047
		5	-.439	.924	1.000	-3.139	2.262
	3	1	-.798	.872	1.000	-3.347	1.751
		2	-.359	.693	1.000	-2.384	1.666
		4	-2.909*	.803	.006	-5.255	-.562
		5	-.798	.872	1.000	-3.347	1.751
	4	1	2.111	.867	.181	-.422	4.643
		2	2.549*	.856	.043	.047	5.052
		3	2.909*	.803	.006	.562	5.255
		5	2.111	.867	.181	-.422	4.643
	5	1	.000	.000	.	.000	.000
		2	.439	.924	1.000	-2.262	3.139
		3	.798	.872	1.000	-1.751	3.347
		4	-2.111	.867	.181	-4.643	.422
15	1	2	-2.302	.924	.157	-5.002	.399
		3	-2.218	.872	.138	-4.768	.331
		4	-.227	.867	1.000	-2.759	2.306
		5	.000	.000	.	.000	.000
	2	1	2.302	.924	.157	-.399	5.002
		3	.083	.693	1.000	-1.941	2.108
		4	2.075	.856	.187	-.428	4.577
		5	2.302	.924	.157	-.399	5.002
	3	1	2.218	.872	.138	-.331	4.768
		2	-.083	.693	1.000	-2.108	1.941
		4	1.991	.803	.162	-.355	4.337
		5	2.218	.872	.138	-.331	4.768
	4	1	.227	.867	1.000	-2.306	2.759
		2	-2.075	.856	.187	-4.577	.428
		3	-1.991	.803	.162	-4.337	.355
		5	.227	.867	1.000	-2.306	2.759
	5	1	.000	.000	.	.000	.000
		2	-2.302	.924	.157	-5.002	.399
		3	-2.218	.872	.138	-4.768	.331
		4	-.227	.867	1.000	-2.759	2.306
20	1	2	2.111	.888	.208	-.483	4.706
		3	1.616	.838	.590	-.834	4.065
		4	1.635	.833	.545	-.798	4.068
		5	.000	.000	.	.000	.000
	2	1	-2.111	.888	.208	-4.706	.483
		3	-.496	.666	1.000	-2.441	1.450
		4	-.476	.823	1.000	-2.881	1.928
		5	-2.111	.888	.208	-4.706	.483
	3	1	-1.616	.838	.590	-4.065	.834
		2	.496	.666	1.000	-1.450	2.441
		4	.019	.771	1.000	-2.235	2.274
		5	-1.616	.838	.590	-4.065	.834
	4	1	-1.635	.833	.545	-4.068	.798
		2	.476	.823	1.000	-1.928	2.881
		3	-.019	.771	1.000	-2.274	2.235
		5	-1.635	.833	.545	-4.068	.798

	5	1	.000	.000	.	.000	.000
		2	2.111	.888	.208	-.483	4.706
		3	1.616	.838	.590	-.834	4.065
		4	1.635	.833	.545	-.798	4.068

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

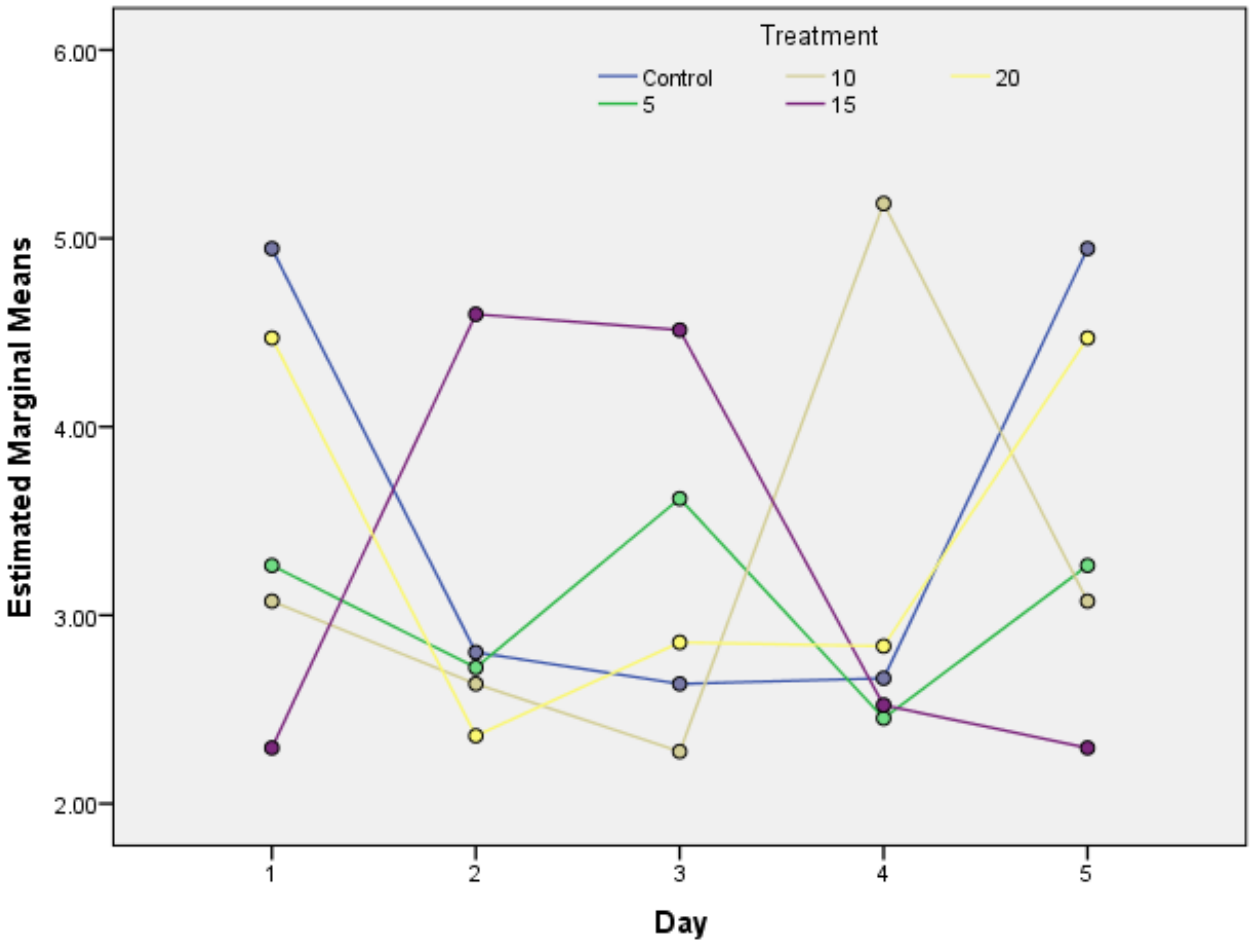
Treatment		Value	F	Hypothesis df	Error df	p-value	Noncent. Parameter	Observed Power[b]
Control	Pillai's trace	.138	2.880[a]	3.000	54.000	.044	8.641	.656
	Wilks' lambda	.862	2.880[a]	3.000	54.000	.044	8.641	.656
	Hotelling's trace	.160	2.880[a]	3.000	54.000	.044	8.641	.656
	Roy's largest root	.160	2.880[a]	3.000	54.000	.044	8.641	.656
5	Pillai's trace	.051	.975[a]	3.000	54.000	.411	2.925	.251
	Wilks' lambda	.949	.975[a]	3.000	54.000	.411	2.925	.251
	Hotelling's trace	.054	.975[a]	3.000	54.000	.411	2.925	.251
	Roy's largest root	.054	.975[a]	3.000	54.000	.411	2.925	.251
10	Pillai's trace	.204	4.605[a]	3.000	54.000	.006	13.815	.866
	Wilks' lambda	.796	4.605[a]	3.000	54.000	.006	13.815	.866
	Hotelling's trace	.256	4.605[a]	3.000	54.000	.006	13.815	.866
	Roy's largest root	.256	4.605[a]	3.000	54.000	.006	13.815	.866
15	Pillai's trace	.158	3.376[a]	3.000	54.000	.025	10.128	.733
	Wilks' lambda	.842	3.376[a]	3.000	54.000	.025	10.128	.733
	Hotelling's trace	.188	3.376[a]	3.000	54.000	.025	10.128	.733
	Roy's largest root	.188	3.376[a]	3.000	54.000	.025	10.128	.733
20	Pillai's trace	.101	2.027[a]	3.000	54.000	.121	6.080	.492
	Wilks' lambda	.899	2.027[a]	3.000	54.000	.121	6.080	.492
	Hotelling's trace	.113	2.027[a]	3.000	54.000	.121	6.080	.492
	Roy's largest root	.113	2.027[a]	3.000	54.000	.121	6.080	.492

Each F tests the multivariate simple effects of Day within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Profile Plots


Treatment
Control
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20
5
15
Estimated Marginal Means
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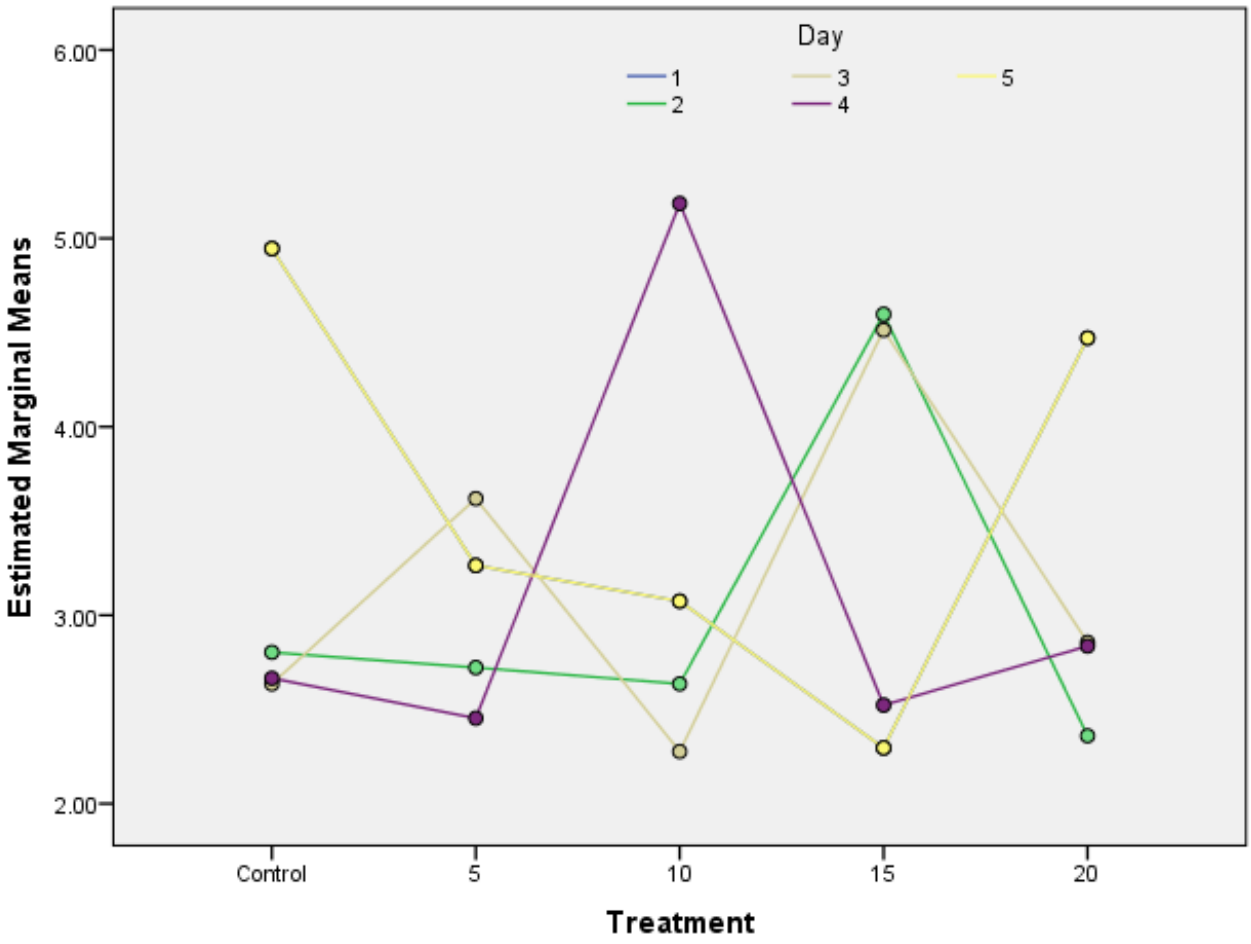
5.00
4.00
3.00
2.00
1
2
3
4
5
Day


Day
1
2
3
4
5
6.00
5.00
4.00
3.00
2.00
Estimated Marginal Means
Control
5
10
15
20
Treatment