ENVIRONMENTAL ASSESSMENT OF HEAVY METALS AND ORGANOTIN COMPOUNDS IN CAPE TOWN HARBOUR: MONITORING, GEOCHEMISTRY AND TOXICITY

HUSSEIN KEHINDE OKORO

Cape Peninsula
University of Technology

Environmental Assessment of Heavy metals and Organotin Compounds in Cape Town Harbour: Monitoring, Geochemistry and Toxicity

By

HUSSEIN KEHINDE OKORO

Thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Technology: (Chemistry)

in the Faculty of (Applied Sciences)

at the Cape Peninsula University of Technology

Supervisor: Prof .O.S.Fatoki

Co-supervisor: Dr. BJ Ximba; Prof. RG Snyman

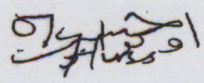
External Co- Supervisor: Prof F.A Adekola

Cape Town

September 2012

DECLARATION

I, HUSSEIN ***KEHINDE OKORO***, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



25-10-2012

Signed **Date**

ABSTRACT

Analytical methods for speciation of targeted organotin compounds (TBT and TPT) in water samples using SPE cartridge and liquid-liquid extraction has been carried out. Also, sediment analysis using methanol - acid digestion and acid- sonication extraction methods were also developed. Different parameters affecting extraction and peak resolution were optimised. Also, three derivatisation procedures were optimised. The accuracy of the extraction procedure was also verified on certified reference material (BCR - 462) certified for TBT (54 ± 15 µg/kg) and DBT (68 ± 12 µg/kg). Freeze – dried mussel tissue (ERM – CE 477) certified for TBT (2.20 ± 0.19 mg/kg), DBT (1.54 ± 0.12 mg/kg) and MBT (1.50 ± 0.28 mg/kg). Good recoveries were obtained with methanol - acid digestion. The results were validated by analysing the real water and sediment samples collected from Cape Town harbour and the compounds were detected in both water and sediment samples, respectively.

The annual distribution of Organotin Compounds (OTCs) in the seawater of Cape Town harbour was investigated. The concentration of OTCs varies for locations in Cape Town harbour. The concentration ranges from 0.067 ± 0.01 to 111.290 ± 32.20 x 10^{-3} µg/l for TBT while that of TPT ranges between ND to 23008.0±0.03 x 10^{-3} µg/l respectively between locations. Seasonal variation in TBT and TPT concentrations with a higher level in summer than in winter and spring was observed. Apparently, the observed high or low values recorded for TBT in Cape Town harbour could be the result of an increase or decrease in the traffic of ships and boats. In addition, dilution effect due to increase in water volume could also account for the decrease in concentration of TBT. TBT was detected in all the sediments samples analysed except for location 9 (entrance to harbour, the two control sites (which are located far away from the inner harbour where boating activities is taking place), and location 12 (Robinson dry dock 2) where the samples were not found at all. The results indicated that TBT is present throughout the seasons but is predominantly present in this order summer > winter > spring. High variation recorded during summer is associated with a steady flow of water during summer which enhances siltation of TBT in water column into the sediment. The least value recorded for TBT and TPT in winter could be as a result of erosion due to an increase in water flow which removes OTCs from sediment.

The toxicity effects of tributyltin were also investigated. Responses of lysosomal membranes of hemocytes of the mussel, *Mytilus galloprovincialis,* as a biomarker of stress due to exposure to tributyltin was used for this study. The neutral red retention time assay was employed for this purpose. Two groups of mussels were exposed to different environmentally relevant concentrations of tributyltin (0.1 µg/l and 1.0 µg/l). A third group served as control. The experiment ran over four weeks. Two groups exposed to TBT exhibited significantly increased (P≤ 0.05) whole body TBT concentrations (0.08 ± 0.00010

µg/g and 0.70 ± 0.00030 µg/g dry mass, respectively and significant shorter (P≤0.005) NRR (Neutral Red Retention) times (14.00 ± 3.005 min and 10.00 ± 2.006 min, respectively) after four weeks of exposure. For the control group, no TBT was detected but the NRR times were significantly higher (24 ± 10.00 min) when compared to the exposed groups. Apparently, from this study, for both exposures group NRR times became progressively shorter as TBT concentration increase with time. This study has revealed that the two contributing factors influencing lysosomal responses are exposure concentration and exposure time of TBT. Regression analysis was performed on the three treatment group, the two exposed group showed a decrease trend with R^2 values, of 0.850 and 0.971 for the 0.1 µg/l exposed group and the 1.0 µg/l exposed group, respectively. The NRR time assay could be considered as a useful technique and lysosomal membrane destabilization a useful cellular biomarker of stress due to tributyltin exposure.

Distribution of possible chemical forms of metals in marine sediments of Cape Town harbour was investigated using modified Tessier's sequential extraction procedure. Si, Al and Zn were mostly associated with Fe-Mn oxides, whereas Sn and Hg were mainly bound to residual and organic matter. Pb, Sn and Hg exhibited similar binding behaviour which indicated an anthropogenic point source from the shipyard waste. The mobility of metals followed the order: Si > Zn > Fe > Cu> Al> Cd> Pb > Sn > Hg. Geochemical assessment of marine sediments collected from Cape Town harbour was carried out using Inductively Coupled Plasma-Mass Spectrometry, Fourier-Transform-Infrared and X-ray- Diffractrometry techniques. The clay mineral phases consist of biotites, kaolinites and halites. The enrichment factors of Tin, Lead, Zinc, Iron, Cadmium, Aluminium and Mercury revealed anthropogenic inputs of these metals into the marine environment. The geomineral analyses revealed the presence of quartz, pyrite, and calcite and carrolite minerals as the main constituents of the marine sediments. One year monitoring of priority heavy metals As, Cd, Hg, Pb and Sn in seawater from Cape Town harbour with respect to their seasonal variations and their pollution levels were studied. The concentrations of heavy metals were determined using ICP-MS instrument. High significant variation of P (≤0.05) was observed for all metals between seasons and across locations. Seasonal variations of heavy metals showed that Sn and Cd are more prevalent during summer while Hg, Pb and As are more prevalent during winter season. Heavy metal concentrations in the mussels (*Mytilus galloprovincialis*) collected from the Cape Town harbour were determined using Energy Dispersive X-ray Fluorescence (EDXRF) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). EDXRF showed that tissue portions of the mussel contained K, Ca, Fe, Cu, Zn, Si, Sr, Al and Au, while the shell portion contained K, Ca, Fe, Cr, Zn, Si and Sr. Due to poorer detection limits of EDXRF, ultra- trace elements (Mn, Pb, As, Hg, V, Cr, Sn, Cd, Ni and Co) were determined in mussels using ICP-MS.

ACKNOWLEDGEMENTS

- First and foremost, i would like to acknowledge the almighty Allah, the Glorified, the Lord of Dominion and Celestial Domains, the All-knowing for seeing me through my Doctoral programme. A word is insufficient for me to thank you.

- I would like to thank my supervisors; Prof .O.S. Fatoki, Dr. BJ Ximba, Prof. R.G Snyman and my external co-supervisor Prof. F.A Adekola for their unrelentful efforts day in and day out despite their other engagements to see towards the completion of this programme. I say a big thank you to you all. May you leave long to reap the fruit of your labour (amen).

- I also acknowledge my caring and loving parents Alhaji Imam Zakariyah Adeola Okoro and my mum, Mrs Oladutemu Okoro for their efforts on me right from childhood up to this moment and for being wonderful parents. I also thank my brothers Mr Adeola Kamar Okoro, Adeola Idowu Okoro. Appreciation to my late sister Mrs Falilat Olayinka Olufawoye whose sudden death came as shock to me while am away. May your soul rest in peace. To chief Jamiu Ekungba and Prof Ishaq Oloyede, Prof EO Odebunmi, Dr MRS Elleta I thank you for your encouragements and supports.

- Thanks also go to my dearest and loving wife Mrs Tawakalit Aderonke Okoro, for her understanding and full supports towards the success of my Doctoral programme. Acknowledgement also goes to my children Lateefah Ayodeji Okoro and AbduLateef Abidemi, Okoro who was born in my absence during the course of my research work in South Africa. Daddy loves you.

- Special thanks to Erick O. Jondicko, Dr Rasheeda Toefy and Pof Van Schalkwyk for the assistant you rendered for my stastical analysis. I also thank Dr Olatunde Olatunji, Dr Opeolu and my postgraduate colleagues namely: Lanre Olujimi, Daso Peter, Akinsoji Olatunbosun and Ayanda Sola for your cooperation and useful advice. God bless you all

- Acknowledgement also goes the Technical staff of Chemistry department, CPUT namely; Zandile Mthembu, Gillian FennessyYon, Nohako Khayinsa for your cooperation and for being there always. My thanks to all academic and non-academic staff of Chemistry department, CPUT. Acknowledgement also goes to Mr Sebastian Brown, Alistair Aldonis and Ashley Davids of CSIR, South Africa for being part of the

sampling team. I thanked Michelle Yvonne Slabber of Ecotoxicology lab, CPUT, for helping me in sampling and for her assistance all times during toxicology experiment.

- I wish to thank the management of Cape Peninsula University of Technology, Cape Town, South Africa for the URF grant offered for this study. I also acknowledge University of Ilorin, Ilorin, Nigeria for supplementation staff development award.

- Finally I thank all my friends and especially members of Al-Ameen Society and Cape Duah Forum, South Africa for their prayers and supports.

DEDICATION

This thesis is dedicated to the Almighty Allah for seeing me through my programme. I will forever be grateful to you O "ALLAH".

Table of Contents

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

AAS	Atomic absorption spectrometry
ASE	Accelerated solvent extraction
AChE	Acetylcholenesterase
DBT	Dibutyltin
TBT	Tributyltin
TBTO	Tributyltinoxide
EPA	Environmental Protection Agency
EC	European Commissions'
EQTC	Environmental Quality Target Concentration
EVISA	European Virtual Institute for Speciation Analysis
FAL	Facilitation of International Maritime Traffic
GC- FPD	Gas Chromatography- Flame Photometry Detector
GC	Gas Chromatography
GFAAS	Graphite furnace atomic absorption spectrometry
HPLC	High Pressure Liquid Chromatography
ICP-MS	Inductive coupled plasma –Mass spectrometry
IR- RP	Ion – Pair Reversed Phase Chromatography
IMO	International Maritime Organization
IR	Infra-red
LLE	Liquid-Liquid Extraction
NRR	Neutral Red Retention
ND	Not Detectable
NPA	National Port Authority
MBT	Monobutyltin
MS	Mass spectrometry
MeHg	Methyl Mercury
OTC	Organotin Compounds
PVC	Polyvinchloride
RSD	Relative Standard Deviation
RPL	Relative Pennis Length
TEA	Triethylamine
TMHA	Tetramethylammoniumhydroxide
TPrT	Tripropyltinchloride
TPT	TriPhenyltins
TBTCL	Tributyltinchloride

TBT	Tributyltin
TDI	Tolerable Daily Intake
WHO	World Health Organisation
STEB	Sodiumtetraethylborate
SPSS	Statistical Package for Social Science
SAMSA	South Africa Maritime Safety Authority
UV-Visible	Ultraviolet visible spectrometry
USEPA	US Environmental Protection Agency.
NRRT	Neutral Red Retention Time

PUBLICATIONS FROM THIS RESEARCH WORK

Okoro, H.K, Fatoki, O.S, Adekola, F. A, Ximba, B.J, Snyman, R.G (2011) "Sources Environmental Levels and Toxicity of Organotin in Marine Environment- A Review" ***Asian Journal of Chemistry.*** Vol. 23 (2); 473 – 482, Published by Asian Journal of Chemistry. Available online at www.asianjournalofchemistry.co.in

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2011), "Human Exposure, Biomarkers and Fate of Organotins in the Environment". ***Review of Environmental Contamination and Toxicology, Springer-Newyork.*** Published by Springer Science + Business Media B.V. 2011: Vol 213: pp 27 -54.

Fatoki, O.S, **Okoro, H.K,** Adekola, F.A, Ximba, B.J, Snyman, R.G (2012). "Bioaccumulation of Metals in black mussels (*Mytillus* galloprovincialis) in Cape Town Harbour, South Africa". ***The Environmentalist***. 32: 48-57 Published by Springer Science + Business Media LLC 2011: (DOI 10.1007/s10669-011-9370-5.

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012) "A Review of Sequential Extraction and Instrumental Techniques for Heavy Metals Speciation in Soil and Sediment Matrices. ***Open Access Scientific Reports***. Published by Omics group. 1(3): 181. DOI: 10.4172/Scientific reports.181.

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012) "Development of an Analytical Methods for the Speciation of Organotin Compounds in Seawater, Sediment and Mussel's Samples Using GC-FPD and GCMS-TOF. ***Polish Journal of Environmental Studies. Published by Hard Publishing Company, Poland.*** Vol. 21, No. 6***: 1743-1753.***

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012) "Mineral Analysis of an Artificial Pond Sediment Samples from Western Cape Province, South Africa. Manuscript accepted on 26 August, 2012 by **International Journal of Physical Sciences**. 7(34): 5304-5307.

Okoro, H.K, Fatoki, OS, Adekola, F.A, Ximba, B.J, Snyman, R.G Mdokwana, BW (2013). "The Effect of Particle Sizes on Metal Accumulation in the Marine Sediments of Cape Town harbour, South Africa. ***Journal of the North Carolina Academy of Science.*** *Article in press.*

Okoro, H.K, Fatoki, OS, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012). Fractionation, Mobility and Multivariate Statistical Evaluation of Heavy Metals in Marine Sediments of Cape Town Harbour, South Africa." Manuscript Submitted to ***Arabian Journal of chemistry.*** **Elsevier, Science Direct**. Revised Edition Submitted.

Okoro, H.K, Fatoki, OS, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012). "Geochemical Assessment of Sediment from Cape Town Harbour, South Africa." Manuscript Submitted to ***International Journal of Environmental Science and Technology, Springer***, Revised Edition Submitted.

Manuscripts under Preparation

Okoro, H.K, Fatoki, OS, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012). Measurement of lysosomal membrane stability of the black mussel *Mytilus galloprovincialis*, after TBT exposure, using the neutral red retention time bioassay. Under preparation.

Okoro, H.K, Fatoki, OS, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012). Physico-chemical characteristics and One year monitoring of heavy metal pollution in Seawater from Cape Town Harbour and their Seasonal Variation. ***Fresenius Environmental Bulletin, Germany.*** Manuscript *Under* review.

Okoro, H.K, Fatoki, OS, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012) Spatio-temporal Variation of Organotin Compounds in Seawater and Sediments from Cape Town Harbour, SouthAfrica using Gas Chromatography with Flame Photometric Detector (GC-FPD) . ***Arabian Journal of chemistry.*** **Elsevier, Science Direct**. Manuscript *under* review

Book Chapter

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2014) ".Environmental and Clinical Toxicology of Organotin Compounds" ***Encyclopaedia of Toxicology, Third Edition.*** Chapter in print.

International Conference presentation

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2011) "Mineral Analysis of Lake Sediment Samples of Pentech, Cape Town, South Africa Proceeding Of the Bondo University College, Kenya and Cape Peninsula University of Technology, South Africa (BUC-CPUT, 2011) Joint Conference on Food Security and Environmental Sustainability in a world of changing Climates pp 51- 52, Anyango B and Ndakidemi P (editors), Published by Bondo University College, Kenya.

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2011) "An assessment of Heavy Metal Pollution in Marine Sediments of Cape Town Harbour, South Africa" Proceeding Of the University of Ilorin, Nigeria and Cape Peninsula University of Technology, South Africa (UNILORIN- CPUT, 2011). Proceeding, under review.

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2011) "Mineralogy and heavy metal mobility of Marine Sediment of Cape Town Harbour, South Africa". SETAC MEETING, BOSTON, USA.

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012) "*In situ* Derivatisation Method for the analysis of Organotin compounds in Environmental Samples "Proceeding Of the University of Ilorin, Nigeria and Cape Peninsula University of Technology, South Africa (UNILORIN- CPUT-Bondo Conference. 2012).

Okoro, H.K, Fatoki, O.S, Adekola, F.A, Ximba, B.J, Snyman, R.G (2012) "Hemocytic lysosome response in the mussel *Mytillus galloprovincialis* after exposure to Tributyltin. Society of Environmental Toxicology and Chemistry (SETAC). 34TH Annual Conference. Long Beach, California, USA.

CHAPTER ONE

Introduction

1.1: Background

Organotin compounds are organic derivatives of tin (Sn^{4+}) characterised by the presence of covalent bonds between three carbon atoms and a tin atom. They are designated as mono, di, tri and tetraorganotin compounds with the general formula (n-C_4H_9), Sn-X, where X is an anion or a group linked covalently through a hetero-atom (Dubey and Roy, 2003). Organotin pollution in the aquatic environment is of global concern. Two triorganotin compounds, tributyltin (TBT) and triphenyltin (TPT) are toxic to aquatic life (Fent, 1996) and are used worldwide, not only as biocides in antifouling paints, but also as preserving agents for wood and timber, and as fungicides in agricultural activities. This results in direct release into the water with consequent uptake and accumulation in aquatic fauna (Harino *et al.,* 2000; Okoro *et al.,* 2011a). Due to its widespread use as an antifouling agent in ship boat and paints, organotin is a common contaminant of marine and freshwater ecosystems.

Organotin studies became of interest when antifouling paints were realised to be responsible for the worldwide decline of marine molluscs in coastal areas (Blanca, 2008). The first hints dated back to early 1970's when the phenomenon of imposex was reported for *Nucella lapillus* in the UK (Blanca, 2008). In awareness of the undesired impacts of TBT, efforts have been undertaken globally in order to find a solution to this problem and legal requirements have been enforced to protect the aquatic environment. Thus, the use of TBT in small boats was prohibited in many countries since the mid-1980 (Konstantious and Albanis, 2004, Okoro *et al.,* 2011b). Many analytical methods for organotin compounds have been developed (Morabito and Quevanviller, 2002). The most successful ones are those that involve separation of TBT and its degradation products by gas chromatography, due to their high resolving power and easy coupling to sensitive and selective detectors, as mass spectrometry (Federico et *al.,* 2007). Organotin compounds have been reported to have various effects on aquatic organisms and humans. These include larvae mortality (Bella *et al.,* 2005) and impairment in growth, development, reproduction and survival of many marine species (Haggera et *al.,* 2005).

Several animal experiments have suggested that the spectrum of potential adverse chronic systemic effects of organotins in humans are quite broad and includes primary immunosuppressive, endocrinopathic, neurotoxic metabolic, and enzymatic activity, as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary gastrointestinal, blood dyscrasias, reproductive/ developmental, liver, kidney, bio-accumulative and possibly carcinogenic activity (WHO-IPCS, 1999; EU-SCOOP, 2006; Nakanish, 2007). The fate of

organotin compounds and chemical characteristics of heavy metals have been investigated in developed countries. Limited studies have been considered to be the prevalence of organotin compounds in South African harbours.

1.2: Statement of the research problem

Ships are often painted with antifouling substance, i.e. tributyltin (TBT), to protect them from rusting in order to extend their life span, but these substances leach into marine water especially in high traffic areas such as harbours and marinas and usually affect aquatic animal life adversely. South Africa has one of the world's busiest shipping routes and the ever increasing activity in South African harbours has made it imperative to have reliable data on the chemical speciation of organotin compounds in conjunction with biomonitoring. Organotin contamination has been recognized as pollution problem in some major South African harbours. Imposex in marine invertebrates due to TBT exposure has been reported in water from Durban and Richards Bay harbours and Knysna lagoon (David and Anisha, 2003). TBT and triphenyltin (TPT) pollution has been reported in environmental water samples collected from industrially polluted areas in South Africa, sea water from Port Elizabeth harbour as well as river and dam water samples from the areas around Johannesburg (Ewa *et al.*, 2004).

In Johannesburg, high concentrations of dibutyltin (DBT), monobutyltin (MBT) and TBT were recorded in groundwater and sediment samples. Bioaccumulation of organotins was also identified in algae and plant samples (Hermogene *et al.*, 2009). Cape Town harbour is used as the study area. This harbour is one of the busiest ports in South Africa. It handles the largest amount of fresh fruit for export and has a major repair and maintenance facility which is used by several large fishing boats and the West African oil industry. The harbour is located within the coordinates of 33°54'S 18°26'E. The port has evolved greatly over the centuries and currently consists of several main docks. The Ben Schoeman Dock is the largest outer dock of the port, where the container terminal is situated. The sediment samples collected at this site were very muddy. The Duncan dock is the smallest and the older inner dock. It contains the multipurpose and fruit terminals as well as a dry dock, a repair quay and a tanker basin. Both water and sediment samples at this site were very muddy and oily. The synchrolift dry dock is where the ships are lifted up for repair. There is therefore the need for comprehensive research on environmental assessment of heavy metals and organotin compounds in Cape Town Harbour; monitoring, geochemistry and toxicity, which has not been studied before.

1.3: Research questions

1. Are organotin compounds present in Cape Town Harbour?
2. If confirmed, at what concentrations are the various species of organotin compounds present in Cape Town Harbour?
3. Is TBT toxic to the mussel *Mytilus galloprovincialis*?
4. How does TBT bioaccumulate in marine mussels?
5. What is the toxicity of TBT to mussels?
6. Can this toxicity be measured by means of the NRRT assay?
7. Can lysosomal destabilization be used as biomarker of TBT exposure in these mussels?
8. What are the concentrations and environmental impacts of heavy metals in Cape Town Harbour?
9. What are the mineral phases present in the Cape Town Harbour?
10. How does the physicochemical parameter of water and sediment samples in Cape Town Harbour varied seasonally?

1.4: Research objectives

1.4.1: Broad objectives

The broad objective is to investigate methods for organotin analyses with a view to improve on them and using the improved method to analyse OTCs in water and sediment in Cape Town harbour and then investigate the use of biomarker to test for the toxicity of these compounds using mussel *Mytilus galloprovincialis*. In addition, the geochemistry of the sediment, visa a viz speciation, mobility and bioavailability of the metals in sediment and heavy metals in seawater will be monitored.

1.4.2 : Subsidiary objectives

The study will focus on chemical speciation and toxicity assessment of organotin compounds in Cape Town harbour. The major objectives of the study are given below:

- To assess spatial distributions of chemical components especially (TBT and TPT) in marine water and associated sediments. This would allow interpretation in terms of relative degrees of contamination and location of sources by determining total concentration and concentration of TBT and TPT in sediments.
- Determination of organotin compounds in marine water.
- Chemical speciation of organotin compounds in sediment, to support the effects of contaminants on marine invertebrates.

- To assess temporal changes in chemical composition and physical properties of surface sediment at a specific location through repeated sampling. This would detect changes in sediment quality.
- Determination of TBT accumulation in mussels under laboratory condition.
- Determination of the toxicity of accumulated TBT on lysosomal membrane integrity of Haemocytes of the mussels.
- Geochemical assessment of sediment in Cape Town Harbour.
- Monitoring of heavy metals and organotin compounds in Cape Town Harbour and their seasonal variations.
- Assessment of seasonal variation of some physicochemical parameters in both water and sediment samples from Cape Town Harbour.

1.5: Delineation of the research

There is a generally limited study on the environmental assessment of heavy metals and organotin compounds in many African harbours, and in particular Cape Town Harbour, while yet there is vast increase in docking of ships painted with organotin – containing paints. Water and sediment samples were collected from Cape Town harbour for organotin and heavy metals analysis. Mussels' (*Mytilus galloprovincialis)* were sampled from a clean site at "Scarborough" for biomarker studies. However, in the course of this research study, tests for the toxic effects of heavy metal were not investigated due to time limitation. The technique used for toxicology study was limited to one biomarker, others were not investigated. This study was limited to Cape Town harbour.

CHAPTER TWO

2.1: LITERATURE REVIEW

Organotin compounds (OTCs) have been extensively used in boat paints since 1960 because of their excellent and long lasting antifouling properties. A considerable number of studies have been conducted on the effects of organotins on aquatic organisms, their concentration and their distribution in aquatic environments. The results show that organotins have high toxicity towards aquatic organisms (Okoro *et al.,* 2011b). Owing to the potential environmental accumulation and harmful biological effects, OTCs are of growing public concern (Chen-Fengchem *et al.,* 2010, Okoro *et al.,* 2011b). OTCs such as tributyltin and triphenyltin are used mainly in antifouling paints for ship hulls to inhibit growth of algae, barnacles or mussels which are killed upon contact with the paint. In addition, butyltins are used as fungicides, biocides, pesticides, biocides, wood preservatives and stabilizing agents in polymers and catalysts (Harino *et al.,* 2003; Puri *et al.,* 2004).

Owing to the usefulness of organotins as antifouling agents in boat paints, they are common contaminants of marine and freshwater ecosystems. Fent and Muller (1991) detected concentrations of selected organotin species in a wastewater treatment plant in Zurich, Switzerland. It was discovered that municipal wastewater and sewage sludge contain considerable amounts of organotin species: TBT), butyltins (BT), dibutyltins (DBT), and monobutyltins (MBT). MBT and DBT occurred as degradation products of TBT, and they are known to have entered the treatment plant as a contaminant of municipal wastewater. Moreover, the leaching and weathering of polyvinyl chloride (PVC) materials that contain OTCs may also result in their release on a large scale (Becker *et al.* 1997).

Organotin first became a topic of broad interest when it was discovered that antifouling paints were causing the decline of coastal marine molluscs. Such reports first surfaced in the 1970's, when the phenomenon of imposex was reported for *Nucella lapillus* in the UK (Blanca 2008). As awareness of the effects of TBT has grown, global efforts to address the problem have increased, and measures have been taken by authorities to protect the aquatic environment from organotins. Hence, the use of TBT on small boats was prohibited by many countries beginning in the mid-1980s (Konstantious and Albanis, 2004). Because detection of environmental contaminants is so critical to their regulation, many methods have been developed to analyze for the OTCs in environmental media (Morabito and Quevauviller 2002).

The most successful methods are those that involve separation of TBT and its degradation products by gas chromatography (GC) GC is sensitive and has both high resolving power, and selective detection, when coupled with mass spectrometry (Delucchi *et al.,* 2007). Sentosa *et al,.* (2009) used an ion-pair reversed phase chromatography (IR-RP)

technique to analyse for speciation of DBT, TBT, and triphenyltin (TPhT). These three species were successfully resolved using an ion-pair-reversed chromatography column. The eluates were detected on line by using a hydride generation-quartz furnace atomic absorption spectrometry (HG-QFAAS) method. The eluent consisted of a mixture of methanol, water and acetic acid that had a composition of 80: 19:1, and contained 1.0 mol L^{-1} of decane sulfonate acid as the ion pairing reagent. The pH of the eluent was adjusted to 1.0 mol L^{-1} H_2SO_4. All species were successfully resolved under these conditions. The capacity factors (k^1) for DBT, TBT, and TPhT were 0.27, 2.54 and 5.92, respectively. The resolution (R_s) values for DBT-TBT and TBT-TPhT were 9.76 and 3.50, respectively. These values demonstrate the effectiveness of this chromatographic system to resolve the OTCs.

Aquatic organisms exposed to the OTCs have shown various effects. In many marine species, such effects include larval mortality (Bella *et al.,* 2005a), and impairment in growth, development, reproduction and survival (Haggera *et al.,* 2005). Moreover, the results of several experiments have indicated that there is or may be a spectrum of potential adverse chronic systemic effects of organotin exposure in animals and humans. The type of damage that has been sustained by exposure to organotin in animal testing includes immunosuppression, endocrine effects, neurotoxic effects, and effects on enzymatic activity. In addition to being bioaccumulative, exposure to organotins may also produce the following types of damage: ocular, dermal, cardiovascular, pulmonary, gastrointestinal, blood dyscrasias, reproductive-developmental, liver, kidney, and possibly carcinogenic effects (WHO-IPCS 1999; EU-SCOOP 2006; Nakanish 2007). Although the fate and chemical characteristics of the organotin compounds have been much investigated in developed countries, only limited data are available from Africa.

2.2 : Occurrence of organotin compounds

Organotin compounds or stannes occur in nature as chemical compounds based on tin with hydrocarbon substituent. Organotin chemistry is part of the wider field of organometallic chemistry (Sander *et al.,* 2005). The first organotin compound was diethylin dioxide, discovered by Edward Frankland in 1849. An organotin compound is commercially applied as a hydrochloric acid scavenger or heat stabilizers in polyvinylchloride (PVC) and as a biocide. Tributyltin oxide (TBTO) has been extensively used as a wood preservative. TBT compounds are used as marine ant-fouling agents and concerns over toxicity of these compounds [some reports describe biological effects to marine life at a concentration of 1 nanogram per litre] have led to a worldwide ban by the International Maritime Organization (IMO). Butylin trichloride is used in the production of tin oxide layers or glass bottles by chemical vapour deposition (Sander *et al.,* 2005). The physical characteristics of some OTCs are presented in Table 2.1

Table 2.1: Physical properties of selected organotin compounds

Organotin compounds	MeltingPoint (°C)	BoilingPoint (°C)	Density (g/cm^3)	Solubility (mg/dm^3
Bu_4Sn	-97	145/1.3kPa	1.06	n.i
Bu_3SnCl	-16	172/3.3kPa	1.21	50[b] 5-17[c]
Bu_2SnCl_2	39-41	135/1.3 kpa	-[d]	4-50[b] 92[+]
$BuSnCl_3$	n.i[a]	93/1.3kPa	1.69	
Me_3SnCl	37-39	154	-	n.i
Me_2SnCl_2	106-108	188-190	-[d]	20 000[b]
MeCl3	48-51	171	-	n.i

[a] n.i. = no information

[b]Solubilty in seawater

[c] Solubility in distilled water

(Adapted from Okoro et al., 2011a)

2.3: Routes of Human Exposure to the Organotins

The OTCs constitute a large class of compounds that have widely varying properties and that have been used for many purposes. The global production, in 2003, was approximately 40,000 t (EVISA 2010). Annual production at such levels, the wide spread use of the OTCs, and their high stability in marine water have led to their presence as contaminants in various ecosystems. Consumption of contaminated drinking water, beverages, and, in particular, marine food is an important route of human exposure to TBT (Forsyth and Jay 1997; Azuela and Vasconcelos 2002; Chieu *et al.,* 2002). Marine fishery products have been reported to contain high concentrations of OTCs. Therefore, the human diet is expected to have some amounts of the OTCs that will result in human tissue and blood residues (Lo *et al.,* 2003; EFSA 2004; ATSDR 2005; EU-SCOOP 2006). Recent results have shown that fish and fish products are generally the main source of OTCs in the diet. They were detected in whole blood samples of fishermen and their family members, and an association existed of the levels found with age, gender, and level of fish consumption (Pann *et al.,* 2008). These researchers concluded that their results give strong support to the hypothesis that fish constitute the main source of TPhT for humans in Finland. A general source of OTCs for human exposure is represented in figure 2.1.

Sadiki and Williams (1999) analyzed Canadian drinking water samples that had been distributed through PVC (polyvinylchloride) pipes. These authors confirmed the presence of OTCs in some drinking water samples collected from residential houses and commercial

buildings that were supplied by recently installed PVC piping. The contamination levels detected ranged up to 291 nanogram (ng) (Sn) L^{-1} MMT (Monomethyl), 49.1 ng (Sn) L^{-1} DMT (Dimethyl-tin), 28.5 ng (Sn) L^{-1} MBT and 52.3 ngL^{-1} (Sn) DBT. Takahashi *et al.* (1999) reported that several household commodities composed of polymethane, plastic polymers and silicones, such as diaper covers, sanitary napkins, certain brands of gloves, cellophane wrap, sponges and baking parchments, contained amounts (up to the μgg^{-1} level) of several organotin compounds. DBT was detected in treated turkey livers at levels between <0.2 and 6 μgg^{-1}, when DBT derivatives were used as an anthelmintic and coccidiostat in poultry production (Tsuda *et al.*, 1995).

In the UK, a survey showed that organotin levels were generally low in commercial species sampled from many locations throughout the country, and it was suggested that levels found did not present a health risk (FSA 2005). Lo *et al.*, (2003) conducted a study in Germany, using eight human volunteers (4 males and 4 females; aged 18-54). The serum of the tested individuals exhibited levels of organotin that were below the limits of detection, and TBT and TPhT were found at concentration ranges between 0.02-0.05 μgL^{-1} and 0.17-0.67 μgL^{-1}, respectively. Alzieu (2000) reported that contact exposure to TBT causes irritation of the eyes and skin, potentially leading to severe dermatitis. Because of these properties it is difficult to guarantee a safe environmental level for TBT. Therefore, use of TBT as a biocide in aquatic systems may well be incompatible with the protection of the ecosystem, and with certain marine activities such as oyster farming.

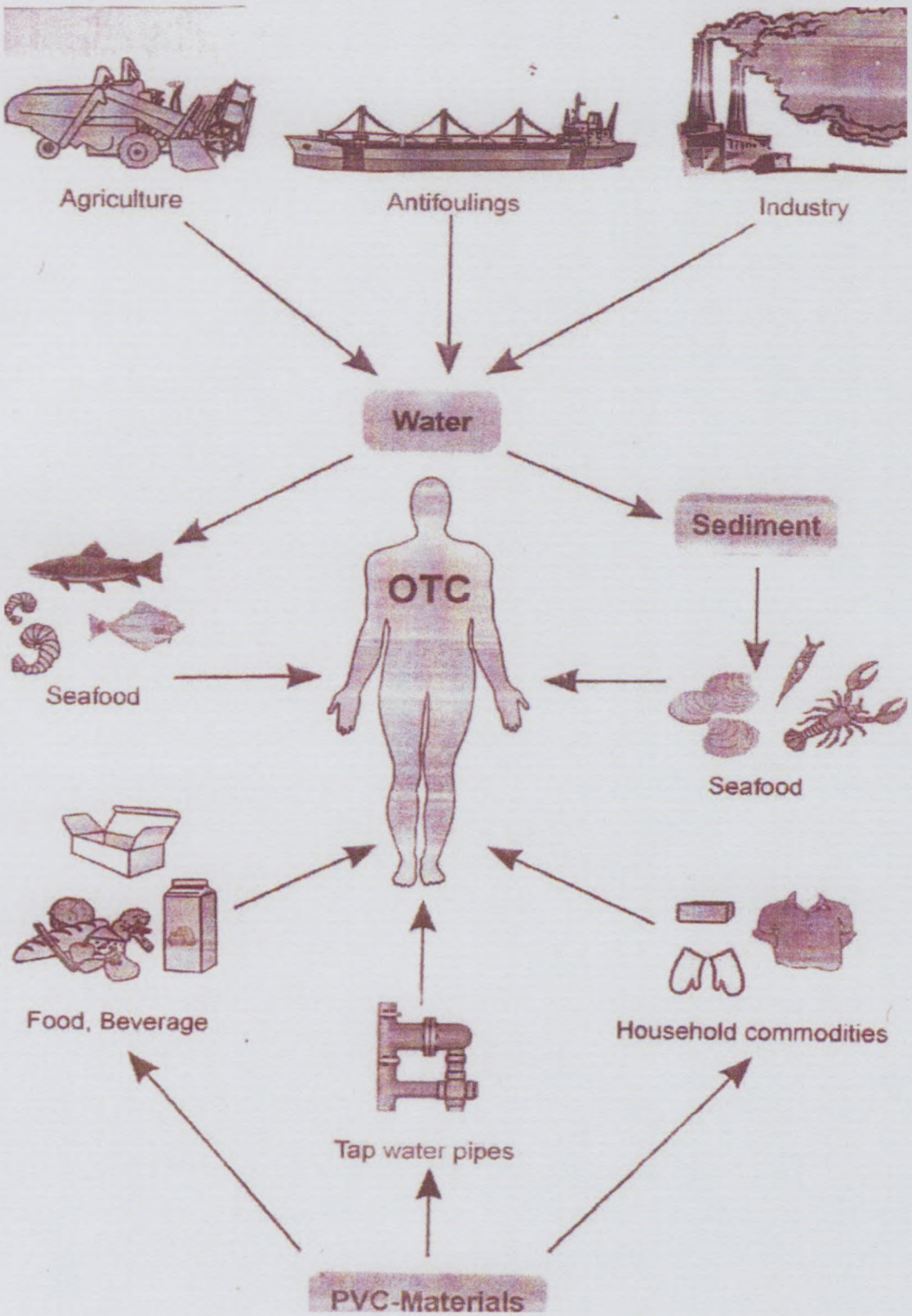


Figure 2.1: General sources of organotin compounds for human exposure

(Adapted from M. Hoch, 2001)

2.4: Distribution of the Organotins in the Environment

Because of the extensive use of organotins in numerous human activities, large amounts of the OTCs have been introduced to various ecosystems (Blunden and Evans 1990). Significant concentrations of the organotins and their metabolites have been detected in all phases of the aquatic environment: waters, suspended matter, sediments and biomass. The levels of organotins detected in the atmosphere are very low (Blunden and Evans 1990). Among the OTCs, even trace levels in the environment for TBT may be of concern, because it has been considered among the most hazardous compounds to marine organisms (Wagner 1993; Maguire 1996).

2.4.1: Organotin in Aquatic Systems

OTCs are of concern, because of their high toxicity, widespread use, direct input into the environment and their relatively high persistence. The OTCs enter the aquatic system by many routes. To date, organotin research has been restricted mainly to regions having high shipping volumes, harbours and/or shipyards, because the primary ways in which organotins reach the environment is through use as antifouling agents. TBT in antifouling paints is directly emitted into water, resulting in contaminated water, marine sediments, lakes and coastal areas (Hoch 2001). As expected, the butyltins have also been detected as residues in marine mammals. The concentrations of hepatic butyltin reported in fireless porpoise, collected from the Seto Island Sea, Japan, were as high as 10,000 ngg^{-1} wet wt (wwt), whereas the levels in crustaceans taken from the Japanese coastline ranged from 110 to 5200 ngg^{-1} wwt. Evidence exists to show that legislation introduced to govern the use of TBT in antifouling paints has reduced aquatic concentrations of this contaminant (Dowson et *al.*, 1993; Fent and Hunn 1995). Datas reported in different regions in the world is shown in table 2.2.

Table 2.2: Butyltin compound in seawater (ng SnI^{-1}) reported for several regions in the world

Sampling location	Year	MBT	DBT	TBT
American harbours and marinas				
West and east cost, Canada	1995	<d.1, -460	<d.1,-270	<d.1,-500
Asian an Oceania harbour and marinas				
Coast , Korea	1997-1998	<d.1-13.4	<d.1-22.3	<d.1,-4.5
North coast of Kyoto, Japan	2003	2.5-23	2.1-13	3.9-27
European harbours and marinas				
South west coast , Spain	1993	<d.1.-51	6.8-20	9.1-79
South east coast , France	1998	-	-	<0.015-0.12
Coastal waters , Greece	1998-1999	<d.1.-19	<d.1.-159	<d.1.-70
North west coast , Spain	Not provide	0.8-11.6	0.3-33.7	0.4-196.6

(Adapted from Okoro et al., 2011a)

2.4.2: Organotin in Sediments

Triorganotin compounds have low aqueous solubility and low mobility and are easily adsorbed onto suspended particulate matter (SPM). The deposition of SPM leads to the accumulation of considerable amounts of trisubstituted organotins and their degradation products in sediment (Hoch 2001). Several studies have been conducted on organotin pollution of river, lake and harbour sediments. Brack (2002) investigated OTCs in sediments from the Goteborg harbour in Sweden, and reported that their levels ranged from 17 to 366 ng/g dry weight for TBT and from 1.5 to 71ng/g dwt for TPT. These results were similar to those recorded from other harbours and marinas, and from an earlier study in the Goteborg harbour, which is located in the estuary (Brack 2002). DBT, MBT, DPT (diphenyl-tin) and MPT (monophenyltin), which are the degradation products of TBT and TPT, were also found in this harbour. TBT concentrations are the highest in the inner harbour and in the upper ca. 10cm sediment layer. This indicates that there is a risk of TBT mobilization from the sediment surface, which may be exacerbated by the frequently disturbed harbour environment. Table 2.3 presents various datas reported worldwide on BT in sediments.

Takahashi *et al.,* (1997) studied the chemical speciation of OTCs that exist in sediments at a Marina in Tokyo, Japan. These authors reported that >20 OTCs, including biodegraded ones, existed at the sampled site, and their identity was confirmed against authentic standards using gas chromatography mass spectrometry (GCMS) and a GC atomic emission detection (GC- AED) system. Eleven OTCs were found in the tributyltin-trichloride (TBTCl). Among them were unexpected OTCs, such as di-n-butyl (2-methylhexyl) tin chloride and di-n-butyloctyltin chloride

.

Table 2.3: Butyltin compounds in sediments around the world

Sampling Location	Year	MBT	DBT	TBT
American harbours and marina				
West and east cost, Canada	1995	<d.1-330	<d.1-1100	<d.1-5100
Crystal Lake, US	2001-2003	21.3-320[a]	59-350[a]	1.5-14,000[a]
Asian an Oceania harbour and marinas				
Port of Osaka, Japan	1995-1996	<d.1	<d.1	10-2100
Coast, Malaysia	1997-1998	5.0-360[a,b]	3.8-310[a,b]	2.8-1100[a,b]
Great Barrier Reef World Heritage Area, Australia	1999	<d.1-1.61	<d.1-7.1	<d.1-1275
Alexandra harbour, Egypt	1999	<0.1-186	<0.1-379	1-2076
Mumbai harbour, India	2000-2001	<d.1-131[b]	n.a	4.5-1193[b]
Fishing harbours, Taiwan	2001-2004	n.a	n.a	2.4-8548[b]
Sanricu coast, Japan	2005	<d.1-3300	<d.1-3400	2-14,000
West coast ,France	1993	25-74	9-29	7-30
River Thames, UK	1994	12-172	12-219	1-60
Tagus Estuary , Portugal	1998-1999	n.a	n.a	5.4-35[b]
North west sicilian coast, Italy	1999-2000	<d.1	<d.1	3-27

(Adapted from Okoro et al., 2011a)

The half-life of TBT in sediments is in the range of years. The accumulation of organotin on suspended particulates or sediments makes them available to filter- or sediment-feeding organisms. Resuspension of contaminated sediment offers an additional risk to aquatic organisms (Hoch 2001). The accumulation in sediments of butyltin and phenyltin species constitutes an ongoing pollution source, because residues of these compounds are slowly released into aquatic systems (Ceulemans and Adams 1995; Kuballa *et al.,* 1996 Chiron *et al.,* 2000).

2. 4.3: Organotin in Organisms

Previous studies have revealed that high concentrations of toxic organotin compounds exist in some fish and in aquatic invertebrates, such as gastropods and filter-feeding organisms. The presence of high concentrations of the toxic organotin residues in invertebrates results in imposex (possession of male features by female organisms). Little is known about the accumulation and toxic effects of organotin in high tropic-level vertebrate predators; hence, their ability to disrupt endocrine system of organisms worldwide is of concern. Humans are also exposed to the OTCs. The major route of such exposure is through food ingestion or exposure to household materials containing or contaminated by the organotins. (Hoch 2001; Okoro *et al.,* 2011b). Hu et *al.,* (2006) studied tropic magnification of TPT in a marine food web of Bohai Bay, North China. Five benthic invertebrate species and six fish species were investigated. The concentrations f TPT detected in marine fish were, as expected, higher than those of TBT. A positive relationship was also found between trophic level and the concentration of TPT, indicating tropic magnification (TMF) of TPT in this food web.

Analysis of organotin residues in water and surface sediment samples from the bay revealed low environmental inputs of TPT, which indicated that the high concentrations of TPT found in fish from Bohai Bay resulted from food web magnification. The species in the study were primary producers (phytoplankton/Seston and Zooplankton), and comprised the following: five invertebrates: crab (*Portunus trituberculatus*), burrowing shrimp (*Upogebia sp*), short-necked clam (*Ruditapes pluillippinarium),* veined rapa whelk (*Rapama venosa),* and bay scallop (*Argopecter irrandians*). The other six species included the Lesser weever *Echiichthys vipera* catfish, (*Chaetu-richthys stigmatias*), bantail flathead (*Platycephahis indicus*), flower croakers (*Nibea albiflora*), wolfish (*Obentamblyopus rubicundus)* and mullet *Mugil cephalus.* Hu et *al.,* (2006). Zhang *et al.,* (2003) worked on the butyltins in sediments and biota collected from the Pearl River Delta, in South China. Both sediment and biota samples were collected and assessed using GC-AED analysis. The concentrations of TBT detected in the sediments ranged from 1.7 to 379.7 ng/g. Shipping activities in the bay were thought to be responsible for the spatial distribution of the detected residues. A good linear

relationship was observed between the concentrations of DBT, TBT and MBT samples taken from the Pearl River and associated estuary, and from the West River, suggesting a common source for the residues. All TBT concentrations in fish, mussel, and shrimp samples, which were collected in the study, retained residues that were below the seafood tolerable average residue level (TARL).

Meng-Pei *et al.,* (2003) investigated the accumulation of OTCs in pacific oysters (*Crassostrea gigas),* and both butyltin and phenyltin residues were quantified in this species. These oysters were collected during different seasons at several aquaculture sites, located along the west coast of Taiwan. BT compounds were detected in oyster samples at all but one site. MPT and DPT were not detected in any of the samples. The average concentration range of MBT, DBT, TBT and tetrabutyltins (T_4BT) in the sampled oysters were from non-detectable (n.d) - 406 ± 12.7, n.d- 280.9 ± 15.3, n.d- 417.2 ± 11.2 and n.d - 85.8 ± 8.3 ngg^{-1} (wwt), respectively. The concentration of TBT compounds detected in the oysters varied both spatially and temporally.

Lisicio *et al.,* (2009) used two different analytical methods to determine levels of OTCs in marine organisms. Both methods involved extraction by tropolone, derivatization, and purification on florisil, followed by analysis using GC-MS. The main difference between the two procedures used was in the derivatization step: one employed a Grignard reagent (n-pentylmagnesium bromide), whereas sodium tetraethylborate (STEB) was used in the other method. All compounds analyzed showed lower detection limits with derivatization using STEB, particularly with TBT. Lisicio *et al.,* (2009) also performed an *in vivo* experiment on TBT. He exposed one mussel species (*Mytilus galloprovincialis*) to known amounts of TBT for several days. Both control and contaminated tissues were then analyzed using the STEB derivatization method. Results indicated bioaccumulation of TBT, which accumulated especially in the gills.

Albalat *et al.,* (2002) assessed the levels of organotin pollution along the Polish coast (Baltic Sea), using mussels and fish as sentinel organisms. TBT, MBT, and DBT and TPT were the target compounds for which monitoring was performed. The bioaccumulation patterns found for the butyltin and phenyltin compounds varied substantially. The butyltins were detected in mussels at all sampled stations. Mussels sampled in the Gulf of Gdansk had the highest residue levels (68ng/g wwt, measured as Sn) and had elevated TBT/DBT ratios, which suggested that there had been recent inputs of TBT to the area. Additionally, flatfish were sampled in the Gulf of Gdansk, and several tissues (liver, digestive tube and gills) were individually analyzed. Although TPT residues were not detected in mussels in the Gulf of Gdansk, they were present in fish tissues.

The highest organotin concentrations were observed in the liver (69 ng/g wwt, measured as Sn) of fish caught near the port at Gdansk. Relatively high concentrations were

observed in the digestive tube, suggesting that organotin-contaminated food had been ingested, and food sources comprised an important uptake route of those compounds by mussels. Cooke (2002) studied the effect of organotins on human aromatase activity *in vitro*. TBT, at concentrations of 12 and 59 μM, and DBT at a concentration of 74 μM, inhibited aromatase activity in vitro. Comparison of various studies worldwide on BT compounds in biological samples is shown in Table 2.4.

Table 2.4: Butyltin compounds in biological samples worldwide

Sampling Location	Year	Biological Sample	MBT	DBT	TBT
American harbours and marinas					
Coast , Canada	1995	Mussel	<d.1. -708	<d.1, -1062	20-1198
	1996	Mussel	-	-	<1440[a,b]
Asian an ocean harbour and marinas					
Japan sea, Japan	1991	\Walley pollock	<3 [a]	<2.5 [a]	2.2-6.4 [a]
Bangladesh	1994	Fish	<5.6-170 [a,b]	<0.36-15 [a, b]	0.47-3 [a, b]
Aomori, Japan	1996	Fish	<d.1,-20[a, b]	<d.1, -50[a, b]	<d.1, -240 [a,b]
Coast, Korea	1997-1998	Vivalves	<d.1, -461	23-699	16-1610
Coast , Korea	1997-1998	Starfish	51-2860	8-139	7-323
Coast, Malaysia	1998	Fish	2.3-7.4 [a, b]	<1.3-13[a,b]	2.4-190 [a, b]
Aquaculutrearea, Taiwan	2002	Oyster	<3.3- 407 [a, b]	<3.9-281[a,b]	<3.8-417 [a, b]
North coast of Kyoto, Japan	2003	Mussel	0.8-2.9[a]	0.8-3.1[a]	0.8-11[a]
Coast, Vietman	2003	Clam	2.8-18	4.4-27	3.8-15
Coastline of Hong Kong, China	2004	T.clavigera	<d.1,-336	<d.1,-19.7	<d.1,-18
Coastline of Hong Kong, China	2004	T.luteostoma	<d.1, -51	<d.1. -8.5	3.8 -170
Sanrieu coast, Japan	2005	Mussel	4-32	3-92	3-287
European harbours and marinas					
Northwestern MediterraneanSpain	1996	Deep sea fish	<d.1-54 [a]	4.0 -67[a]	1.0-52[a]
River Elbe and North Sea	1993	Fish	<d.1,89[a,b]	<d.1, -55[a, b]	66-490 [a, b]
The Netherlands	1993	Fish	23-41 [a, b]	13-183 [a, b]	9.2-67 [a, b]
South west Coast, Spain	1993-1994	Oyster		59.3±21.3	269±96
Strait between Denmark and Sweden	1997	Vivalve	2.5-15 [b]		200-300[a,b]
Baltic Sea , Poland	1998	Mussel	<1.4-4.7[a]	<1.4-24[a]	2.2-39 [a]
South west coast, Spain	1999	H.trunculus	63	85	48
Coast, Portugal	1999-2000	Mussel	<7.9-41	<2.5-18	<5.7-489
Northwestern Sicilian coasts, Italy	1999-2000	H.trunculus	<d.1,-167	<d.1, -316	<d.1,-91
West coast, Portugal	2000	Mussel	<10-605	<10-345	11-789
Aegean Sea, Greece	2001-2003	Bivalves	<d.1,151	<<d.1-366	<5.7-489
North west coast, Spain	2005	Oyster	0.4-12.9	7.6-441	74-193
North west coast, Spain	2005	Mussel	52.8-96.1	20.2-25.7	52.8-96

MBT: monobutyltin; DBT; dibutyltin TBT: tributyltin; <d.1: below detection limit; n.a: no data available

[a] Wet weight

[b] ng organotin instead Sn

(Adapted from Blanca Antizar Ladislao, 2008).

2.4.4: Organotin in Soils

TPT acetate and TPT hydroxide have increasingly been used as soil-treatment fungicides worldwide to treat a variety of crops. Such treatments have resulted in increasing levels of TPT acetate and TPT hydroxide in soils. Few studies have been conducted in which the abundance and persistence of TPT in soil has been measured. Kannan and Lee (1996) conducted a study on the foliage and soils of Pecan trees after application of TPT hydroxide. Their study results revealed that total phenyltin (MPT, DPT, and TPT) levels in foliage and soils ranged between 72 and 76 $\mu g g^{-1}$ (Sn) dwt. In addition, TPT residues were reported in fish (blue gill, largemouth bass and channel cat fish) taken from a pond near a recently treated Pecan orchard (Visoottiviseth *et al.,* 1995). The vapour loss during field spraying of TPT hydroxide is negligible because of its low vapour pressure (1×10^{-7}mm Hg at 25°C). But TPT is photolytically degraded in soils only if it is near the soil surface, where light can penetrate (Visoottiviseth *et al.,* 1995).

2.4.5: Effects of Organotins in the Environment

The European Food Safety Authority (EFSA 2004) has assessed the health risk to consumers associated with exposure to the OTCs. It was concluded that the critical toxicological endpoint is immunotoxicity. Because different OTCs are similar to one another, they are grouped for risk assessment purposes. The tolerable daily intake (TDI) for the group was established as 250 ng/kg body wt, and applied to the sum of residues that contain TBT, DBT, TPhT and di-n-octyltin (DOT). Alzieu (2000) reported that contact exposure to TBT causes irritation of the eyes and skin, potentially leading to severe dermatitis. Because of these properties, it is difficult to guarantee a safe environmental level for TBT. This means that its use as a biocide in aquatic systems could be incompatible with protecting ecosystems, and preventing damage to certain marine activities, such as oyster farming.

OTCs produce various known effects on aquatic organisms when they are exposed to these substances. These effects include larval mortality (Bella *et al.,* 2005a), growth impairment, developmental and reproductive effects and survival reduction in many marine species (Haggera *et al.,* 2005). In addition, the results of animal experiments have suggested what the spectrum of potential adverse chronic effects to humans of the organotins may be. Among effects that could be damaging to humans are primary immunosuppression, endocrinopathy, neurotoxicity, metabolic effects, and effects on enzymatic activity. OTC exposure may also induce adverse effects to the eyes, skin, blood (dyscrasias), liver and kidney, as well as to the following organ systems: cardiovascular, upper respiratory, gastrointestinal, and reproductive /developmental systems. Moreover, there is a risk of bioaccumulation and possibly carcinogenicity from OTC exposure (WHO-IPCS 1999; EU-SCOOP 2006; Nakanish 2007).

2.5: Fates of Organotins in the Environment

There have been several investigations into how the OTCs are distributed, and degraded in the natural environment, and such information is both useful and important (Hoch 2001). The OTCs enter ecosystems after marine or agricultural applications or after industrial use and release. However, research to date has focused only on tributyl- and triphenyl-tin pollution, because these compounds directly enter the environment through industrial use of organotin biocides. Recently, sewage sludge, municipal and industrial waste water and landfill leachates have also been discovered to constitute major sources of environmental organotins (Hoch 2001). Once these compounds become ecosystem pollutants, they may persist for long periods. The period of persistence is a function of the status of various removal mechanisms. Removal mechanism include physical ones (adsorption to suspended solids and sediments), chemical means (i.e., chemical and photochemical degradation processes), and biological ones (i.e., uptake and biological degradation) (Hoch 2001)

2.5.1: Degradation

The degradation of organotins in the environment occurs as a progressive elimination of organic groups from Sn cations. As successive organic groups are removed, toxicity is generally reduced. Degradation is achieved by both biotic and abiotics factors. Photodecomposition by ultra-violet (UV) light is the most important abiotic degradation process. In aquatic and terrestrial ecosystems, biological processes are the most important factor effecting degradation of the OTCs. Research has shown that organotin degradation is mediated by microorganisms; however, little information is available about the mechanism by which such degradation occurs. Also lacking is an understanding of the mechanism by which microbes are tolerant to the OTCs, or the role played in degradation by anionic radicals (Dubey and Roy 2003). Biotic processes probably represent the most significant mechanisms by which TBT degradation occurs in soil, fresh water, marine and estuarine environments.

Research interest on the bioaccumulation and biodegradation of organotin in the water column, in sediments and in marine organisms has been stimulated by the paucity of data available in these areas. OTCs are known to be present in three main compartments of aquatic ecosystems: the surface microlayer, the water column and at the surface layer of bottom sediments (Clark *et al.,* 1988). TBT degrades rapidly to DBT and MBT, with half-lives of several days (Dubey and Roy 2003). The half-life value for the decline of TBT (0.03 μg^{-1}) from a clean water site was 9 and 19 days for light and dark treatments, respectively (Dubey and Roy 2003). A first-order multistep kinetic model was used to describe the sequential degradation rate and pattern for TBT to form DBT, MBT and tin (IV). Using this model, the

half-lives of TBT, DBT and MBT were 2.1, 1.9 and 1.1years, respectively (Sarradin *et al.*, 1995).

Abiotic degradation processes constitute other potential pathways for the degradation of TBT from soil, sediments and water columns. Such abiotic processes may attack the Sn-C bonds by several different processes. Examples are: UV irradiation-facilitated breakdown, chemical cleavage, gamma irradiation and thermal cleavage. Only UV radiation (300-350 nm), in which the energy level corresponds to about 300 $KJmol^{-1}$, is likely to cause direct photolysis of TBT. Because UV light does not penetrate deeply, photolysis is expected to occur only in the upper few centimetres of the water column (Clark *et al.*, 1988). Maureen and Willingham (1996) reported that TBT degradation process may be explained as a sequential loss of alkyl groups from TBT to form toxic inorganic tin, as shown in the following equation:

$$R_3Sn^+ \longrightarrow R_2Sn^{2+} \longrightarrow RSn^{3+} \longrightarrow \quad Sn\ (IV).$$

TPhT has low mobility, low solubility and a strong ability to bind to soil and sediment in the aquatic environment (Blunden *et al.*, 1986). For unbound organotins that can be reached by chemical action, chemical cleavage may be mediated by mineral acids, carboxylic acids and alkali metals. These agents are capable of heterolytically cleaving Sn-C bond, through both nucleophilic and electrophilic reactions (Blunden and Evans 1990). Albalat *et al.*, (2002) have studied the biodegradation of the organotins. They monitored levels of TBT, MBT, and DBT at 10 stations along the Polish coast (Baltic Sea). One mussel (*Mytilus edulis*) and one fish species (*Platicht flesus*) were used as sentinel organisms. The bioaccumulation patterns of butyltin and phenyltin compound varied substantially. BT compounds were detected in mussels from all sampled stations. TPT was not detected in mussel but was found in fish, which indicated that ingesting organotin-contaminated food was an important uptake route of these compounds in *P. flesus*. Paton *et al.*, (2006) investigated the microbial and chemical degradation and toxicity of phenyltin compounds in soil. These authors discovered that the degradation of organotins was significantly slower in sterile soils than in non sterile soils. In non sterilized soils, the half life of TPT was 27 and 33 days at amendment levels of 10 and 20 $mgkg^{-1}$ Sn, respectively. There was an increase in observed toxicity as the degradation of triphenyltin proceeded. This phenomenon proved that the metabolite formed is either more bioavailable or more toxic than is the parent compound, or both.

2.5.2: Bioaccumulation

Lipophilicity is a criterion for the environmental persistence of organotins. Among the organotins, TBT is considered to be an important pollutant because of its extreme toxicity to several organisms, and because of its tendency to bioaccumulate. Bacteria have been reported to display a remarkable ability to accumulate TBT. Marine bivalves are also able to accumulate significant amounts of TBT (up to 5 $\mu g g^{-1}$). However, fish and crustaceans accumulate much lower amounts, owing to their possession of efficient enzymatic mechanisms to degrade TBT (Laughlin 1996). Absorption in mice is also low, and TBT is mainly excreted unchanged via the faeces. Mammals and birds accumulate high levels of the butyltins in their organs and tissues (Iwata *et al.,* 1995). In mammalian species, TBT compounds may be metabolized to DBT and related metabolites. An undetermined amount of this compound is known to remain in fat, liver, and kidney (Adebayo *et al.,* 2003). Other researchers have undertaken studies to evaluate the bioaccumulation of organotins (Harino et al. 2005; Strand *et al.,* 2005; Azumi *et al.,* 2007). Similar results were also recorded by Adebayo *et al.,* 2003).

2.5.3: Sorption of the Organotins and their Biological Effects

In recent years, restrictions have been placed on the use of TBT on pleasure boats in Europe. Although considerable progress has been made in reducing TBT effects, they still continue to be observed in marine ecosystems. An essential source of contamination of TBT along the German North Sea and the Baltic Coast has been remobilization (by desorption) of the high TBT concentrations present in sediments (Langston and Popoe 1995). A comparison of the burden of TBT in sediments to which snails and mussels are exposed, gives rise to concern for conducting any future dredging and disposal of TBT-contaminated sediments (WWF 1995). Because suspended matter has a high affinity for OTCs, any perturbation of sediments by dredging may remobilize TBT, and thereby substantively increase TBT residue levels in the water column. Presently, desorbed or actively remobilized TBT-contaminated sediment in harbours and in some coastal areas constitutes the main source of biologically available TBT (Langston and Popoe 1995). Hongwen *et al.,* (1996) investigated adsorption behaviour of eight organotin species and Sn^{4+} ($SnCl_4$) on estuarine sediments. They found that adsorption of the organotins varies greatly, and depends on molecular structure. The order of adsorption coefficient for tin compounds in the studied sediment samples were: tetra > mono > di > triorganotins. Correlations of the log K values (using eight different structural parameters) showed that the electronic properties of the Sn atom constitute the principal factor controlling their adsorption behaviour. The mechanism by

which the organotins are adsorbed is mainly through an ion exchange process, and involves little lipophilic partitioning (Hongwen *et al.*, 1996). Hermosin *et al.*, (1993) reported the adsorption mechanisms for MBT to various clay minerals, and found that its adsorption capacity for all clays was higher than the corresponding cation exchange capacity (CEC value). Adsorption onto clay is important to the environmental distribution and fate of organotins, because research has shown that large proportions of organotin contaminants are associated with the clay fraction of particulate matter. Thus, soils and sediments may serve as traps for these toxic contaminants. Unfortunately, the number of studies conducted on the remobilization of adsorbed organotin from environmental media is still few (Hoch 2001).

2.5.4: Biomethylation

Methyltin compounds can be formed by processes that involve biomethylation. Several biotic and abiotic methylation agents exist. Methylcobalamin (CH_3B_{12}), the methyl co-enzyme of vitamin B_{12}, is a carbanion donor that is able to convert inorganic Sn (IV) to several methyltin species (Hoch 2001). Methylcobalamine has been demethylated by $SnCl_2$ in aqueous HCl solution in the presence of an oxidizing agent (Fe^{3+} or Co^{3+}) to form a monomethyltin species. Methyliodide (CH_3I) can also methylate tin species, whereas tin (IV) compounds do not react. Chemical or biological processes are capable of methylating inorganic tin (II), Sn (IV) and methyltin derivatives under stimulated environmental conditions. Recently, methylation of butyltin species in sediments has been reported (Hoch 2001), and may arise from biological methylation of anthropogenic butyltins in the aquatic environment. Selected possible reactions of Sn-C include the following:

$$2Bu_3Sn^+ \rightarrow Bu_2Sn^{2+} + Bu_4Sn \qquad (1)$$

$$Bu_2Sn^{2+} + Bu_3Sn^+ \rightarrow BuSn^{3+} + Bu_4Sn \qquad (2)$$

$$Bu_3Sn + Me^- \rightarrow Bu_3MeSn \qquad (3)$$

Biomethylation processes are of great ecological relevance, because some methylated metals have higher toxicity to aquatic organisms than does the inorganic metal (Hoch 2001).

2.6: Fate of Organotins in Marine Invertebrates

2.6.1: Bioaccumulation in Marine Invertebrates

Most research on TBT accumulation by marine invertebrates was concentrated on molluscs (bivalves) and crustaceans (decapods), because these groups dominate the ecological habitat and serve as important seafood resources (Laughlin 1996). Research conducted on TBT accumulation by marine invertebrates revealed that marine bivalves are able to accumulate significant amounts of TBT (up to > 5 μgg^{-1}) (Laughlin 1996). Azumi *et al.,* (2007) studied the accumulation of organotin compounds at aquaculture sites in Korea. High concentrations of butyltin compounds (mono, di, and tributyltin) were detected, especially in the gills, hepatopancreas, and digestive tracts of Sea squirt (*Halocynthia roretzi)*.

Meng-Pei and Shin-Mei (2003) investigated levels of OTCs in pacific oysters (*Crassostrea gigas*) collected from aquaculture sites. Butyltin compounds were detected in most samples, whereas no MPT and DPT compounds were detected. The average concentrations of monobutyl-, tributyl-, triphenyl- and tetraphenyl-tins ranged from non detectable (nd) to 406.6 ± 12.7, nd to 28.09 ± 15.3, nd to 417.2 ± 11.2 and nd to 85.8 ± 8.3 ngg^{-1} (wwt), respectively. The accumulation of OTCs also occurred in deep sea organisms, namely gastropods (*Collilоconcha nankaiensis*), sea cucumbers (*Psychropotes verrucosa)*, galatheid crabs (*Munidopsi Albatrossae* and *Munidopsis subsquamosa), and* bivalves *(Clyptogena Isubasa* and *Clyptogena nautilei)*. High concentrations of BT and PTs (phenyltin) were observed in gastropods and sea cucumbers. The composition of BT in deep sea organisms were calculated, and an increase in the MBT proportion was recorded, while a decline in DBT proportion was observed at higher trophic levels (Harino *et al.,* 2005). Accumulation of organotins in marine invertebrates has also been reported by Harino *et al.,* (2008).

The concentration of OTC in seven species of dolphin (bottlenose, finless porpoise, Indo-Pacific humpbacked, long-backed common, Pantropical spotted, spinner and striped), which were stranded on the coast of Thailand, were measured. The ratio of the average of BT and PT compounds in tissues and organs was 16:1; average residue levels in tissues and organs for the dolphins were 152 μgkg^{-1} and 62 μgkg^{-1}, respectively. The highest concentration of TBT was generally observed in the liver. No significant difference in the concentration of OTC between genders was observed. The concentrations of BTs in all organisms were high and following order: whales > dugongs > dolphins. The concentrations of PTs in whales were higher than those in dolphins and dugongs. In general, it has been observed that species with a high rate of uptake or a low rate of metabolic conversion and elimination display relatively high bioaccumulation ratios (Meador and Rice 2001).

2.6.2: Toxicity to Marine Invertebrates

TBT causes impairments in growth and development, and induces reproductive failures, shell anomalies and gel formation. It also causes chambering, high mortality, disturbs the energy metabolism of bivalves, and inhibits the activity of many enzymes. These effects reduce the survival of many species (Beaumont and Budd 1984; Haggera *et al.,* 2005). TBT, as early as the 1970s, was known to be very toxic to many aquatic organisms (Blabber 1970; Smith 1981). The high toxicity of TBT is attributed to its effects on mitochondrial function (Blabber 1970; Smith 1981). The embryonic and larval stages of marine invertebrates are less tolerant to toxicants than are adults, and this difference has been used to assess the biological quality of marine water and sediments (Fent and Muller 1991).

TBT is known to have other toxic endpoints (Horiguchi *et al.,* 1998), e.g., acute lethal toxicity in rock shell larvae (*Thais clavigera*). However, growth impairment is a much more sensitive endpoint for measuring exposure to TBT than is mortality (Meador and Rice 2001). TBT is known to inhibit oxidative phosphorylation, which affects cell metabolism by stimulating the production of adenosine diphosphate, and results in mitochondrial membrane malformation. TBT affected larval development of bivalves *(Clyptogena isubasa),* and caused sexual disturbances in gastropods (C*ollilоconcha nankaiensis*) at ngL^{-1} levels in seawater. At a level of 1.0 ngL^{-1} TBT caused masculinization in many female gastropods (*Collilоconcha nankaiensis*), a phenomenon known as imposex. It also limits cell division in phytoplankton and reproduction of zooplankton. At a level of 2 ngL^{-1}, TBT has been reported to induce shell calcification anomalies in the oyster *Crassostrea gigas*, and at 20 ngL^{-1} to disturb the reproduction of bivalve molluscs (Bella *et al.,* 2005a). Ruiz et *al.,* (1995) investigated the effect of TBT exposure on Veliger larval development of the bivalve *(Clyptogena isubasa). They* found that TBT contributed to the demise of clam populations by preventing successful and timely development of Veliger larvae. TBT also affects the abundance and relative growth rates of male and female Whelks around marinas (Gil *et al.,* 2000).

2.7: The Role of Biomarkers

Pollution of the marine environment is a global concern because of the adverse effects caused by various contaminants, whose levels are growing at an alarming rate. Residues of many contaminants, such as the OTCs, continue to enter the natural environment and continue to accumulate in many organisms. Therefore, it is crucial that means to track both the presence and effects of such contaminants be developed. Biomarkers offer one important way in which environmental contaminants effects can be monitored.

The idea behind biomarkers is not a new concept, but is a new name for a pre-existing monitoring principle (Adams 1990). Biomarkers are defined as the measurements of body fluids, cells or tissues that indicate, in biochemical or cellular terms, the presence of contaminants or the magnitude of the host response (Bodin *et al.,* 2004). According to Van Gestel and Van Brummelen (1996) "biomarkers" are any biological response to an environmental chemical that is measured inside an organism or its products (urine, faeces, hairs, feathers, etc.), and indicates a departure from the normal status. A response may result from a biochemical, physiological, histological and/or morphological (including appearance, pigmentation, surface deformation, etc) measurement of health, although behavioural effects are excluded. Hence, biomarkers cannot be used to measure effects in intact organisms, or cause affected organisms to deviate from their normal status (Van Gestel and Van Brummelen 1996). Therefore, one can discern that biomarkers are potentially sensitive tools of immense importance for measuring biological effects that affect environmental quality (Sarkar *et al.,* 2006).

Some authors claim that biomarkers may also be accommodated into whole animal studies (Ross *et al.,* 2002; Magni *et al.,* 2006), and may be specific to one pollutant, or may be altered in response to either pollutant effects or the presence of natural stressors (Pfeiffer *et al.,* 2005). What is certain is that they are potentially very useful as prognostic and diagnostic early warning tests and offer the potential of specificity, sensitivity and application to a wide range of organisms (Sarkar *et al.,* 2006). The use of properly researched biomarkers are not limited to laboratory use, but may be applied to field studies too. However, the initial development of biomarkers usually involves laboratory experimentation to first identify potential responses, and to establish causal mechanisms before application to field use (Sarkar *et al.,* 2006).

2.7.1: The Significance and Utility of Biomarkers

Biomarkers are used to evaluate the exposure effects to many different contaminants (i.e., metals, organic xenobiotics and organometallic compounds (Ross *et al.,* 2002, Depledge and Fossil 1994). The most significant features of the use of biomarkers are summarized below;

1. Biomarkers are a means to achieve sensitive detection of selected chemical stresses within organisms.
2. They generate insights on possible harmful effects that cannot be obtained from chemical analysis alone (Depledge and Fossil 1994).
3. They may be used to predict effects on invertebrate populations and communities (Lagadic *et al.,* 1994), and may help assess types or degree of environmental damage, or formulation of regulations to control such damage (Sarkar *et al.,* 2006).

4. They offer means to identify interactions between contaminants and organisms and measure sublethal effects (Sarkar *et al.,* 2006).

5. They offer alternative ways of detecting the presence of both known and unknown contaminants (Sarkar *et al.,* 2006).

6. They constitute a temporally and spatially integrated measure of the degree to which pollutants are bioavailable (Sarkar *et al.,* 2006).

7. They may be used to establish important routes of exposure by application to species from different tropic levels, and aid in designing strategies for intervention and remediation (Sarkar *et al.,* 2006).

2.7.2: Biomarkers for TBT in Marine Invertebrate

Useful biomarkers have been developed to help monitor the effects of contaminants in marine invertebrates. Among these are the following biomarkers that have been used to assess the toxicity of TBT: metallothionein induction, acetyl cholinesterase inhibition, imposex, lysosomal enlargement, lysosomal membrane destabilization, peroxisome proliferation, lysosomal activity, genetic or molecular biomarkers, TBT-sensitive immunological biomarkers, apoptosis induction, phagocytic index and amoebocytic index. Some of these biomarkers are more useful than others (Depledge and Fossil 1994; Ross *et al.,* 2002; Sarkar *et al.,* 2006). The following are detailed discussions of these biomarkers:

2.7.2.1: Methallothionein (MT) induction

MTs are cysteine rich peptides that exist in the cytosol and the nucleus and in lysosomes. They are non-enzymatic proteins that have low molecular weight, no aromatic amino acids and are heat stable (Olsson *et al.,* 1998; Roeva *et al.,* 1999). MT-like proteins has been reported in many aquatic invertebrates, but occur mainly in molluscs (Isani et *al.,* 2000). Mussels, used worldwide in environmental pollution assessment, are good candidates for monitoring MT for assessment of metal contamination (Petrovic *et al.,* 2001; Mourgand *et al.,* 2002; Raspor *et al.,* 2004; Leinio and Lehtonen 2005). The use of MT as a biomarker has been validated in many *in situ* studies (Lionetto *et al.,* 2001; Petrovic *et al.,* 2001; Rodriguez-Ortega *et al.,* 2002; Ross *et al.,* 2002; Mourgand *et al.,*2002). Such studies have generally found MT to work well for the intended purposes.

Fafandel *et al.,* (2003) investigated molecular response to TBT stress in marine sponges (*Suberites domuncula).* Proteolytical cleavage and phosphorylation of stress response KRS-SD protein kinase, in control and TBT treated sponges were investigated. Exposure of sponges to TBT resulted in alteration of KRS-SD1 and KRS-SD2 expression levels and their phosphorylation state. KRS-SD induction, its phosphorylation and proteolytical cleavage

during TBT stress, suggests that mechanisms similar to ones present in human cells exist in sponge cells in which KRS/MST protein kinase is involved in promotion of apoptosis following oxidative stress.

2.7.2.2: Acetyl cholinesterase (AChE) inhibition

AChE enzymes are responsible for hydrolyzing the neurotransmitter acetylcholine into choline and acetic acid. AChE is usually located in the membranes of erythrocytes of both vertebrates and invertebrates. AChE controls ionic current in excitable membranes and plays an essential role in nerve condition at the neuromuscular junction (Pfeiffer *et al.*, 2005, Magni *et al.*, 2006). AChE biomarkers may be less useful in fish because fish have higher levels of tolerance to AChE inhibition. Measurements of AChE inhibition are most frequently used where a biomarker for organophosphate insecticide exposure is required (Matozzo *et al.*, 2005). However, AChE biomarkers have also been used with the OTCs. Rebeiro *et al.*, (2002) evaluated TBT subchronic effects in tropical freshwater fish (A*styanax bimaculatus* Linnaeus*). A. binaculatus* adult fish were acclimatized in a laboratory and isolated into groups of eight individuals. Two groups were used as controls and one group was exposed to TBTCl and dissolved in corn oil (0.0688 ± 0.0031µg TBT g^{-1}), every 6 days for 32 days. A muscle fragment was excised for the determination of the acetylcholinesterase activity and blood smears were obtained for differential white cell counts. The results indicated nuclear irregular shapes, chromatin condensation, presence of intranuclear lipid bodies, and degenerative nuclei. AChE activity was not affected by TBT exposure. The increasing number of metrophylls metrophilis may represent cytotoxic and stress conditions facilitating the invasion of the opportunist.

2.7.2.3: TBT- Sensitive immunological biomarkers

Several xenobiotics alter immune function and the immune system. TBT has been observed to have adverse effects on cellular immune functions of haemocytes. The three indices established as TBT pollution biomarkers are amoebyocytic index, phagocytic index and lysosomal activity index (Chima *et al.,* 1999).

2.7.2.4: Lysosomal biomarkers

Matozzo *et al.,* (2002) studied the effects of TBT on circulating cells from the clam *Tapes philippinarum.* They found that exposure of haemocytes to 0.05 µm caused a significant increase ($p < 0.05$) in neutral red dye uptake into the lysosomes, compared with controls, whereas no differences resulted after exposure to TBT. Enlarged lysosomes were observed in haemocytes exposed to TBT. Moreover, in haemocytes treated with 0.05µm and 0.1µm of TBT, superoxide chromatase activity significantly decreased ($p < 0.05$ and $p < 0.1$) with respect to that of the control. A significant decrease in lysozyme activity was also observed in haemocytes exposed to 0.05 and 0.1 µm TBT. Lysozyme is a lysosomal enzyme that may be secreted by haemocytes in the haemolymph during phagocytosis. Reduced lysozyme activity suggests immunosuppression, resulting in lowered resistant bacteria challenge (Matozzo *et al.,* 2002).

2.7.2.5: Molecular (genetic) biomarkers

Measurement of certain molecular biomarkers may have obvious advantages for detecting early chemical effects because pollutants interact with the receptors of organisms at the molecular level to cause their effects (Nicholson and Lam 2005). Schroth *et al.,* (2005) utilized a strategy that identified molecular biomarkers and linked the study of abiotic stress to evolutionary history. These authors used the Moon jelly fish, *Aurelia* spp., as a model species. The authors used complementary DNA subtraction analysis to identify genes that were differentially regulated after exposure to the chemical stressor TBT. They also identified differential expression patterns following exposure to TBT at different temperatures. Results suggested that the identified genes were involved in response to the chemical as well as to heat-induced stress.

2.7.2.6: Apoptosis

This is a form of genetically programmed cell death which can be initiated by an internal clock, or by exposure to extracellular agents such as hormones, cytokines, killer cells and a variety of chemical and viral agents. These methods that are applied when using apoptosis as a biomarker are normally characterized by morphological and biochemical criteria (Micic *et al.,* 2001). Micic *et al.,* (2001) investigated the induction of apoptosis by tri-nTBT in gill tissue of the mussel *Mytilus galloprovincialis*. These authors used the terminal dUTP nick-labelling technology (TUNEL) to detect cells displaying DNA fragmentation within gill structures. Genomic DNA fragmentation was detected as characteristically ladder-like patterns of DNA fragments that were induced by a single injection directly into the pallial fluid of different doses of TBT below the mantle after one day of incubation. After 1.5 h of TBT incubation, DNA degradation of a higher order DNA structure, and a reduced G_o / G_1 cell cycle region, were detected. The effect of TBT on the cell cycle in the mussel (*Mytilus galloprovincialis)* gill was dose related and exposure time dependant. In this study, three types of investigation were performed: (a) detection of internucleosomal fragmentation by conventional gel electrophoresis, (b) identification of DNA fragments of higher chromatin organization by pulsed field gel electrophoresis, and (c) the detection of apurinic sites in gills sections of TBT treated mussel (*Mytilus galloprovincialis)* using TUNEL (Micic *et al.,* (2001).

2.7.2.7: Imposex

Imposex is characterized by the development of morphological features (i.e., penis vas deference) in female gastropod molluscs, or superimposition of male morphological features onto females. Imposex results from exposure of certain invertebrates to organotin antifouling paints (Marshall and Rajkumar 2003). Imposex serves as a useful morphological biomarker for measuring organotin contamination of marine ecosystems. High incidences of imposex were characterized by lower female to male ratios, suggesting that sterility and female mortality were TBT related (Marshall and Rajkumar 2003). In other studies, organotins were found to accumulate in the tissue of marine invertebrates. TBT generally shows the greatest accumulation among the butyltin compounds, and is the primary cause of imposex (Bryan *et al.,* 1988; Barreiro *et al.,* 2001). The induction of imposex by TBT may account for a sizable portion of the decline of certain coastal marine molluscs (Gibbs and Bryan 1996). Pessoa *et al.,* (2001) studied the occurrence of organotin compounds in Portuguese coastal waters and found that acute effects from TBT were induced at concentrations as low as 1µg/ L in aquatic organisms. Moreover, imposex was induced at levels below 0.5ng/L of TBT (as Sn). TBT at 20ng/L (as Sn), caused sterility, and this was followed by the disappearance of the most sensitive neogastropods on a given shore. The

authors concluded that the use of imposex was the most sensitive indicator of exposure to TBT of all known non-target pathological conditions.

2.7.2.8 Neutral Red Retention Time technique

The neutral red retention time bioassay (NRRT) is a useful technique for monitoring the destabilization of the lysosomal membrane caused by heavy metals and organic pollutants. The retention of neutral red within the lysosomal compartment, over time, is used to measure the level of change to the lysosomal membrane. This technique has served as early warning system because it can identify low level of contamination the moment it occurs (Svendsen and Weeks, 1995). NRR time technique has been used widely for hemocytic and digestive gland cell lysosomes of the mussel exposed to different hydrocarbon and metals (Lowe and Pipe, 1994; Lowe *et al.*, 1995a, 1995b).This technique has been used as biomarker in order to test for the toxic effects of organic pollutants (Lowe and Pipe, 1994; Fernley *et al.*, 2000) and on heavy metal as well (Svendsen and Weeks, 1995; Weeks and Svendsen, 1996; Svendsen and Week, 1997; Reinecke and Reinecke, 1999). Other applications of NRR technique includes field application (Fernley *et al.*, 2000). Ringwood *et al.* (1998) used digestive gland cell of the oyster *Crassostrea virginica* as models to test for copper exposure. Snyman *et al.* (2000) also used NRRT to investigate the toxicity of the fungicide copper oxychloride for the common garden snail *Helix aspersa.*

Mytilus galloprovincialis has been used widely in various studies (Micic *et al.*, 2001; St.Jean *et al.*, 2002; Bekri and Pelletier, 2004; Hagger *et al.*, 2005; Koukouziza and Dimitriadis, 2005; Magi *et al.* 2008; Gopalakrishnan *et al.*, 2011). *Mytilus galloprovincialis* was chosen for this study because it occurs commonly in Cape Town harbour, South Africa. The response of *Mytilus galloprovincialis* to TBT using NRRT has not been extensively studied. The aim of this work is to determine the toxicity of TBT for the mussel *Mytilus galloprovincialis* by investigating TBT bioaccumulation and the resultant destabilization of hemocytic lysosome membranes. In addition, to identify the relationship between NRR times and TBT concentrations over time in order to assess the use of lysosomal membrane destabilization as biomarker of stress resulting from exposure to TBT.

2.8: The Regulation of Organotin Compounds

The presence of tributyltin in the environment has attracted the most regulatory attention, because of the volume of its use in antifouling paints to coat boat hulls or harbour edifices. When biocides are released from paint over time, it forms a thin layer of concentrated TBT in the vicinity of its immediate use area. This contaminated area repels or kills organisms such as barnacles (Huggett *et al.*, 1992). However, TBT diffuses from the application area to contaminate adjacent water, sediments and non-target organisms. As

previously mentioned, TBT contamination causes morphological aberrations in oysters and mussels (Wadlock and Thain 1983). These effects and other associated environmental impacts of TBT had led the authorities of many countries to target TBT for regulation (Abbott *et al.,* 2000).

According to the USEPA (United States Environmental Protection Agency) (2001) TBT restrictions apply in many countries around the world. For example, the European Union, Canada, Scandinavia and South Africa have banned the use of TBT on vessels that are less than 25 m in length. As a result of increasing awareness of the undesired effects of TBT, global efforts have been made to solve this problem, and, increasingly, legal requirements have been enforced to protect the aquatic environment from TBT (Konstantinous and Albanis 2004). France, in 1982, was the first country to ban the use of organotin in antifouling paints for application to boats of less than 25 m in length (Alzieu *et al.,* 1986). This ban was sequel to the collapse of the oyster industry in France' Archon Bay in the late 1970's and early 1980's (Alizieu *et al.,* 1989, 1991). The enhanced TBT concentrations in seawater and the frequency of oyster shell anomalies were the cause of the collapse. Subsequently, comparable regulations as those imposed in France also were passed, after 1988, in North America, UK, Australia, New Zealand, Hong Kong and most European countries (Alzieu *et al.,* 1989; De Mora *et al.,* 1995 Champ 2000, 2003;).

The International Maritime Organization (IMO) campaigned for a global treaty to ban the application of TBT-based paints starting January 1st, 2003 as a result; a total prohibition took place by January 2008 (IMO 2001). In Europe, the current Water Framework Directive is the major community instrument for controlling port and diffused discharges of dangerous substances. Decision no 2455/ 2001/EC (20 November 2001) of the European commission parliament amended the water policy directive 2000/ 60/EC and defined 11 priority hazardous substances, including TBT compounds, that were subject to cessation of emission, discharge and lose to water. In addition, regulation No 782 /2003 of the European Parliament and of the council of 14 April 2003 was aimed at prohibiting organotin compounds on all ships entering European seaports TBT monitoring was also mandated by legislation from several European Commissions, including: the Council Decisions 75/437/EC (marine pollution from land–based sources), 77/585/EC (Mediterranean Sea), and 77/586/EC (River Rhine) and the Council Directive 80/68 EC (groundwater) (Champ 2000).

In 1985 the government of the United Kingdom (UK) prohibited the application of TBT-based antifouling paints to small vessels. In 1986, an Environmental Quality Target Concentration (EQTC) was set for TBT at a level of 20 ngL^{-1} in 1986. This value was based on the lethal concentrations that were effective for control of selected commercially important molluscs. Because of the high toxicity value of the TBT, this value was reduced by a factor of 10 one year later to achieve improved environmental protection (Takahashi et al. 1997). In

Spain, a Royal Decree (995/2000) established the concentration limit of organotin species in waste discharges to continental surface waters. The value selected was less than 20 ngL^{-1}. Legislation that addresses concentrations in sea water samples has yet to be approved.

The United States enacted the organotin antifouling paint control Act in 1988. A leaching rate of organotins from the application sites was limited to 4 $\mu gcm^{-2}d^{-1}$ (USA 1988). Moreover, the Occupational Safety and Health Administration (OSHA), American Federal Agency and the National Institute for Occupational Safety and Health (NIOSH) have established workplace exposure limits of 0.1mg m^{-3}. The Food and Drug Administration (FDA) has also set a limit for the use of tin as a food additive (ATSDR 2005). In addition, the water quality criterion of the USEPA is that aquatic life and the uses to which aquatic life are put should not be unacceptably affected.

In 1989, the Canadian government regulated TBT (under the Canadian Pest Control Products Act) by stipulating a maximum daily release rate for antifouling paints of 4 µg TBT per cm^3 of boat-ship hull surface. In Australia, the evidence for establishment of a relationship between deformities in oysters and the presence of TBT in oyster tissue led to the banning of TBT-based paints (Takahashi *et al.,* 1997). Japan also restricted TBT usage on antifouling coatings of boats and aquaculture nets by implementing limits in 1990. But TBT is still used as an antifouling agent for ocean liners and deep-sea fishing boats (Takahashi *et al.,* 1997). Similar actions on the usage of TBT in paints were taken by Switzerland, the Netherlands, Sweden, New Zealand, South Africa and most European countries (Sergi *et al.,* 2005). However the legislative restrictions on the use of TBT-based marine paints in Tanzania are less clearly defined. As a result of legislation restricting the use of TBT-based antifouling paints some reduction in the levels of TBT has been reported, particularly in areas in close proximity to recreational shipping activities (Hawkins *et al.,* 2000; Rees *et al.,* 2001). However, in areas near industrial shipping activities (e.g., ports), TBT levels remain high (Peachery 2003; Valkirs *et al.,* 2003; Horiguchi *et al.,* 2004; Harino *et al.,* 2006).

South Africa is positioned along a primary shipping route between Europe, the Americas, and Asia. South African harbours provide infrastructural support to the global shipping industry, with some of the largest and busiest African harbours being located on the eastern seaboard of South Africa. The Constitution of the Republic of South Africa (ACT 108 of 1996) and the Bill of Rights enshrine basic human rights such as having access to sufficient water and a safe and healthy environment. The two Acts that enable the South African government to fulfil these rights (through the Department of Water affairs) are the Water Services Act of (Act 108 of 1997) and the National Water Act, 1998.

In South Africa, the Maritime International organization (IMO) held an international convention on the control of harmful antifouling systems in 1990. The convention was

adopted in 2001, and South Africa was a signatory. The convention required prohibition or restriction of the application of antifouling systems and they listed the substances to be controlled. The convention also required signatory states to ensure that controlled substance application or removal was done appropriately, and required such states to perform surveys of their own ships. The regulations required that any ships in violation of the convention standard were subject to being warned, detained, dismissed or excluded from a country's port (IMO 2001). This convention required the South African government to develop new legislation to effect provisions of the convention. Annexture1 of the convention included a list of organotin compounds. The waste resulting from the removal of these toxins, as stated in article 5 of the convention, should be disposed of in accordance with permits from the Department of Water affairs (DWA) and Environmental Affairs (DEA). The South African Maritime Safety Authority (SAMSA) became responsible for enforcing and implementing the legislation. The provision of waste disposal was taken over by the National Port Authority (NPA) (IMO 2001).

The Facilitation of International Maritime Traffic (FAL) 1991 amendments to the convention were passed to prevent unnecessary delays in maritime traffic. This required the port authority to inspect foreign ships to verify that their condition, manning and operation were in compliance with international rules and the regulating act of the South African Maritime Authority. Several other conventions for protection of coastal and marine ecosystems are in force, and are indirectly related to organotin contamination. For example, the Ballast Water Convention requires that pollution checks be made of the maritime environment resulting from discharges of oil and other hazardous waste generated outside Africa into African countries. The Lome IV Convention also banned the export of hazardous waste from European countries to Africa (EC report 2007). In general, despite the ban on, or regulation of, TBT usage in some countries, TBT contamination continues in the aquatic environment. Therefore, environmental concerns for this contaminant remain high, and warrant continued assessment and monitoring. Continued diligence is needed, particularly in countries that do not restrict the use of TBT-containing antifouling paints. Moreover, further research is necessary on elucidating the pathways, kinetics, and persistence of organotin compounds.

2.9: Industrial application of organotin compounds and its pollution sources

According to Ross, (1965) there are three main areas in which organotin compounds have product and process utility; (1) heat stabilizers; (2) catalytic agents; (3) and biocidal compounds. Organotin derivatives account for the fourth largest production of organometallics amounting to about 3 to 4 million pounds per year as compared with about

485 million pounds per year for organolead compounds Ross (1965). Diorganotins have no antifungal activity, low toxicity and low antibacterial activity except for diphenyltins. They are used in polymer manufacturing as PVC stabilizers. Monoorganotins are mostly used for this since they have no biocidal activity and their toxicity to mammals is very low. Methyltin, butyltin, octyltin and manostertiss are also used as PVC heat stabilizers (Gummy et *al.* 2008). Organotins are remarkably varied in their physical, chemical and biological properties. Therefore, Sn has a large number of its organometallic derivatives in commercial use than any other element. This is reflected in their divergent industrial applications (as shown in table 2) and is mainly restricted to compounds of the types R_4Sn, R_3SnX, R_2SnX_2 and $RSnX_3$. Tetraorganotin compounds do not have any large scale commercial outlets, but are important intermediates in the production of less alkylated derivatives.

2.9.1: Manufacture

Organotin compounds can be synthesized by several methods, namely Grignard route, Wurtz route, alkylaluminium route and direct synthesis. These routes to produce organotin halides involve two reaction steps. The first step is a reaction of tin tetrachloride ($SnCl_4$) with suitable reagents to form various tetralkytins compounds (R_4Sn). In the second step, R_4Sn reacts with $SnCl_4$ in a redistribution reaction to form less alkylated organotin chlorides, like R_3SnCl, R_2SnCl_2 or $RSnCl_3$ (Blunden and Evans 1990). From these organotin chlorides, various Sn derivatives can simply be produced. The commercial production of organotin compounds by using the Grignard reagent (nRMgCl) began in the USA and the Metal and Thermit corporations plant in Rath way, New Jersey, in the late 1940s (Bennett,1983). The process of commercial production of organotin by Grignard reagent gives high yield but the use of high solvent is required while manufacturing of organotin compounds by alkyl aluminium route started in Germany at Schering industrial chemical divisions, Bergkaman, in 1962 Bennett (1996). Organotin halides can also been directly synthesised by a reaction between Sn metal or Sn alloys and alkylhalides. The order of reactivity of alkyhalides with tin follows RI > RBr > RCl. Methyltin stabilizers are produced by direct synthesis in USA while direct synthesis routes using alkylhalides and bromides was developed in Japan in the early 1950s.

2.9.2: PVC stabilizers

About 70% of the total annual organotin production is applied as derivatives for thermal and heat stabilizer in the plastic industry and as catalysts for polyurethane foams and silicones. PVC decomposes easily upon heating (80-200°C) or on prolonged exposure to sunlight due to loss of HCl from the polymer. The results are embrittlement and discoloration. To avoid this kind of degradation, certain organotins, mainly mono and

dialkylated derivatives, are added to the PVC at a level of 5-20 g kg^{-1} (Lawson 1986). Organotin –stabilized PVC has numerous applications including packaging materials, foils, piping of potable water, wastewater and drainage water, window frames and coating materials. Leaching of organotins from PVC pipes with a length of 46 m lead to a concentration of 35 mg (Sn)/ m^3 in water after first use, and to a subsequent constant release of 1mg (Sn/m^3). Several studies have shown that leaching of organotin ingredients from PVC and related materials lead to the contamination of foodstuffs, beverages, drinking water, municipal water and sewage sludge (Forsyth *et al.* 1992; Fent, 1996; Forsyth and Jay, 1997). Also an ordinary plastic product produced was analyzed by Takahashi *et al.* (1999) and BT was detected.

BT in product from supermarket was detected in 50% of the plastic product samples including baking parchements made by silconized paper, gloves made of polymethane, sponges for dish washing and cell phone film for foodstuffs. The transfer of this pollutant to foodstuffs was confirmed by analyzing the cookies which were baked on the investigated baking parchment. MBT, DBT and TBT were detected by the authors in these cookies. This means that high temperature is not sufficient enough to eliminate BT compounds. It is also worthy of note that significant amount of BT remained in the baking parchment after cooking in the oven. The ever growing production and use of PVC and its consequent disposal will lead to an accumulation of mono-alkylated organotin derivatives in the environment and possible long term effect on man and biota Quevanviller *et al.* (1991). This is because we have little knowledge especially in Africa about chemical leaching of OTCs mobilized by degradation of PVC materials in dumping sites.

2.9.4: Agriculture

Until recently, investigations concerning environmental pollution by OTCs have focused primarily on TBT used in antifouling paints. Freshwater and soil contamination by OTCs has received less attention. However, the agricultural and biocidal application of OTCs probably gives rise to a significant portion of the pollutants in the environment due to their direct input into soil, water and air by spraying, leaching and runoff. Since 1960 both triphenyltin hydroxide and triphenyltin acetate have been used to control fungal diseases causing potato blight (leaf spots) on sugar beets, carrots, onions and rice and used also to prevent tropical plant diseases in peanuts, pecants, coffee and cocoa (Chapman & Seligman,1996). In general, the agricultural use of organotin containing pesticides represents also another potential source of environmental pollution. They are mostly applied by spraying, which means that the surrounding can also be contaminated. It is well known that triorganotins are strongly adsorbed onto soil particles. Not much is known about the

degradation rates and desorption processes of these compounds under soil conditions. An entrance of OTCs into surface water due to runoff has to be taken into account Fent (1996).

2.9.5: Antifouling coatings

OTCs have been used extensively in boat paints since 1960 because of their excellent and longer lasting antifouling properties. Gibbs *et al.,* (1991a). Due to biocide components which are released only at the paint surface, the releasing rate is low and this results in antifouling lifetimes of 5-7years. Surfaces treated with modern TBT-based copolymer paints are designed to reach a constant TBT leach rate of 1.6 µg (Sn) cm^{-2} per day. The leach rate of freshly painted surfaces will be as high as 6 mg (Sn) cm^{-2} per day and is reduced in several weeks to the desired constant rate Bartley (1996). For example during a 3- day stay in a harbour, a commercial ship, leaching TBT at the constant leach rate, can release more than 200 g TBT into water. If freshly painted, this can result in dissolved TBT contamination of the surrounding water ranging between 100 and 200 ngl^{-1} or about 600 ng (Sn) l^{-1} respectively (Bartley 1996). These are the reasons why major harbours or other facilities where shipbuilding, repairing, and repainting have been contaminated by TBT pollution. This acute toxic chemical (TBT) compound which is the active ingredient in antifouling paints thus affects the aquatic organisms ever introduced into the water.

2.10: Toxicity of organotin compounds

Tributyltin compounds are extremely toxic substances belonging to the organotin group. Besides, the toxicity evaluation of commercially important organotin stabilizers found in PVC medical devices, there have been many investigations concerned with the toxicity of all types of OTCs. The toxicity of alkyl and aryltin derivatives has been recognized for a long time and is primarily due to the solubility of these organotin fluids. Triethyltin derivatives were identified as the toxic contaminant in stalinon which resulted in neurological symptoms in many of the afflicted patients. Triethyltin appeared to be the work active, producing muscular weakness (Stalinon 2009). TBT is extremely toxic to aquatic organisms. It may cause imposex and calaficati abnormalities in mollusc Alzieu et *al.,* (1989).

Toxic lesions found among laboratory and process workers handling di - and tributyltin compounds were typical acute skin burns, caused by the colourless di or tributyltin dichloride Lyle (1958). Ingestion of fruit juices containing high concentrations of tin has the following major symptoms and signs: nausea, vomiting, diarrhoea fatigue, and headache Horio (1967). Inhalation of triphenyltin caused acute intoxication in man. Tetraalkytin also caused muscular weakness and paralysis followed by respiratory failure (Stoner 2009). Tetraorganotins are very stable molecules with low toxicity and low biological activity. They can be metabolized to toxic triorganotin compounds. Triorganotin are very toxic also .Tri-n-

alkyltins are phytotoxic and therefore cannot be used in agriculture. Depending on the organic groups, they can be powerful bactericides and fungicides. Both Diorganotins and monoorganotins have low toxicity to mammals (Gumy *et al.,* 2008; Gomez et *al.,* 2008).

Despite the toxicity and lots of environmental effects of organotins. There are comparatively few clinical observations and epidemiological data concerning the effects of inorganic tin compounds on man and even fewer on the effects of organotin compounds. Therefore the EC banned TBT-containing paints due to the evidence of their extremely hazardous nature. Bottom-dwelling organisms in particular, are exposed to this contamination. Tin in its inorganic form is generally acceptable as being non toxic, but the toxicological pattern of organotin is very complex. However, the biological effects of the substances depend on both the nature and the number of the organic groups bound to the Sn cation. TBT is an agent showing a high toxic effect to aquatic life. Even at low nanomolar aqueous concentrations (1-2ng l^{-1}) TBT causes chronic and acute poisoning of the most sensitive aquatic organisms, such as algae, Zooplankton, molluscs and the larval stage of some fish (Gibbs and Bryan 1996). Lethal concentrations are in the range of 0.04-16 $\mu g l^{-1}$ for short term exposure, depending on the aquatic species (WHO 1990).

2.11: Organotins: levels of contamination

Despite the global ban on the application of organotin compounds as biocides as it was scheduled by IMO to begin in January 2003, the convention has, however, not yet been enforced and the use of the compounds continues in several countries especially in Africa where increase in usage of organotin products still continuing to date. Since the first reports on the environmental contamination of OTCs in the early 1970s, considerable amount of research has been performed worldwide on OTCs in the environment. However, only a handful of studies on organotin contamination have been carried out in the gulf region (De Mora *et al.*, 2003). The biological effects of OTCs have been described in many areas of the Brazilian coasts. It has been always filed that OTCs are present in many centres where maritime activity such as harbours, shipyards, and marinas are carried out. Relatively high concentrations of OTCs have been detected in sediments and organisms in the two most important areas of maritime activity in Brazil (Godol *et al.,* 2003; Castro *et al.*, 2004, Camilla et *al.* 2004; Fernandez *et al.,* 2005). This situation shows the need to assess the possible health risks for human populations arising from ingestion of contaminated sea foods.

In the North West Mediterranean, Borghi *et al.,* (2002) worked on OTCs present in deep sea fish. It was shown that OTCs are even present in deep sea marine organisms (Borghi *et al.,* 2002; Ikeda *et al.,* 2002). They found that the concentration of TBT in different tissues of several deep sea fish species collected between 1000 and 1800 m depth in the

North West Mediterranean are comparable to levels found in coastal fish. In addition, deep-sea fish contained much higher levels of phenyltins, particularly TPT, than previously reported concentrations in shallow water organisms Ikeda *et al.,* (2002) Their results established the long -range transport of OTCs to the deep- sea environment, and the subsequent exposure of fish inhabiting non-point source areas. They attributed the high residual levels of TPT detected in deep-sea organisms to the use of TPT in agriculture or as an anti-fouling agent, its transport to the deep-sea environment associated with particulate matter, and the non-biodegradable nature of TPT in the food chain (Ikeda *et al.,* 2002).

In France, Ruiz *et al.,*(1995) found that the oyster farming industry at Arcachon Bay nearly collapsed due to the effects of TBT on the reproductive cycle of oysters. France is the first country to introduce legislation prohibiting the application of TBT paints to small (L25 meter) vessels in 1982 after establishing the link between TBT and the fall of oyster production at Arcachon Bay from 10,000 - 15,000 tons per year in the mid seventies to 30,000 tons in 1981 (Ruiz *et al.,* 1996). Donard *et al.,* (2001) pointed out that OTCs affect all facets of the ecosystem and that they should be listed as global pollutants similar to polychlorinated biphenyl, Hg and polychlorinated dibenzodioxins owing to their efficiency as endocrine disrupters, even at very low concentrations. They suggested that trisubstituted OTCs should be at the top of the list of priority pollutants (Donard et *al.,* 2001).

Sentosal *et al.*, (2009) recently noted the usage of IP-RP techniques for the speciation of DBT, TBT and triphenyltin (TPhT). OTCs were detected and the capacity factors (k1) for DBT, TBT, and TPhT species were 0.27, 2.54, and 5.92, respectively, While the selectivity for DBT-TBT and TBT-TPhT were 9.76 and 3.50, respectively. Federico *et al.*, (2007) reported levels of organotin pollution. They carried out systemic measurements of TBT and DBT, in sediments along different locations in the inner zone of Bahia Blanca estuary. Two samples were taken near the main dry dock facility at Peutro Belgrano naval base, in Argentina. TBT concentrations from non-detected to 170.3 ng Sng^{-1}were measured in the inner region of the estuary, and higher one of 3.288 ng Sng^{-1} near the dry dock at Peuto Belgrano. DBT values ranging between non-detected and 7.52 ngSng^{-1} were obtained along the principal channel, but extreme concentration of 1.645 ng g^{-1} was measured at Puerto Balgrano. These values show that this estuary is affected by organotin pollution, mainly in areas of heavy shipyard activities.

Jianying *et al., (*2006) reported field studies on trophic magnification factors (TMF) of TBT and TPT in a marine food web. TBT, TPT, and their metabolites in plankton benthic invertebrate species, and six fish species collected from Bohai Bay, North China were determined and it was found that the concentration of TPT in marine fish were unexpectedly higher than those of TBT. A positive relationship between trophic levels and concentration of TPT indicates trophic magnification of TPT in this food web. Analysis of organotin in the

water and surface sediment from Bohai Bay revealed low inputs of TPT to the environment, which indicated that the high concentrations of TPT found in fish from Bohai Bay, were due to the food web magnification of TPT. This information will be useful to know the level of distribution and to evaluate the harm caused by these pollutants.

2.12: Analytical methods

Sample preparation techniques for speciation analyses generally consist of several steps (Morabito, 2003) The necessary steps depend on the physico-chemical properties of the analytes to be determined and of matrix (water, sediment and biological materials) to be analyzed. However, the suitability of the sample preparation steps with the chosen determination technique must also be assured. Each analytical step needed in such determinations (e.g. derivatization, extraction, separation and detection) can affect the accuracy and precision of the final quantitative speciation results (Adams and Slaets, 2000; Moraibito *et al.,* 2000; Dietz *et al.*, 2007). To provide time resolved introduction of the analytes into the detector, a selective and sensitive detector coupled with some chromatographic separation step such as i.e. high performance liquid chromatography (HPLC), gas chromatography (GC) and gel electrophoresis (GE), are required (Donard and Ritsema 1993). A significant number of various instrumental techniques is reviewed in the literature for the determination of organotin compounds (Donard and Ritsema, 1993; Pereiro and Diaz, 2002; Morabito, 2003). The most commonly applied techniques over the past year have been based on gas and liquid chromatographic separation followed by different types of detectors (Morabito 2000).

Speciation of OTCs in sediment and biota may present difficulties during extraction because such process of isolating the targets chemical compounds from complex cell structures and biomolecules, and the number of possible errors is much higher. Generally extraction procedures of organotins in solid samples involve soxhlet extraction, mechanical shaking, use of sonication bath and microwave and pressurised liquid extraction (PLE) (Dietz *et al.,* 2007). The most frequently adopted methods for organotin extraction from sediment are leaching with acids (acetic or hydrochloric acid) or acid polar solvent (methanol) mixtures (Abalos *et al.,* 1997). Organotin compounds are present in water at ngl^{-1} levels and hence their quantification requires highly sensitive techniques, and collection of larger sample volumes together with the application of pre-concentration methods. The high salt content of seawater may pose difficulties in the determination step, and the complete validation of organotin analysis in seawater samples is far behind achievement sequel to reproductivity problems. Brunori *et al.,* (2005). Generally, the applied method for organotin analysis in seawater are (i) direct derivatization with organocarbonates or hydride in an acidic medium followed by liquid-liquid extraction (ii) (LLE) with non –polar solvents (toluene

, dichloromethane) alone or in mixture and in the presence of acidic conditions and subsequent derivatization (Brunori *et al.,* 2005).

After derivatization, GC separation of OTCs is followed by different detection techniques such as GC-MS , GC-MS-MS, GC-FID, ICP-MS or flame photometry (GC-FPD) for the determination of the species (Jiang , 2000; Vella *et al.,* 2000; Ikonomou *et al.,* 2002;Tsunoi *et al.*, 2002). Furthermore, good enhancement is achieved from extraction of organotin from the aqueous to organic phase by the addition of a complexing agent such as tropolone or carbamates (Pellegrino *et al.,* 2000; Brunori *et al.,* 2005; Dietz *et al.* 2007). In addition, LLE is the less preferred method for solvent extraction because its procedure is time consuming and achievement preconcentration factors is very low. LLE can be applied to non-filter samples and does allow transfer of analyte to organic solvent (e.g. hexane, toluene) for subsequent analysis.

Solid phase extraction (SPE) involves passing the liquid sample through a solid adsorbent that retains the analytes by mechanism of adsorption, chelation, ion-exchange or ion-pair; and subsequent recovery upon elution with an appropriate solvent. The advantages of SPE are that it is fast and sensitive, robust, easy to use, less solvent consumption, possible integration of columns and cartridges in on-line flow injection systems and possible application as species storage device for field sampling. SPME is based on the partition equilibrium of target analytes between a polymeric stationary phase attached onto a fibre and the sample matrix, combining analyte extraction and preconcentration in a single step. The analyte is then desorbed from the fibre at very high temperature into an appropriate separation and detection system (Donard *et al.,* 1995; Snaz-Medel, 1998),

Presently, SPME application consists in analyte ethylation and headspace extraction. Thus, SPE and SPME meet modern requirements for analysis following point sampling. Following extraction methods for the determination of OTCs should provide sufficient sensitivity and selectivity. Most reported techniques so far combine a separation technique such as gas chromatography (GC) coupled to element – specific detection systems such as atomic absorption spectrometry (AAS) (Donard *et al.,* 1995; Snaz-Medel, 1998), flame photometric detection FPD (Lalere *et al.,* 1995; Tao *et al.,* 1999), pulsed flame photometric detection PFPD (Bravo *et al.,* 2005) or inductively coupled plasma mass spectrometry ICP-MS (Moete *et al.,* 1997; Monte *et al.*, 1999; Encinar *et al.,* 2000).

For GC analysis, a derivatization step is necessary prior to separation, due to the low volatility of the target compounds. The conversion of ionic alkyltins into species that can be analyzed by gas chromatography can be into two categories, those based on in-situ hybridization with sodium borohydride ($NaBH_4$) or alkylation with sodium tetraethyl borate ($NaBEt_4$) (Brunori *et al.,* 2005). In situ hydride generation with $NaBH_4$ as described in Cai *et al.,* (1993) considers only methyl-and butyltins with appropriate recovery rates. Compounds

with higher boiling points such as phenyltins cannot be analyzed after using $NaBH_4$ (Kuballa *et al.,* 1996). $NABEt_4$ has recently become very popular as a derivatization reagent. This method makes the sample preparation faster and easier because it enables an in-situ derivatization and followed by extraction of the ethylated OTCs into an organic phase (hexane, isooctane) which is subsequently analyzed. The exact method and description of sample preparation is given elsewhere (e.g. Tutschku *et al.,* 1996; Carlier- Parnassian *et al.,* 2002). Recently, sodium tetra (n-propyl) borate ($NaBPr_4$) was introduced as a derivatization agent De Smaele *et al.,* (1998). Comparison of $NaBEt_4$ and $NaBPr_4$ as derivatising agents gave similar derivatization reagents (Schubert *et al.*, 2000).

In general, proper validation of the sample treatment has been the remaining problems for a variety of biological environmental samples due to the lack of matrix that matched certified reference materials (CRM). Currently, there are six CRM for tin species: BCR-CRM 646 for fresh water sediment and certified for MBT, DBT, TBT, monophenyltin (MPhT), diphenyltin (DPhT) and triphenyltin (TPhT), BCR 462 for a coastal sediments and certified for DBT and TPhT, one for harbour sediment (NRCC-PACS-2) certified for MBT, DBT and TBT as Sn, one for marine sediment (NIST-SRM 1941b) certified for MBT, DBT and TPhT, and one for one mussel tissue (BCR-CRM 477) certified for MBT, DBT and TBT (IEA, 2003; Dietz *et al.*, 2007). Recently, Lisico *et al.,* (2009) used two analytical methods for the determination of butyltin compounds in mussels. Both methods include extraction with methanol containing tropolone, derivatization, purification on florisil and GC-MS analysis. The main difference between the procedures is in the derivatisation step: one employs a Grignard reagent (n-pentyl-magnesium bromide) while the other uses sodium tetraethylborate (STEB). Quantitative determinations were carried out in single ion monitoring using tripropyltin as internal standards. The accuracy of the procedures was verified on a certified reference material (ERM 477), providing good results for both methods.

All the considered compounds showed lower detection limits with STEB derivatization; in particular for TBT the difference between the methods overcame one order of magnitude. An in - vivo experiment was then performed exposing mussels *Mytilus galloprovincialis* to known amount of TBT for 7days. Control and contaminated tissues were analyzed using the STEB derivatisation method. Results showed the accumulation of TBT, especially in the gills. New sampling approaches are required to provide large scale time weighed average data on tin distribution in order to assess long term and diffuse contamination. In Brunori *et al.,* (2005), Dietz *et al.,* (2007) and Nemanic *et al.* (2007) wide and full detailed information on organotin sample preparation can be obtained. A lot of review has been reported on methods of analyses of organotin compounds in which I have cited a few.

2.12.1: Gas chromatography

GC is used frequently for the analysis of organotin compounds than liquid chromatography (LC) owing to its ability to give higher resolution. GC-techniques provide versatile detection methods, well developed analyte modification strategies, high separation efficiency and a wide range of sample pre-treatment procedures. The main demerit of GC methods for organotin analysis is that they require the OT species to be volatilised prior to separation and detection. An additional step is therefore necessary in the speciation procedure. Various detectors are available for use with GC systems depending on the type of sample to be analyzed. The main limitations and drawback of most detectors used for OT analysis are lack of selectivity and sensitivity towards analytes of interest. The conventional GC detectors, which are incorporated into the GC without coupling, are electron capture detector (ECD), flame ionisation detector (FID) and thermal conductivity detector (TCD). Detectors that are coupled to GC are flame photometric detector (FPD), pulsed flame photometric detector (PFPD), reactive flow detector (RFD), mass spectrometry, atomic emission detection (AED) and inductively coupled plasma mass spectrometry (ICP-MS).

2.12.2: Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS technique has the ability to detect a wide variety of compounds by ionizing into charged species. The differently sized charged fragments are separated according to mass charge ratio. Most chemicals have unique fragmentation patterns called mass spectra. The data station contains a library of known mass to identify an unknown compound exhibiting a particular spectrum. Authentic compounds are used for confirmation after tentative identification is made. The application of MS with isotope dilution can produce superior accuracy and precision compared to more common calibration strategies provided that the first solid-liquid extraction step is quantitative or true isotope equilibration between the added spike and the analyte is achieved. Since the quantitation is done by ratio measurements, subsequent analyte recoveries do not affect the final result (Centineo *et al.* 2006). Recently micro plasma (MP) ion source was developed for GC-MS instruments. GC-(MP) MS has been found to be useful instrument for organotin speciation in environmental samples, it detect tin species down to the low pictogram level. Another form is GC-ITDMS (Gas chromatography–ion trap detection mass spectrometry which is useful as GC- MP earlier mentioned.

2.12.3: Gas chromatographic- Flame Photometric Detector (GC-FPD)

FPDs system is based on the emission of tin species in a hydrogen-rich flame. Selectivity for tin is obtained at 610 nm using a cut-off or interference filter. However, the FPD signal may be disturbed by the co extracted sulphur species. Dual flame FPD had been introduced to improve the selectivity of the technique, but it provided lower sensitivity than the single-flame FPD. The new generation FPDs in which the continuous flame is replaced by a pulsed flame, the pulsed FPD (PFPD) detector, has recently been introduced. The main advantages are the increased sensitivity and lower matrix dependency. In general, FPDs have been used widely for the detection of tin species due to the fact that they are rugged and inexpensive in design. They are used for the determination of OT after ethylation with $NaBEt_4$ (Brunori *et al.*, 2006).

2.13: Heavy metals

Heavy metals are among the most serious environmental pollutants due to their high toxicity, abundance and ease of accumulation by various plant and animal organisms. Persistent increase of heavy metals in harbour sediments can be attributed to the contribution of effluents from waste water treatment plants, industries, mining, power stations, and agriculture (Guevara *et al.*, 2004) which carry run-offs to the harbour (Fatoki *et al.*, 2012). The increase in urbanisation and industrialisation also could lead to an increase in marine discharges and therefore results in total loads of pollutants discharges to the sea. These discharges may contain heavy metals among other pollutants (Fatoki and Mathabata; 2001). In addition, ship traffic and repair activities, especially in and close to the harbour, are also suspected to be indicative of elevated concentrations in the upper reaches of harbours.

Metal concentration in sediments can be traced to high concentration in living organisms and humans and therefore put public health at risk. The bioavailable metal load in sediments may affect the distribution and composition of benthic assemblages (Capalat *et al.*, 2005) and this will cause increase in concentration of these pollutants in living organisms (Kress *et al.*, 2004). High concentrations of heavy metals in living organisms can result in morphological abnormalities, neurophysiological disturbances, genetic alteration of cells (mutation), tetratogenesis and carcinogenesis. Moreover, heavy metals can affect enzymatic and hormonal activities, as well as growth rate and an increase in mortality rate (Bubb and Lester; 1991). Metals accumulate in sediments from both natural and anthropogenic sources and sediments act as scavenger agents as well as an adsorptive sink for heavy metals in an aquatic environment. Sediments can therefore be described as appropriate indicators of heavy metal pollution (Sakai *et al.*, 1986).

The accumulation of metals in sediments from both natural and anthropogenic sources occurs in the same way, thus making it difficult to identify and determine the origin

of heavy metals present in them (Idris *et al.*, 2007). Moreover, the total concentration of metals often does not accurately represent their characteristics and toxicity. It is thus helpful to evaluate the individual fractions of the metals in order to overcome the above mentioned obstacles and to fully understand their actual and potential environmental effects (Tessier's *et al.*, 1979). Single extractions are thus used generally to provide a rapid evaluation of the exchangeable metal fraction in soils and sediments (Quevavauviller, 2002; Sahuquillo *et al.*, 2003). However, various complicated sequential extraction procedures were used to provide more detailed information regarding different metal phase associations (Tessier *et al.*, 1979; Bordas and Bourg, 1998; Templeton *et al.*, 2001).

A wide range of techniques is available whereby various extraction reagents and experimental conditions are used. These techniques involve a 5-step (Tessier *et al.*, 1979), 4step (BCR, Bureau Commune de Reference of the European Commission) and 6-step (Kerstin and Frostier, 1986) extraction, and are thus becoming popular and adopted methods used for sequential extraction (Cuong *et al.*, 2006; Pardo *et al.*, 2008). Several analytical methods have been used for the determination of heavy metals contents in marine environments. These include; flame AAS, (Dapaah *et al.*, 1999; Gomez-Ariza *et al.*, 1999), atomic fluorescence spectrometry (Cheam *et al.*, 1992), anodic stripping voltametry (Fischer and Van den Berg, 1999; Morales *et al.,* 1999), ICP- AES (Hiraide *et al.,* 1980) and ICP-MS (Ridout and Jones, 1988; Sakao *et al.,* 1999). Heavy metal mobility and bioavailability depend strongly on their chemical and mineralogical forms in which they occur (Baeyens *et al.*, 2003). Several speciation studies have been conducted to determine study different forms of heavy metals rather their total metal content. These studies reveal the level of bioavailability of metals in harbour sediments and also confirm that sediments are bio-indicators of heavy metal pollution in marine environment (Esslemont, 2000; Guevara *et al.,* 2004; Wepener and Vermeulen; 2005; Idris *et al.*, 2007;).

Although several studies have been conducted on heavy metal pollution of harbour sediments there are no data are available on heavy metals speciation in Cape Town harbour.

2.14: Heavy metals as marine pollutants

Major and trace elements occur naturally in the environment (Guerra- Garcia *et al.,* 2005). This natural occurrence of metals in the environment due to various particle sizes for instance, complicates assessments of contaminated marine sediments because measurable quantities of metals do not automatically infer anthropogenic enrichment (Guerra- Garcia *et al.,* 2005). In addition to shipping traffic especially in and close harbours Industrial activities, vehicle emissions, agricultural activities and domestic waste can all act as a source of heavy metal pollution in the marine environment (Idris *et al.,* 2007). Many adverse effects have

been done on human health by the environmental pollution of heavy metals. Heavy metals condition is problematic due to their persistence and non-degradability in the environments (Yuan *et al.,* 2004) Metals distribution and association in marine sediments occur in various ways which include ion exchange, adsorption, precipitation and complexation. They are not permanently fixed by sediments (Yuan *et al.,* 2004). Heavy metals pollution in aquatic environment and their uptake in the food chain by aquatic organisms and humans, put public health at risk. In general, heavy metals are stable and persistent environmental contaminants of marine sediments. Interest in metals like Zn, Cu, Fe, and Mn which are required for metabolic activities in organisms depends on their nutritional value and their toxicity. Metals like Cd, Hg, Cr, Pb and As may exhibit extreme toxicity even at lower concentration under certain condition. Thus this makes regular monitoring of aquatic environment to be imperative and necessary. Thus this makes regular monitoring of Cape Town harbour to be imperative and necessary.

2.15: Occurrence of heavy metals in marine sediments

Heavy metals are stable and persistent environmental contaminants of coastal sediments. In recent years there has been growing concern over increased contamination of estuaries and harbours from various anthropogenic sources (Wepner and Vermulen; 2005). Sediments serve as the ultimate sink for many contaminants and as a result, they pose the highest risk to the aquatic life as a source of pollution (Williamson *et al;* 1996). Bruder-Hubscherv *et al.* (2002) worked on metal speciation in coastal marine sediments from Singapore and confirmed that sediments are the main repository and source of heavy metals in the marine environment and that they play a major role in the transport and storage of potentially hazardous metals. A number of factors have been attributed to pollutant accumulation in harbour sediments. The design of the harbour to minimize hydrodynamic energy, industrial activities (ship repairs and traffic, accidental spills, loading and unloading), agricultural activities and urban (waste water) activities can all acts as sources of heavy metal pollution in marine environment (Forstner and Wittmann 1981; Bubb and Lester; 1991; Fatoki and Mathabata; 2001; Guevara- Riba *et al.*, 2004). Heavy metal accumulation in marine sediment is due to a highly dynamic nature of the marine environment which allows rapid assimilation of these pollutants into sediments by processes such as oxidation, degradation, dispersion, dilution and ocean currents.

Phytoavailability of heavy metals depends on the characteristics of the sediment, the nature of the metal species, the interaction with sediment matrix and the duration of the contact with the surface binding. Heavy metal availability in marine organisms can be traceable to sediment characteristics such as pH, organic matter content and type, and then moisture (Iwegbue *et al.,* 2006). In general, increase in population growth, rapid unplanned

industrialization, urbanization, exploration and exploitation of natural resources and newly introduced modern agricultural practices are the major contributory factors responsible for the presence of heavy metals in marine sediments.

2.16: Heavy metals in water, soil and sediments

Heavy metals refer to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentration. Heavy metals occur naturally in the ecosystem with large variations in concentration. Nowadays, anthropogenic sources of heavy metals i.e. pollution, have been introduced to the ecosystem (Fatoki and Mathabata; 2001). These metals are a cause of environmental pollution (heavy-metal pollution) from a number of sources, including lead in petrol, industrial effluents and leaching of metal ions from the soil into water bodies by acid rain. Toxic metals can be present in industrial, municipal and urban runoff, and by definition they are harmful to humans and aquatic biota. Increased urbanization and industrialization have increased the levels of trace metals, especially heavy metals in water ways. There are over 50 elements that can be classified as heavy metals, but only 17 that are considered to be both very toxic and relatively accessible. Mercury, lead, arsenic, cadmium, selenium, copper, zinc, nickel and chromium, however, should be given particular attention in terms of water pollution and discharge effects. Toxicity levels depend on the type of metals, its biological role, and the type of organisms that are exposed to it (Akan *et al.*, 2010).

Zinc

Zinc is one of the numbers of trace elements considered essential to plant growth and the physiological function of organism. The permissible limit for dissolved zinc in aquatic ecosystem according South Africa water quality guildelines is 2.0 ppb (DWAF, 1996). The highest concentrations are found in the urethra tract and the prostrate (ATSDR, 1994). It has been found that various parts of the body contain zinc, relatively high concentrates are present in the skin, while the visceral organs contains approximately 30-50 µg/g of fresh tissue. Most of the body zinc is in the bones where its concentration is approximately 200 µg Zn/g. Excessive intake of Zn may lead to vomiting, dehydration, abdominal pains, nausea, lethargy and dehydration (ATSDR, 1994).

Cadmium

Cadmium is also one of the heavy metals found in soil and water samples. It is a by-product of the mining and smelting of lead and zinc. It is used in nickel cadmium batteries, PVC plastic and paint pigments. It can be found in soils because insecticides, fungicides sludge, and commercial fertilizers that use cadmium are used in agriculture. Cadmium may be found in reservoirs containing shell fish. Inhalation accounts for 15-20% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Cadmium toxicity is generally indicated when urine levels exceed 10 µg/dl and blood levels exceed 50 µg/dl. Cadmium sulphide and selenide are commonly used as pigments in plastics (Eaton, 2005).

Aluminium

Although aluminium is not a heavy metal (specific gravity of 2.55 -2.80), it makes up about 8% of the surface of the earth and is the third most abundant element. It is readily available for human ingestion through the use of food additives, antacids, buffered aspirin, astringents, nasal sprays and antiperspirants from drinking water (Bakare-Odunola, 2005). Studies suggested that aluminium might have a possible connection with developing Alzheimer's and Parkinson's disease when researchers found what they considered to be significant amounts of aluminium in the brain tissue of Alzheimer's patients. Aluminium also causes senility and presenile dementia (Pe'rez et al., 1991; Bakare-Odunola, 2005).

Copper

Copper is an essential substance to human life, but in high doses it can cause anaemia, liver and kidney damage and stomach and intestinal irritation. Copper normally occurs in drinking water from copper pipes, as well from additives designed to control algal growth (Pe'rez et al., 1991). In humans exposure to lead can result in a wide range of biological effects depending on the level of duration of exposure (Gomez et al., 2000). Various effects occur over a bound range of doses, with the developing foetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys and acute of chronic damage to the nervous system. Some studies suggest that there may be a loss of up to 21Q points for a rise in blood lead levels from 10 to 20 µg/dl in young children. Average daily lead intake for adults is estimated at 1.6 µg from air, 20 µg from drinking water and 28 µg from food. Copper is generally remobilized with acid based ion exchange or oxidation mechanism (Gomez-Ariza *et al.,* 2000).

Mercury

Mercury is a toxic substance which has no known function in human biochemistry or physiology. It is a global pollutant with complex and unusual chemical and physical properties (Fatoki and Mathabata; 2001). The major natural source of mercury is the degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water. The usage of mercury is widespread in industrial processes and in various products, (e.g. batteries, lamps and thermometers). Toxicity of mercury results mental disturbance and impairment of speech, hearing, vision and movement (Pe'rez et al., 1991; Hammer and Hammer, 2004). It is also widely used in dentistry as an amalgam for fillings and by the pharmaceutical industry.

Concern over mercury in the environment arises from the extremely toxic forms in which mercury can occur. Natural biological processes can cause methylated forms of mercury to form which bio-accumulate over a million fold and concentrate in living organisms especially fish. These forms of mercury: monomethylmercury and dimethylmercury are high toxic causing neurotoxicological disorders. The main pathway for mercury to humans is through the food chain and not by inhalation (Hammer and Hammer, 2004).

Table: Analytical methods used for speciation of heavy metals

Locations	Extraction techniques	Analytical methods	References
Barcelona harbour, Spain	BCR-3STEP Sequential Extraction	ICP-MS, ICP-AES and Single-beam-flame AAS.	Guevara-Riba *et al.*, 2004
Kranji and Pulau Tekong harbour Singapore	Modified BCR-3STEP Sequential Extraction	GFAAS	Cuong and Obbard; 2006
Townsville harbour, Queensland,	Modified Tessier Extraction	AAS	Esslemont,2000
Richards Bay Harbour	Tessier Extraction step	Flame AAS	Wepener and Vermeulen; 2005
East China Sea	BCR-3STEP Sequential Extraction	ICP-MS	Yuan, 2004
Norwegian Sea and Baltic Sea	4- Step Sequential Extraction	AAS	Pemopkowiak *et al.*, 1999
Huelva Estuarine	3-Step Sequential Extraction	AAS	Usero *et al.*, 1998
Southwest Coast of Spain	BCR-Sequential Step	GFAAS	Morillo *et al.*, 2003
Urban and suburban agricultural soils from China	BCRSEP Optimized	ICP-MS	Zhang and Wang, 2003
Polluted soil and sediments from Morocco	BCRSEP optimized	FAAS	Elass *et al.*, 2004
Soil affected by an accidental spills in Spain			
Agricultural soil from Chile	BCRSEP	ICP-MS	Pueyo *et al.*, 2003
	New SEP developed	AAS	Fuetes et al., 2004b

Table 2: Speciation of the analyzed heavy metals in sediment of different coastal system

Cr	**Acheloos river estuary, Greece**	**2**	**1.5**	**7.1**	**1.1**	**88.3**	**Dassenakis *et al.*, (1995)**
	Cadiz Bay	**0**	**6.9**	**6.4**	**8.3**	**78.4**	**Izquierdo *et al.*, (1997)**
Fe	Acheloos river estuary, Greece	0.3	5.2	12.2	4.2	78.1	Dassenakis *et al.*, (1995)
	Coastal sediment, Baja, USA	0	0.4	6	1.6	92	Villaescusa *et al.*, (1997)
	Barbate River Salt marshes	<0.1	0.8	17.4	2.8	79	Dassenakis *et al.*, (1995)
Cu	Humber estuary, UK	2	6.1	8.2	53.1	30.6	Comber *et al.*, (1995)
	Mersey estuary, UK	1.8	12.9	10.5	37.4	37.4	Comber *et al.*, (1995)
	Barbate Rivers salt marshes						
Zn	Vigo estuary, Spain	0.0	10.0	34.0	11.0	39.0	Belzunce-S *et al.*, (1997)
	Huelva estuary, Spain	15.7	18.9	28.8	12.9	24.3	Perez *et al.*, (1991)
Cd	Humber estuary, UK	31.9	37.7	26.1	4.3	0	Comber (1995)
	Mersey estuary, UK	34.2	29.1	22.8	6.3	7.6	Combe *et al.*, .(1995)
	Barbate River Salt marshes						
Pb	Mersey estuary, UK	5.3	16.8	55	9.2	13.7	Comber *et al.*, (1995)
	Vigo estuary, Spain	0	9	31	8	53	Belzunce-Segara (1997)
	Barbate River Salt marshes				7		
Mn	Acheloos River estuary, Greece	1.1	72.1	11.2	13.4	13.4	Dassenakis *et al.*, (1995)
	Huelva estuary, Spain	9.3	0.25	25	58.6	58.6	Perez *et al.*, (1991)
	Cadiz	11.7	41.1	16.2	22.8	22.8	Izquierdo *et al.*, (1997)

Key: Results are expressed in % the total metal concentration. F1 to F5 represents fractions of the sequential extraction techniques of Tessier et al., (1979).

2.17 Effects of heavy metals on public health

Sediments house many contaminants and therefore pose the highest risk to the aquatic environment as a source of pollution (Bervoets *et al.*, 1994; Williamson *et al.*, 1996). Environmental pollution by heavy metals impacts negatively on human health. Their remediation proves to be problematic due to the persistence and non degradability of heavy metals (Yuan *et al.*, 2004). High concentrations of heavy metals in biota can be linked to high concentration in sediments. The bioavailable metal load in sediments may affect the distribution and composition of benthic assemblages (Kress *et al.*, 2004), and this can be linked to high concentration recorded in living organisms (Pempkwait *et al.*, 1994). The most obvious effect of pollution is to reduce diversity of biological species that are not able to tolerate the toxicants. Most resistant organisms are often undesirable in human terms. Example is the blue-green algae or sewage fungus that forms slime or scum. Heavy metals are dangerous because they tend to bio-accumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Heavy metals can cause serious health effects with varied symptoms depending on the nature and the quantity of the metal ingested (Adepoju-Bello and Alabi, 2005).

Antimony is a metal used in the compound antimony trioxide, a flame retardant. There is a little information on the effect of long term antimony exposure, both lead and

antimony are suspected human carcinogen (Bakare-Odunola, 2005). Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and human. In human, long term exposure is associated with renal dysfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in human and animals. This is an intensely painful disease leading to deformity of bone (Bakare-Odunola, 2005). The biological activity of selenium has been of interest since it is needed by humans and other animals in small amounts, but in larger amounts can cause damage to the nervous system, fatigue and irritability. Selenium accumulates in living tissue, causing high selenium content in fish and other organisms, and causing greater health problems in human over a lifetime of over exposure. Acute exposure to lead is also more likely to occur in the work place, particularly in manufacturing processes that include the use of lead symptoms include abdominal pain, convulsion, hypertension, renal dysfunction. Etc. Chronic exposure and accumulation of lead may result in birth defects, mental retardation, and autism. Lead also depresses sperm count (Anglin-Brown *et al.,* 1995).

Arsenic is a highly toxic metalloid element. It is a key additive in rat poison, and with constant exposure, it is thought that arsenic may affect the chromosomes of humans and their health. However, very small amounts of arsenic could be good for humans to live and even be able to breathe. The inorganic form of arsenic found in contaminated meats, weed killers and insecticides, however can be very toxic (Pizzaro *et al.,* 2003). Chromium is used in metal alloys and pigments for plants, cement, paper, rubber and other materials. Low level exposure can irritate the skin and cause ulceration. Long term exposure can cause kidney and liver damage, and damage to circulatory and nerve tissue. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium. However, under certain environmental conditions and certain metabolic transformations, chromium (III) may readily be oxidized to chromium (VI) compounds that are toxic to human health (ATSDR, 2000, Awan *et al.,* 2003). The vast increase in environmental pollution by heavy metals puts public health at risk. Various effects of heavy metal pollution in humans are morphological abnormalities, neurophysiological disturbances, genetic alteration of cells (mutation), tetratogenesis and carcinogenesis. The presence of heavy metals affects enzymes and hormonal activities as well as growth and in mortality rate (Idris *et al.,* 2007).

2.18: The influence of salinity on heavy metal mobility of harbour sediments

Trace metals are among the most common contaminants bound to estuarine sediments. The bioavailability and toxicity of these metals to aquatic organisms depend on the physical and chemical forms of the metal as well as several physicochemical parameters such as temperature, pH, salinity, dissolved oxygen and particulates matter composition. In fresh water, pH is the controlling factors while salinity is stated as one of the controlling factors affecting the partitioning of contaminants between sediments and water in sediments in marine or estuarine environment due to the great variability of this parameter in them (Chapman and Wang, 2001). Several studies relating the effects of salinity and pH on heavy metals mobility in estuarine and marsh sediments are reported (Liang and Wong, 2003; Riba *et al.,* 2003, Riba *et al.,* 2010). A decrease in the salinity of dredged harbour sediments may lead to a different partitioning coefficients of (ratio between metal in sediment and the interstitial water, Kd) heavy metals but depends on several predominant processes such as mobilisation of metals through complexation with seawater anions (Cl^- and SO_4^{2-}) (Chapman and Wang, 2001) . Changes in salinity play a major role in metal distribution in dredged harbour sediments, especially when washing procedure is applied as a remediation technique or when dredged harbour sediments are deposited in the open air.

In related studies, Riba *et al* (2004) investigated the influence of pH, and salinity on the toxicity of heavy metals in sediments to the estuarine calm Ruditape Phillippinarium. They found out that heavy metals tend to be more bioavailable at lower salinity than at higher salinity value and this may be more toxic to the exposed organisms. They were able to establish that the effect of the salinity varies from metal to metal depending on the relative important of two counteracting processes, desorption from sediments to water or coagulation, flocculation and precipitation. From their results, sediments collected in area affected by chronic heavy metal contamination tend to be more efficient in trapping Zn, Cu and Pb at low salinity values. They found out that Cd tends to be more mobile as salinity increases. In another study, Guevara- Riba *et al* (2005) worked on the effect of chloride on heavy metal mobility of harbour sediments. Modified BCR- SEP was applied to harbour mobility in order to assess the extent trace element mobility (Cd, Cr, Cu, Ni, Pb and Zn) could be influenced by chloride content in sediments. Washed and non-washed sediment were compared respectively. The relative mobility order found for the six trace metals studied was not seen to be influenced by the presence of chloride in the sediments. An increase in mobility was observed for Cd and Zn (the most mobile metals) when chloride was present in the sediments. This was in agreement with findings from Riba *et al* (2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Cleaning of glassware

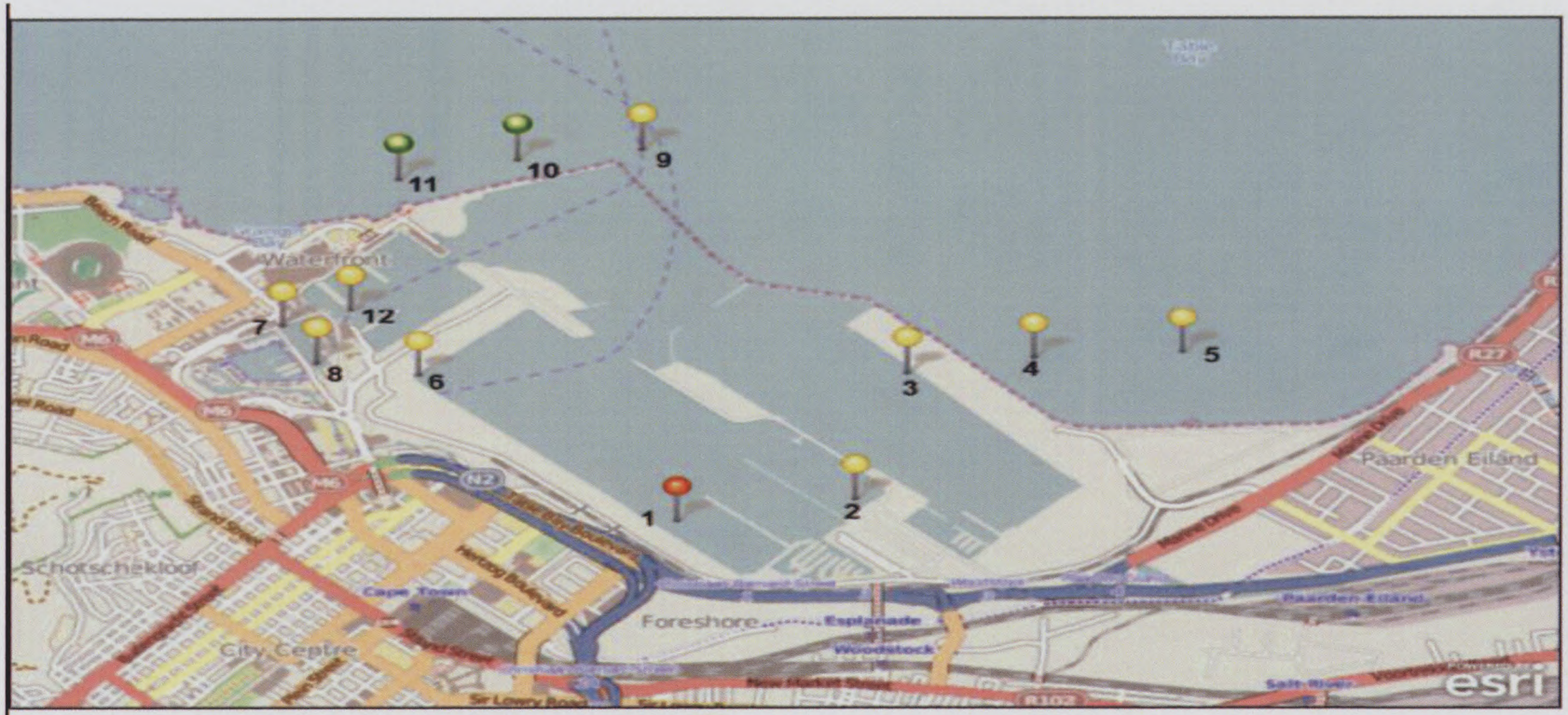
Deionised water (MilliQ 18.2 MΩ cm - Millipore Bedford, MA USA) deionising system was used for the preparation of solutions throughout the study. All reagents used were of analytical grade and were supplied by Merck and Sigma of Cape Town, South Africa. The polypropylene (PP), high density poly ethylene (HPDE) bottles were prewashed with laboratory grade detergent followed by adequate rinsing with deionised water, and soaking in 0.1M HNO_3 overnight followed by thorough rinsing with deionised water. BCR- 277R reference standard material (for trace elements in sediment) was purchased from the European Community Bureau of reference, IRMM, Belgium.

3.2: The study area

Cape Town harbour is used as the study area. This harbour is one of the busiest ports in South Africa. It handles the largest amount of fresh fruit for export and has a major repair and maintenance facility which is used by several large fishing vessels/ships and the West African oil industry. The study area is shown in figure 3.1 while figure 3b represents the sampling points.

Figure 3.1: Map indicating the sampling area and the position of Cape Town Harbour along the Atlantic Ocean and Indian Ocean. (Goggle map)

The harbour is located within the coordinates of 33°54'S 18°26'E. The port has evolved greatly over the years and currently consists of several main docks. The Ben Schoeman Dock is the largest outer dock of the port, where the container terminal is situated. The sediment samples collected at this site were very muddy. The Duncan dock is the smallest and the older inner dock. It contains the multipurpose and fruit terminals as well as a dry dock, a repair quay and a tanker basin. Both water and sediment samples at this site were very muddy and oily. The synchrolift dry dock is where the ships are lifted up for repair. The Victoria and Alfred basin used to be the main pier of the original Cape Town harbour, but now houses the Victoria and Alfred waterfront. However, this basin is still used by smaller commercial vessels such as fishing and pleasure boats (smaller passenger cruise ships). The synchrolift dry dock is where the ships are lifted up for repair. The distances between control sites 10 and 11, sites 7 and 8, sites 6 and 9, sites 3 and 4, sites 4 and 5 are 513 m, 500 m, 265 m, 1400 m, 501 m and 572 m, respectively. The overall distance covered during sampling is 16.4km. The control sites 10 and 11 were chosen based on the previous studies and on-going monitoring programme of the CSIR (Council for Scientific and Industrial Research).The coordinates of all the sampling points and the depths of sampling are presented in table 3.1.



Marine influence Harbour points Control points

Figure 3.2: Map indicating the sampling points on Cape Town Harbour
(Goggle map with modifications)

Table 3.1: Summary of sampling sites with their coordinates and sampling depths

Sampling sites	Coordinates	Sampling depth	Description
1	S33 55.053 E18 26.236	14 m	Duncan Dock
2	S33 54.982 E18 26.707	12 m	Duncan Dock
3	S33 54.571 E18 26.842	14 m	Ben Schoeman Dock
4	S33 54.518 E18 27.184		Inside Sea 500m away from Point 3
5	S33 54.502 E18 27.566	15 m	Inside Sea 500m away from Point 4
6	S33 54.574 E18 25.550	10 m	Duncan Dock
7	S33 54.411 E18 25.190	12 m	Robinson Dry Dock
8	S33 54.535 E18 25.279	14 m	Synchrolift
9	S33 53.827 E18 26.140	8 m	Entrance to harbour
10	S33 53.862 E18 25.809	3 m	Control A
11	S33 53.926 E18 25.496	6 m	Control B
12	S33 54.367 E1825.370	12 m	Robinson Dry Dock 2.

3.3 Development of an Analytical Method for the determination of some Organotin Compounds in Seawater, Sediment and Mussel Samples Using GC-FPD and GCMS-TOF

3.3.1: Instrumentation

Analyses were performed on a Shimadzu GC-2010 plus serie, Gas chromatography coupled to flame photometric detector (FPD), supplied by AAT, Cape Town SouthAfrica. The GC was equipped with a phenomerex ZB5MSi capillary column (30 m x 0.25 mm I.D x 0.25 µm) coated with 5% phenylpolysiloxane. Automated injection was carried out with an auto sampler AOC-20S. The operating parameters for both GC-FPD and GC-MS-TOF are shown in Table 3.2 and 3.3

Table 3.2: GC –FPD analytical conditions

Parameter	Setting
Injection port	Split / Splitless mode : Splitless
Injection volume	1µl
Injection port temperature	280°C
Detector temperature	300°C
Carrier gas – helium flow	1.69 ml / min
Column (Capillary column)	ZB -5MSi (5% phenyl, 95% Phenylpolysiloxane, diameters: 30m x 0.25mm x 0.25µm film thickness
Oven temperature	50°C for 1min then 10°C to 250°C for 4 mins
Detector type	FPD

Table 3.3: Instrumentation and conditions of Analysis for GCMS- TOF

Instrumentation	
Chromatographic system	Waters GCT equipped with CTC CombiPAL Auto sampler
Column	DB_XLB (30 m, 0.25 mm ID, 0.1 □m film thickness)
Experimental conditions	
Injector temperature	280 °C
Carrier gas flow rate	1 ml/min
Injection volume	1 □l
Injection mode	Splitless
Purge flow	50 ml/min
Purge time	1 min
Carrier gas	Helium
MS mode	EI+
Scanning mass range	35 to 650 m/z
Scan time	0.15 min
Inter-scan delay	0.15 min
Oven temperature	50°C for 1min then 10°C to 300°C for 4 mins
Detector type	MS coupled with Time of Flight

3.3.2: Other equipment

The pH was measured using a pH meter with glass electrode from Beckman (Fullerton, USA). Lichrolit florisil SPE Cartridges (1000 mg, 6 ml, 125 -150 m) were obtained from Sigma – Aldrich, Cape Town, South Africa. A vortex mixer made by Scientific industries Vortex Genie 2 supplied by Lasec, South Africa, and a shaker (Orbishake) supplied by Labotech, Magnetic instrument (FMH instrument) were used, respectively.

3.4: Water Samples

MilliQ water and sea water samples collected from Cape Town harbour were used for recovery and validation experiments, respectively. Sea water samples were collected at a coordinate of S33 54.367 E1825.370 in triplicate using a motor powered boat (Waveride DTC 787C - 6.3m Stingray cat hull - supplied by Stingray Marine, powered by Suzuki 90 hp's 4stroke engines) which was equipped with Van Veen Grab sampler. Garmin GPS was used to locate the sampling coordinates. Samples were collected during low tide. The samples were collected from the Synchrolift and Robinson dry dock sites. The samples, in plastic bags were stored in an ice chest and transported to the laboratory for further analysis.

3. 4.1:Standards and reagents

Methanol, n-hexane, isooctane, dichloromethane and tripropyltin chloride (98%) used as internal standard, were obtained from Merck (Germany). All organic solvents were of analytical chromatographic grade. They were doubly distilled prior to use. Tributyltinchloride (95%), dibutyltindichloride (96%), triphenyltin trichloride (95%), sodium tetraethyl borate ($NaBEt_4$, Tropolone 92 and hydroxyl- 2, 4, 6-cycloheptatrienone,. glacial acetic acid (98%), sodium acetate, toluene (99%), hydrochloric acid (32%), silica gel (60 - 200 mm) and anhydrous sodium sulphate were purchased from Sigma – Aldrich, Cape Town, South Africa. High purity gases (helium, hydrogen and medical air - 99.999%) were purchased from Afrox (Pty) Ltd. (South Africa). All glassware used was soaked overnight in 1M HNO_3 to remove sorbed organotin compounds, rinsed with deionised water and dried with acetone immediately before use.

3.4.2: Solutions

Stock solutions of organotin (1000 mg/l) were prepared in methanol and stored in amber bottles in a refrigerator at 4°C. Working standards of 100 mg/l in methanol were prepared weekly. Solutions containing 10 mg/l were prepared daily by dilution in methanol. The sodium acetate buffer (CH_3COOH /CH_3COONa) was prepared by adding an appropriate amount of sodium acetate (4 g) in deionised water followed by pH adjustment with acetic acid (3 ml in 1L) to pH (4.5). The working solution of sodiumtetraethylborate was freshly prepared in methanol and stored at +4°C in the refrigerator. Deionised water was obtained from MilliQ water system (Millipore, USA).

3.4.3: Samples

Freeze-dried coastal sediment (BCR-462) reference standard (certified for TBT- 54 ± 15 µg/kg and DBT - 68 ± 12 µg/kg) was obtained from the Institute for Reference Material and Measurement (IRMM), (Geel, Belgium). Freeze-dried mussel tissue (ERM-CE 477) certified for TBT (2.20 ± 0.19 mg/kg), DBT (1.54 ± 0.12 mg/kg) and MBT (1.50 ± 0.28 mg/kg) was also obtained from IRMM (Geel, Belgium). These certified reference materials were used for method validation.

3.4. 4: Analytical characteristics of the methods

The analytical characteristics of the methods are listed in Table 3.2 and 3.3. There were no traces of organotin compounds found in the procedural blanks and the blank samples. The instrument detection limit (IDL) for both TBT and TPT was determined. The individual standards for TBT and TPT were prepared at concentrations ranging from 0.01 to

1ppm for each analyte. The derivatized organotin standards were run. The instrument was able to detect the compounds up to 10 ppb levels for both TPT and TBT. Method precision was determined by replicate injection of standard mixtures prepared in the laboratory. The standard deviation expressed as the coefficient of variation was recorded. From the literature, various methods for determining the LOD and LOQ were reported. For this study, the LOD (0.01ppm) was calculated as three times the standard deviation while the LOQ (0.003ppm) was calculated as three times the LOD.

3.5: Optimisation of the extraction method

3.5.1: Liquid- liquid (LLE) and solid- phase (SPE) extraction methods

3.5.2: Liquid liquid extraction

Three aliquots of 100 ml of water sample were transferred into volumetric flasks and acidified to pH 2. The mixture was spiked with a known concentration of TBT and TPT standard solution. The spiked samples were shaken manually and left to equilibrate for 15 minutes prior to extraction. This was followed by two consecutive two-minute extractions with 50 ml hexane. The organic layer was collected and derivatized by adding 1ml of sodium acetate buffer at pH 4.5, and 1ml of sodium-tetraethyl borate (STEB) in methanol (1% v/v). The mixture was shaken for 30 minutes. The organic layer was dried over anhydrous sodium sulphate to remove water. The organic extract was concentrated on a water bath. It was purged to dryness using a gentle stream of nitrogen gas and then reconstituted by adding 1 ml of n-hexane. Volumes of 1 µL were injected into the GC-FPD instrument for analysis.

3.5.3: Solid phase extraction procedure

The sample was first filtered through a filter paper to remove suspended particles. A 1 M solution (2 ml) of hydrochloric acid was added to the sample as a preservative. The mixture was stored in a 1 - litre acid – washed amber glass bottles and kept refrigerated at 4°C for later use. The extraction was carried out according to Vidal *et al.* (2003) with some modifications. A volume of 500 ml of water sample was adjusted to pH 2 with HCl. 15 g of NaCl was added in order to simulate seawater samples. Each water sample was aspirated through Strata C_{18} SPE cartridges previously conditioned with a sequence of 5 ml of toluene, 5 ml of methanol and 5 ml of deionised water. Each cartridge was dried for 45 minutes before use. The cartridges were not allowed to dry completely during the extraction process, and air contact with the column was avoided during the extraction process. A vacuum pump connected to PTFE tubing was used to pump the water sample through the column. The sample flow rate was controlled at 8 to 10 ml/min. The analytes were eluted (under gravity)

with 10 ml of toluene from the SPE cartridge, and concentrated to 2 ml by purging with nitrogen gas. The samples were then ready for GC-FPD analysis.

For water samples, the clean-up of the extract was accomplished by an SPE method using florisils. The procedure was very simple owing to the fact that cartridges retained the organotin when extracts were passed through without retaining any potential interference that had been co-extracted with the target analytes. The organotins were then eluted with a mixture of toluene and n-hexane (1:1; v/v). One major advantage of the clean-up step is that it increases the stability of the analytes in the extracts. Extracts obtained without proper cleanup steps showed lower stability than those extracts obtained with clean up.

3.6: Optimisation for derivatisation procedure

Three derivatisation procedures were employed for organotin ethylations. The one with the best yield was employed for the experiment:

3.6.1: Derivatisation method 1

1 ml of organotin standard, 1ml of acetate buffer (82 g/l of sodium acetate in water, adjusted to pH 4.5 with acetic acid) and 50 µl of derivatisation reagent were added. The derivatising agent was prepared by dissolving 2 g $NaBEt_4$ (STEB) in 10 ml methanol (20%). This solution was freshly prepared. The sample mixture was shaken and allowed to react for 30 minutes. After addition of 5 ml of water, the derivatised compounds were extracted in 1ml hexane. The mixture was centrifuged for 10 s and the two phases were allowed to separate. The clear upper layer (apolar hexane phase) was transferred to an auto sampler vial for analysis. The resulting organotin compounds are ethyl derivatives.

3.6.2: Derivatisation method II

1ml of acetate buffer was added to 1 ml of organotin standard. 1 ml of 1 % STEB in methanol followed by 3 ml of isooctane were added to this mixture. The mixture was shaken for 30 mins and dried over anhydrous sodium sulphate and later concentrated to 1ml under gentle stream of nitrogen and then reconsistuted by adding 1 ml of n-hexane

3.6.3: Derivatisation method III

The same reagent was added as stated above but the difference is that after drying over anhydrous sodium sulphate, the extract was then blown to dryness over hot plate and reconstituted with 1ml of isooctane. The difference between method I, II and III was that method II gave very high percentage yield.

3.7: Extraction of organotin compounds from sediments

Three different methods were employed for the extraction of organotins from sediments

3.7.1: Method S1 (Methanol / Acetic acid digestion)

0.2 g of air-dried sediment sample was placed in a reaction vessel. 4 ml of a mixture of acetic acid and methanol (3:1), 3 ml of acetic acid and 1ml of methanol were added. The resulting slurry was exposed to ultrasonic sonication (80 W) for 30 mins. A volume of 1ml of the extract was derivatised as described above.

3.7.2: Method SII (Methanol / Hydrochloric acid digestion)

0.5 g of air dried sediment was placed in a centrifuge tube. 2 g of NaCl, 12 ml of toluene, 7 ml of 0.03% (w /v) tropolone in methanol and 0.7 ml of 32% HCl were added. The capped tubes were shaken for 60mins. The organic layer was collected and concentrated for further analysis.

3.7.3: Method IIII (Mechanical shaking)

10 g of air dried sediment was weighed into a 250-ml round bottom flask. 10 g of sodium chloride, 20 ml of deionised water, 2 ml of concentrated HCl, 20 ml of 0.02 % tropolone in methanol and 100 ml of hexane were added in that order. The flask was covered and shaken vigorously for 12 hrs. The resulting slurry was filtered and collected over anhydrous sodium sulphate (drying agent) to remove the water. The extract was then concentrated on a water bath. It was then loaded on silica column for clean up as described below. Ethylation of the extract was done by adding 1 ml of sodium acetate buffer followed by 1 ml of 1% STEB in methanol, and the mixture was shaken for 10 minutes. It was then dried over anhydrous sodium sulphate. The final extract was dried by purging with a gentle stream of nitrogen, and reconstituted with 1 ml of hexane. 1 µl of the final extract was injected into a GC-FPD for analysis. Figure 3.3 represent overall extraction procedures for water, sediment and mussel samples.

3.8: Optimisation for clean up procedure

Activated silica was spread on an aluminium foil. Some was oven-dried at 180 °C for 24 hrs and the other at 240 °C for 2 hrs before use. The one baked at 240°C for 2 hrs gave efficient cleanup of extract. The column was prepared by first packing anhydrous sodium sulphate at the bottom followed by silica gel at the middle and anhydrous sodium sulphate at the top .The main reason for the clean-up step was to purify the extracts as well as to remove the colour that might be present. This could affect the injector in the GC-FPD

instrument. The main purpose for using activated silica was to trap the analyte of interest, and release it during the elution step. Anhydrous sodium sulphate allows free flow of eluent during elution and also removes water that might be in the eluent after extraction. After the optimised procedure, the derivatised extracts were purified on a column containing activated silica gel soaked with a mixture of n-hexane and toluene (1:1 (v/v). The column was conditioned with 20 ml of hexane and 10 ml elution. The high percent recovery reveals that the solvent is essential for the clean–up process. The eluted samples were then ready for analysis on the GC-FPD instrument.

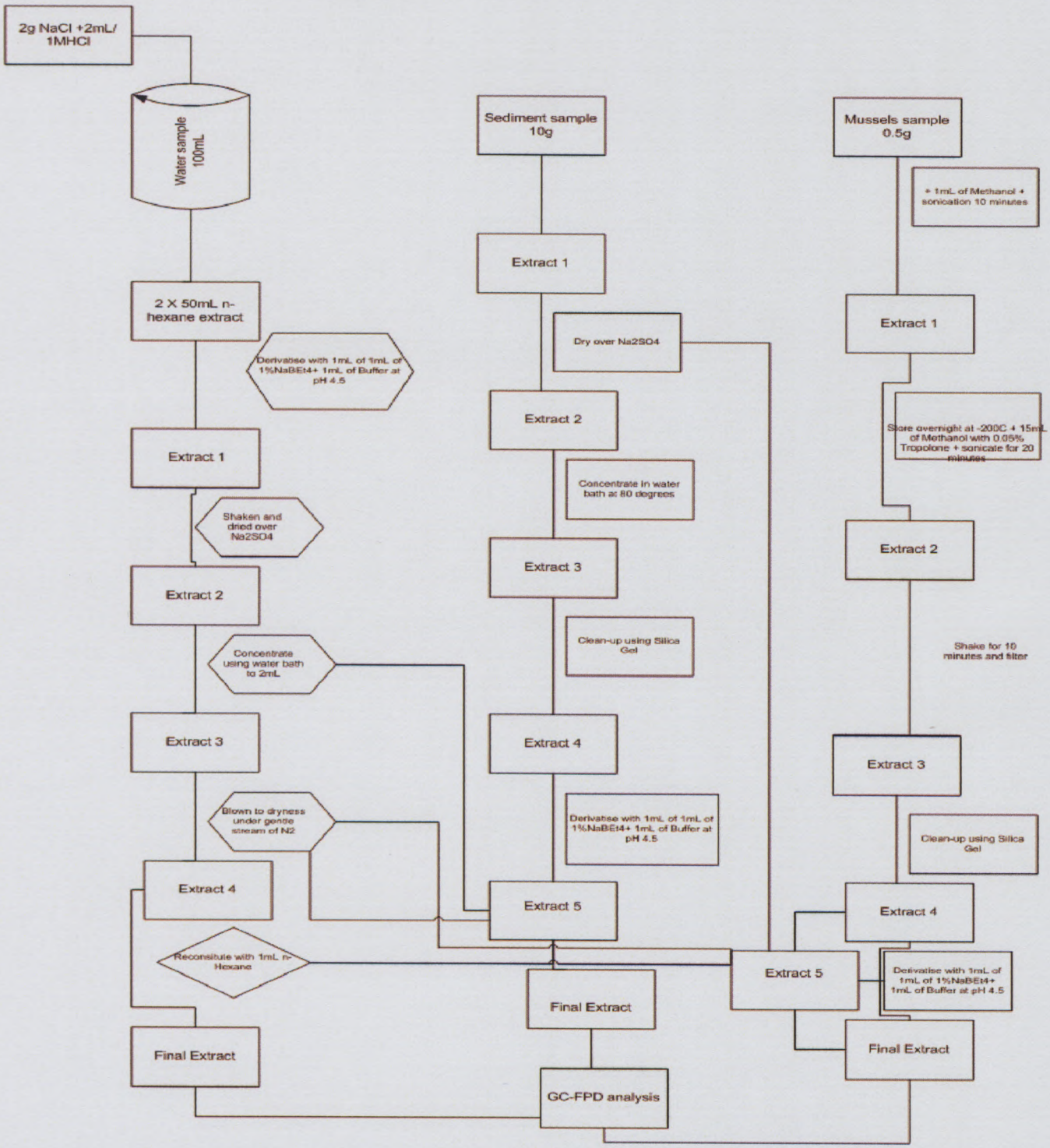


Figure 3.3: A schematic diagram of overall extraction procedures for water, sediment and mussel samples

3.9: Measurement of lysosomal membrane stability of the black mussel *Mytilus galloprovincialis*, after TBT exposure, using the neutral red retention time bioassay.

3.9.1: Experimental design

3.9.1.1: Sample collection and preparation

A total of 75 clean, uncontaminated black mussel (*Mytilus galloprovincialis*) samples were collected from Scarborough Camel Rock on the West Coast on April 20, 2012. The mussels were collected by hand-plucking them from the rocks during low tide. The sample collection method was in compliance with the South African Marine Living Resources Act, 1998 (Act no .18 of 1998) (DWARF (2010). Mussels were transported in individual 20 L buckets with water from their original habitats. In the laboratory, the samples were kept in 20-L plastic containers filled with aerated, clean seawater that had been filtered through an ultra pure filtration system (So-Pure Water Technologies Inc. USA). The mussels were acclimatised to laboratory conditions by maintaining them at a temperature of 15-17°C for two days. They were not fed during this time in order to allow for gut clearance.

3.9.1.2: Exposure experiment

After acclimatisation, the mussels were randomly divided into three groups of 25 animals each. Group 1 represented the control and were not exposed to TBT. Group 2 was exposed to TBT with concentration similar to that measured in seawater in the Cape Town harbour (0.1 µg/l). Group 3 was exposed to a much higher TBT concentration of 1.0 µg/l. Each group was subdivided into three replicates of 25 animals each. These replicates were kept in 20 litter plastic containers, in clean, aerated seawater. Prior to commencement of the 4-week experiment, six individuals from each group were removed and used for neutral red retention time determination, as well as for subsequent metal analysis. For the latter, mussels were frozen and stored at -20 °C until such time as sample preparation could be done. Upon commencement of the exposure experiment, the animals were fed with 3 drops of Microvet invertebrate filter food. After two hours, the water was replaced with clean water, after which the TBT was added to the water. This was done by spiking the low exposure group with 0.1 µg/l (2 µl of TBT) and high exposure group (20 µl of TBT) in that order. Exposures were carried out for four weeks and each week six animals from each group (2 from each replicate group) were removed for analyses.

3.9.1.3: Neutral Red Retention Time (NRRT) Assay

Hemolymph extraction was done by gently opening the mussel valves, and inserting a 1-ml needle to draw 20 µl of hemolymph from an adductor muscle into a sterile syringe containing an equal volume of aliquot mixture of temperature adjusted Ringer solution. The mussel physiological saline Animal Ringer solution was made up of 0.53g NaCl, 0.107 g KCl, and 0.13 g $CaCl_2$ dissolved in 1 L of distilled water as described by Weeks and Svendsen (1996). Two drops hemolymph/Ringer solutions were then placed on a microscope slide. This was followed by the addition of 20 µl Neutral Red working solution to each drop. Neutral Red stock solution was prepared by dissolving 20 mg of dye in 1 ml of dimethyl sulphoxide (DMSO). The working solution was prepared by mixing 10 µl of the stock solution with 2.5 ml of the mussel physiological saline Animal Ringer. The stock solution was prepared freshly every week while the working solution was prepared daily to prevent crystallisation of the non polar Neutral Red in the aqueous Ringer solution. As described by Snyman *et al.* (2000), each hemolymph sample was viewed under a light microscope (model Olympus CX31) with magnification x40. The total numbers of hemocytes, as well as the total number of stained hemocytes were counted under a light microscope at two-minute intervals. After each observation, the slide was placed in a humidity chamber and then returned to the microscope after two minutes. The time, in minutes, at which 50% or more of the total number of the hemocytes were stained, was expressed as the Neutral Red retention time.

3.9.2: Tributyltin analysis

3.9.2.1: Extraction of TBT from mussel tissues

Two different methods were employed for the extraction of TBT from mussels

At the end of the 4-week experiment, all frozen mussel specimens were used for TBT analysis. The first extraction step of the analysis was based on the method described by Liscio et *al.* (2009). Freeze dried certified reference material (ERM-CE 477) for mussel was weighed (0.5 g) into a 50 ml glass tube. A rehumidication step was carried out by adding 1 ml of methanol followed by 10-minute sonication. The extracts contained in the tubes were stored at -20 °C overnight. The resulting slurry formed was further extracted with 15 ml of methanol containing tropolone (0.005%), sonicated for 20 mins, and then the suspension was shaken for 10 mins. The resulting slurry was filtered to remove water and concentrated on a water bath at 80°C and then cleaned up as described above. The extracts were then derivatised by adding 1 ml of sodium acetate buffer, 1 ml of 1%STEB in methanol and mixture was shaken for 10 mins. The extracts were dried over anhydrous sodium sulphate. The final extract was purged to dryness using a gentle stream of nitrogen and

reconstituted with 1 ml of hexane. 1 µl of the final extract was injected into a GC-FPD for analysis.

Extraction Method II: This extraction was achieved by adding 4 ml of a mixture of acetic acid and methanol (3:1 v/v) to 0.2 g of sample, and the resulting slurry was heated in a water bath at 37°C for 1hour. The resulting extract (1 mL) was derivatised and cleaned up as described above.

3.9.2.2 Cell viability

Ten mussels were kept in similar conditions to the test animals, for the purpose of determining whether the process of hemocyte extraction and the NRRT procedure markedly damaged the cells and thus had a significant effect on the outcome of the results. The Eosin Y test was used for this purpose. Dead cells stain red and healthy cells are colourless, thus the percentage viable cells can be calculated (Snyman et al. 2000).

3.10: Quality assurance and quality control (QA / AC)

Solvent blanks and procedural blanks were included in each batch of analyses and they were always analysed after every sample injection. Procedural and spiked water samples were treated in the same manner. A calibration standard solution of known concentration was injected in duplicate to monitor the instrument sensitivity and reproducibility each time prior to sample analysis. Reference standards used for recovery experiments and to confirm extraction efficiency were freeze-dried coastal sediment (BCR-462) certified for TBT (54 ± 15 µg/kg) and DBT (68 ± 12 µg/kg) and freeze–dried mussel tissue (ERM–CE 477) certified for TBT (2.20 ± 0.19 mg /kg), DBT (1.54 ± 0.12 mg/kg) and MBT (1.50 ± 0.28 mg/kg). Both standards were obtained from the Institute for Reference Material and Measurement (IRMM), Geel, Belgium. Both water and sediment samples collected from the Cape Town harbour were used to validate the results.

3.11: Method development for the speciation of heavy metals in marine sediments of Cape Town harbour

3.11.1: Samples collection and preparation

The boat used for sampling was hired from the Council for Scientific and Industrial Research (CSIR) based in Stellenbosch. The boat's description is: Boat Waveride DTC 787C (6.3 m Stingray cat hull) supplied by Stingray Marine and powered by Suzuki 90 hp's 4-stroke engines and equipped with Van Even Grab sampler. Samples were collected in triplicate using the Van Even Grab sampler. Garmin GPS was used to get the sampling coordinates. Samples were collected during low tide except for samples 4 and 5 which were collected at high tide. Eleven samples were collected. Six of them were from inside the harbour, two from the control sites and the last three were collected about one kilometre outside the harbour in the ocean. The samples were collected in zippered plastic bags, placed in ice-packed sample boxes, and transported to the laboratory. In the laboratory, the samples were spread on aluminium foils and air dried at room temperature for a week. The dried samples were ground using mortar and pestle, screened and sieved with a laboratory test sieve of size 500 µm. They were then homogenised and finally stored at -4°C in a refrigerator prior to sequential extraction.

3.11.2: Reagents and instrumentation

Deionised water (MilliQ, 18.2 MΩ cm, Millipore Bedford, MA USA) was used for the preparation of samples in this study. All reagents used were of analytical grade and were supplied by Merck and Kimix of South Africa. The polypropylene (PP), high density (poly ethylene (HPDE) bottles were prewashed with laboratory grade detergent, rinsed with deionised water, soaked overnight in 0.1M HNO_3, and finally rinsed with deionised water. An Agilent 7700 ICP-MS and a Varian Liberty II ICP-AES were used for the determination of heavy metals. A centrifuge (BHG, Rotor Unit II Nr. 7686 V220 A. 0.7 J 487), operated at 4000 rpm for 20 minutes, was used to obtain supernatant extracts at. The sediment samples were then digested using a Milestone GmbH MLS-1200 Mega microwave digestion system configured with a MDR-1000 /6 carousels and TFM vessels.

3.11.3: Sequential extraction procedures

Speciation experiments were performed using the modified Tessier method (Tessier *et al.*, 1979). The main modifications included the use of 1.0 M sodium acetate instead of $MgCl_2$, centrifugation for 20 minutes to get a clear supernatant solution, and microwave

digestion to extract the recalcitrant metals in the residual fractions. The details of the modified Tessier method are described below:

Fraction 1 (Step 1) : A 1.0-g sample was extracted with 8 ml 1M Sodium acetate at pH 8.2 at room temperature and centrifuged at 4000 rpm for 20mins. The residual solid was washed with 5 ml deionised water, and further centrifuged at 4000 rpm for 5 min. The extracted fraction contained absorptive and exchangeable metals.

Fraction 2 (Step 2) : The solid residue from step 1 was extracted further with 8 ml of 1M sodium acetate (pH 5 with glacial acetic acid) and centrifuged at 4000 rpm for 20 min. This was followed by rinsing of solid residue with 5 ml of deionised water and further centrifuged at 4000 rpm for 5 min. This segment contained metals bound to the carbonates.

Fraction 3 (Step 3): The solid residue from step 2 was leached with 8 ml of 0.1M $NH_2OH.HCl$ (in 25% v /v acetic acid) and then centrifuged at 4000 rpm for 20 mins at room temperature. The solid residue was later rinsed with 5 ml of deionised water and further centrifuged at 4000 rpm for 5 mins. The extracted fraction contained metals that are bound to oxides of iron and manganese.

Fraction 4 (Step 4): 3 ml of 0.02M HNO_3 and 5ml of 30% H_2O_2 adjusted to pH 2.0 were added to the solid residue from step 3. It was then centrifuged at 4000 rpm for 20 mins. The solid residue was rinsed with 5ml of milliQ water and then centrifuged at 4000 rpm for 5 mins. The supernatant extract contained metals which are bound to the organic matter.

Fraction 5 (Step 5): The residue from step 4 was finally microwave digested in aqua regia (7.5 ml HCl: 2.5 ml HNO_3). The digestate was then transferred to a 50-ml volumetric flask and made up to mark.

3.11.4.1: Quality assurance

The obtained extracts from each step were analyzed for Sn, Pb, Cu, Zn, Fe, Cd, Al, Si and Hg. All analyses were carried out in triplicate. The blank samples were analyzed after every 10 measurements. Major and trace elements were analysed by a Varian ICP-AES and Agilent 7700 ICP-MS, respectively. For quantification of the element of interest, the instruments were calibrated daily using NIST traceable standards. A quality control standard was analysed prior to the samples to verify the accuracy and precision of the calibration standards, while control standards were used throughout the analysis to monitor accuracy and instrument drift. For the ICP-MS analyses, internal standards were added to all samples and standards in order to correct for instrument drift due to high matrix load. Data acquisition and processing was software-controlled and exported in excel format. In order to check for the accuracy of the sequential extraction procedure, reference sediment materials BCR-277R (for trace elements) were extracted using the modified Tessier technique and analyzed

in triplicate. The reference materials were purchased from the European Community Bureau of Reference, IRMM, and Belgium.

3.12.0: Method development for geochemical assessment of marine sediment of Cape Town Harbour, South Africa.

3.12.1: Sample collection and preparation

A sample collection site is shown in figure 3.2. A boat from Council of Scientific and Industrial Research (CSIR) was used to collect samples in triplicate at each site. All samples were collected with the aid of sample Boat Waveride DTC 787C (6.3 m stingray cat hull) supplied by Stingray Marine powered by Suzuki 90 hp's 4stroke engines and equipped with Van Veen Grab sampler in September 2010. A Garmin GPS was used to locate the sampling points. Samples were collected during low tide except for samples 4 and 5 which were collected at high tide. Sample sites 1 to 9 were collected inside the harbour, while control samples 10 and 11 were collected outside the harbour at distances of 500 m and 1000 m from the harbour, respectively. The distances between control site A and B, site 7 and 8, site 6 and 9, site 3 and 4, site 4 and 5 are 513 m, 500 m, 265 m, 1400 m, 501 m and 572 m respectively. The overall distance covered during sampling was 16.4 km. The coordinates of all the sampling points and the depths of sampling are presented in Table 3.1. The collected samples in plastic bags were covered with ice in a cooler box. The samples were dried for a week at room temperature. The dried sediments were ground using mortar and pestle, sieved with a 500 µm sieve, homogenised and stored at 4°C prior to total metal and geochemical examination.

3.12.2: Reagents

Deionised water (18.2 MΩ cm from Millipore Bedford, MA USA) was used for all sample preparations. All reagents used were of analytical grade: Acetone (Merck), Potassium bromide powder (KBr) (Merck). The sediment reference materials, BCR- 277R for trace element and BCR-462 for butyltins were supplied by the European Community Bureau of reference, IRMM, Belgium and Industrial analytical, South Africa respectively.

3.12.3: Cleaning and pre-treatment of glassware

All glassware were washed with a detergent, rinsed with deionised water, soaked overnight in 0.1M Nitric acid (HNO_3) and thoroughly rinsed with deionised water. Mortar and pestle were oven dried for 24 hours before use; the Potassium bromide (KBr) powder was baked in the oven for 6 hours and stored in the dessicator.

3.12.3: Instrumentation

An Agilent 7700 Inductive Coupled Plasma-Mass Spectrometry (ICP-MS) was used for the determination of heavy metals. A centrifuge (BHG, Rotor Unit II) Nr. 7686 V220 A. 0.7 J 487 was used to obtain supernatant extracts at 4000rpm for 20 mins. A Milestone GmbH MLS – 1200 Mega microwave digestor was used for sample dissolution in aqua regia. Infrared data were obtained using an FT-IR instrument (Spectrum one Spectrophotometer, Perkin Elmer made in USA). X- Ray Powder Diffraction (XRPD) instrument used was a Phillip PANnalytical PW 3830/ 40 X-ray generator with a PW 3710 MPD control. X-ray diffraction (XRD) system which uses the Xpert software programme for data collection and mineral identification. The instrument was operated at 40 kV and 25 mA and sufficient water pressure was maintained at 400-600 kPa. The instrument was coupled with curved Cu-filtered Cu-K_∞ radiation with slow scan speed of 0.040° / s.

3.13: Sample preparation for Total metal analysis

Sample dissolution was done using a microwave- assisted acid digestion procedure (US EPA, Method 3052, 1996). Three replicates of 1g of each were microwave digested with aqua regia (7.5 ml Hydrochloric acid (HCl): 2.5 ml HNO_3). The digested samples were cooled, filtered, and the filtrate was diluted to the mark in a 100-ml volumetric flask. The samples were then analysed using the ICP-MS instrument.

3.14: Sample preparation and Infrared analysis

Each 20-mg sample was mixed with 400 mg of spectroscopic grade KBr in the ratio 1: 20 using a mortar and pestle. Before mixing, a required amount of KBr powder was dried at 120°C for six hours in an oven in order to prevent the broad OH spectral peak of water from interfering with hydroxyls associated with any of the minerals. The mortar and pestle were also dried in an oven for 24 hrs before use. Each sediment sample was weighed in a microbalance and placed in a clean mortar along with the proper amount of dry KBr to prepare a sample pellet. The size of each pellet prepared was 1 mm in thickness and 13 mm in diameter. The prepared pellet was preserved in a moisture-free glass container before it was placed in a suitable sample holder and introduced in the infrared beam for analysis. The Infrared (IR) spectra of the samples were measured at room temperature. For quality control, the instrument was calibrated for its accuracy with the spectrum of a standard KBr powder. Each time before the spectrum of sample is obtain, the spectrum of KBr powder was measured and checked for its accuracy. All spectra of samples were recorded in the range of 450-4000 cm^{-1}. The time duration for each scan was one minute at 4.00 cm^{-1}

resolution. The threshold value was 5.80 % T while the normalization factor from the quantitative prediction was 1.00.

3.15: Sample preparation and XRPD analysis

The XRPD instrument was used to determine the solid/mineral phases of the sediments. 1g (± 0.0001 g) of the surface sediment sample was weighed. This sample was ground to powder using mortar and pestle. Each powdered sample was sieved through a 40 μm mesh in order to remove sand particles, and the prepared sample was ready for XRD analysis. The XRPD measurements were performed at room temperature using cooled dry Nitrogen (N_2 gas). The PXRD profiles were acquired using a step width of 0.02 deg with a counting time of 0.040 °/s step. The total time taken to analyse each sample was 32 minutes and 30 seconds and the total number of steps for each run was 3900. The Xpert graphics collector software was used for data collection and analysis. For most identification a continuous scan from 2 to 80° 2 Θ with a step size of 0.02° 2 Θ was used. Ethanol was used to clean the sample holder after use to avoid any contamination.

3.15.1: Quality control

For quality control the sample holder containing the powdered sample was placed in the middle of the sample chamber so that the surface of the specimen passes exactly through the axis of the goniometer. For most identification a continuous scan from 2 to 80° 2θ with a step size of 0.02° 2θ was used.

3.16: Method development for Seawater analysis

Water samples were collected in 1-litre plastic containers which were initially washed with detergent and rinsed with distilled water. The containers were finally soaked in 10 % Nitric acid. The containers were then rinsed at least three times with deionised water. At the sampling sites, containers were rinsed three times with the water samples before being filled with the samples. The samples were preserved by adding conc. HNO_3 to each sample bottle and the pH adjusted to 2.0 by the use of pH meter. The samples were stored in a refrigerator at about 4 °C, before subsequent analysis. As samples may contain particulate or organic materials, pre-treatment in the form of digestion is required before analysis.

3.16.1: Marine water digestion

100-mL aliquot of well-mixed sample was measured into a beaker. 2 mL of concentrated HNO_3 and 5 mL of concentrated HCl were added respectively. The sample

was covered with a ribbed watch glass or other suitable covers and heated on a steam bath, hot plate or other heating source at 90 to 95°C to the final volume of 15-20 mL. For mercury, 1 ml of conc. H_2SO_4 followed by 1 ml of 5% $KMnO_4$ solutions were added to 40 ml of water sample. The sample was covered with a ribbed watch glass and heated on a steam bath at a very low temperature of 60 °C cooled on ice chest, transferred into a 50 ml standard flask and diluted to a volume of 50 ml.

3.17: Method development for the determination of Bioaccumulation of Metals in black mussels (*Mytilus galloprovincialis*) in Cape Town Harbour, South Africa

3.17.1: Reagents

Deionised water (Millipore Bedford, MA USA) was used for the preparation of solutions throughout the study. All reagents used were of analytical grade and were supplied by Merck and Sigma Aldrich of South Africa. The polypropylene and high density polyethylene bottles were prewashed with laboratory grade detergent, rinsed with deionised water, soaked overnight in 0.1 M HNO_3, and finally rinsed with deionised water. BCR- 277R reference standard material (for trace elements in sediment) was purchased from the European Community Bureau of reference, IRMM, Belgium.

3.17.2: Sampling

Water, sediment and mussel samples were collected in August 2011 from Cape Town harbour inside the harbour area at coordinates of 33°55'31"S 18°25'26"E. The sampling site is named Robinson dry dock. Water and sediment samples were collected in triplicate with the aid of sample Boat Waveride DTC 787C (6.3 m stingray cat hull) supplied by Stingray Marine powered by Suzuki 90 hp's 4stroke engines and equipped with Van Veen Grab sampler. Garmin GPS was used to get the sampling coordinates.

3.17.2.1: Sediment Sample treatments

The sediment samples were placed in plastic poly Zip block bag on ice chest and transported to the laboratory. In the laboratory sediment samples were spread on aluminium foils and air dried at room temperature for a whole week. The dried sediment were grounded using mortar and pestle, screened and sieved with a laboratory test sieve of size 500µm, homogenised and finally stored at 4°C in refrigerator prior to microwave acid digestion.

3.17.3: Sample collection and preparation

Different sizes of mussels (*Mytilus galloprovincialis*) were collected at polluted sites in the Cape Town harbour with the help of the sampling team from the Council for Scientific and Industrial Research (CSIR), University of Stellenbosch, South Africa. The mussel specimens were sorted with respect to their sizes as follows: Group A (Large size), Group B (medium size), Group C (Small size) and Group D (Whole mussel samples) , since size has sometimes been shown to be an important variable (Saavedra *et al.,* 2004; Cevik *et al.,* 2008). Around twenty mussels were selected randomly from each group. After the samples were sorted out, they were dried in an oven at 105 °C overnight before their soft tissues and shells were separated. Dried shells and tissues were ground into fine powders for 20 minutes using a Spex mill. Each powder was then sieved through a 500 mesh sieve.

3.17.4: Digestion of mussel samples

Total digestions of mussel's samples were performed with CEM MARS, model 240/50 microwave digestion system configured with a MDR-1000 /6 carousels TFM vessels. Triplicate samples of 0.5 g of each fine powdered sample were weighed into Teflon digestion vessels and 10 ml of 65% HNO_3 was added before the samples were digested in the CEM MARS microwave oven. The temperature of each sample in the microwave oven was ramped to 200°C at a pressure of 800psi for 25mins and kept at this temperature for a further 15 min. Each residue was diluted to 25.0 mL with deionised water (Pempkowiak, Sikora, & Biernacka, 1999; Bulut *et al.,* 2007). A triplicate of the blank solution was also subjected to microwave digestion.

3.17.5: Determination of essential element plus silicon and strotium

Higher detection limit elements (K, Ca, Fe, Cu, Zn, Si, Sr, Al) were analyzed using Energy Dispersive X-Ray Fluorescence technique (EDXRF) by employing the standard addition method in sample preparation. The mussel samples were stimulated by 55Fe and 241Am radioactive sources. To detect the radiation scattered from the sample, Geometrically Optimized Large Area Drift Detector (GOLD) proprietary detector with 180,000 throughput cps having a resolution of <185 eV and 4μ sec shaping time was used. Four thousand and ninety six channels of a multichannel analyzer (MCA) were employed for the data acquisition. In quantitative analysis, characteristic X-rays emitted by excited atoms of the sample were registered for a time interval of 5000 s (Cevik *et al.*, 2008).

3.17.6: Analysis of trace heavy metals.

Due to poorer detection limit of EDXRF, mussel samples were analyzed for Mn, Pb, As, Hg, V, Cr, Sn, Cd, Ni and Co with an Agilent 7700 ICP-MS. The Agilent 7700 instrument was used with a Meinhart nebulizer and silica cyclonic with continuous nebulisation. The operating parameters were: Plasma RF power: 1550 W; sample depth: 8.0 mm; Carrier gas: 1.08 L/min; Nebulizer pump: 0.10 rps; Helium gas: 5.3 ml/min. ICP-MS and a Varian Liberty II ICP-AES were also used for determination of these elements in water and sediment samples respectively.

3.17.7: Quality assurance

All analyses were carried out in triplicate. The blank samples were analyzed after every 10 measurements. Essential and non-essential elements were analysed by a Varian ICP-AES and Agilent 7700 ICP-MS respectively. For quantification of the element of interest, the instruments were calibrated daily using NIST traceable standards. A quality control standard was analysed prior to the samples to verify the accuracy of the calibration standards, while control standards were used throughout the analysis to monitor accuracy and instrument drift. On the ICP-MS, internal standards were introduced continuously with the samples and standards to correct for drift due to high matrix load. Data acquisition and processing was software controlled and exported in Excel format. In order to check for the accuracy of the extraction procedure, reference sediment materials BCR- 277R (for trace element) were extracted using the above procedure and analyzed in triplicate. These reference materials were purchased from the European Community Bureau of reference, IRMM, Belgium.

3.18. Statistical analyses

The results were statistically analysed using Statistical Analysis Software (SAS, 2002) 9 software (Cary, NC, USA). Pearson's correlation was applied to evaluate the relationships between the variables and correlation coefficient, with $P \leq 0.05$ (99.5%) regarded as significant. Principal Component Analysis (PCA) was used to analyse the analytical data using the same software. The software were also used to do the ANOVA and regression analysis for the toxicology data. PRIMER VERSION 6: Guide to Software and statistical Methods was used to plot the PCA, Cluster analysis graph and MSD plot for the heavy metals in seawater data. NCSS statistical software (NCSS V8) (Hinze, 2012) was used to plot the dot plot diagram for the physicochemical datas.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Organotin speciation on GC-FPD and GC-MS-TOF

All the OTs studied required a derivatisation step prior to GC analysis. The aim of derivatization was to transform the analyte into compounds with higher volatility. Sodium tetraethylborate (STEB) has been developed to minimize the analysis time. The STEB procedure allows a simultaneous extraction - derivatisation in a buffered medium and produces more thermally stable derivatives. In order to achieve high yield, a large amount of STEB was used for the direct ethylation of organotins in sediment and biological samples. This is important to compensate for the consumption of reagents by side reactions with metals and other compounds in the matrix. A typical chromatogram for organotin reference sample for sediment, mussel and organotin standards derivatized using derivatization method II mentioned above (ethyl-derivative with $NaBEt_4$) is shown in Figure 4.1b. The compounds elute according to their boiling points. The mass spectra obtained for TPT in cocktail mixture of TBT and TPT sample is shown in Figure 4.2.

4.1.1 Linearity and precision

For the organotin compounds investigated, the calibration standards were prepared at a concentrations ranging from 0.01 to 2 ppm in order to get better regression (R) value of the linear graph. Calibration data were obtained from peak area measurements. Regression parameters are recorded in Table 4.1 together with the correlation coefficient (r^2). TPT recorded the highest regression parameter while the TBT recorded the lowest value. The slope also varied from 2.122 to 3.268 for TBT and TPT, respectively. Recovery studies were carried out by addition of natural standard of TBT and TPT to deionised water. Deionised water was spiked with known concentration of TBT and TPT (0.5 and 1 ppm). 100 ml of deionised water was spiked with these concentrations of TPT and TBT using solid phase extraction. Quantitative recoveries of 70% were recorded for TPT and 60% for TBT as shown in Figure 4.3. The reference standards of TBT and TPT were ran, TBT came out at 12.85 minutes and TPT at 21 minutes as shown in Figure 4.1a. The calibration plots for TBT and TPT are shown in Appendix C.

For the recovery experiment. Percentage recovery was calculated from the equation below

$$\%\text{Recovery} = \frac{\text{Amount Recovered} \times 100\%}{\text{Amount Spiked}}$$

Amount Spiked = Concentration of the standard recovered × volume of the extract

Concentration of the analyte in the water and sediment samples was calculated from expressed in µg/l and µg/g, respectively.

Concentration = Amount × volume of extract

Volume of sample

Where, amount = injected concentration × volume of extract.

Table 4.1: Analytical method table

Analyte	Retention time	Calibration plot	r2	R	LOD (ppb)	LOQ (ppb)	Mean RF	%RSD
TBT	**12.85**	**Y= 4358x +12619**	**0.998**	**0.90**	**0.01**	**0.003**	**97850.81**	**25, 838**
TPT	**21. 286**	**Y= 10345x-28859**	**1.00**	**0.91**	**0.01**	**0.003**	**8, 1714e-^{06}**	**11,637**

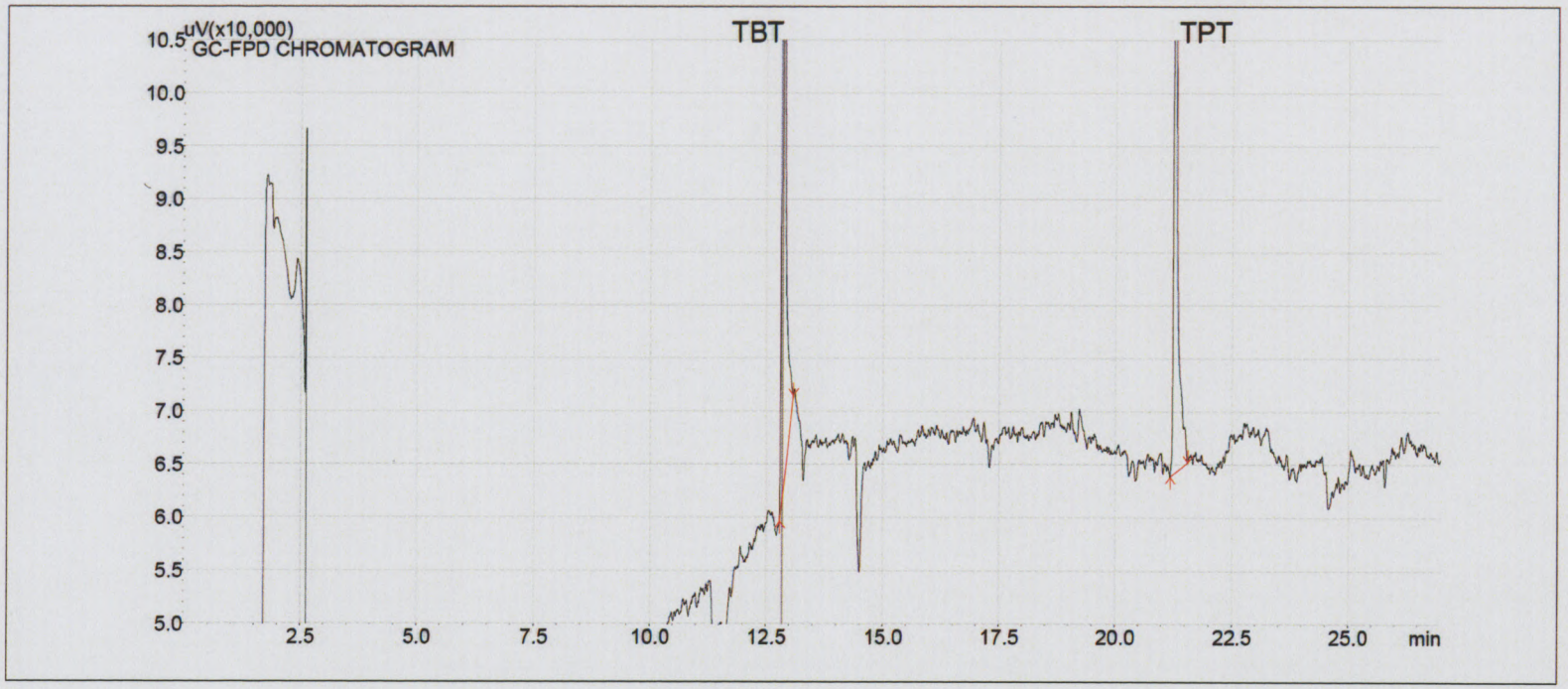


Figure 4.1a: GC-FPD showing chromatogram of reference standards

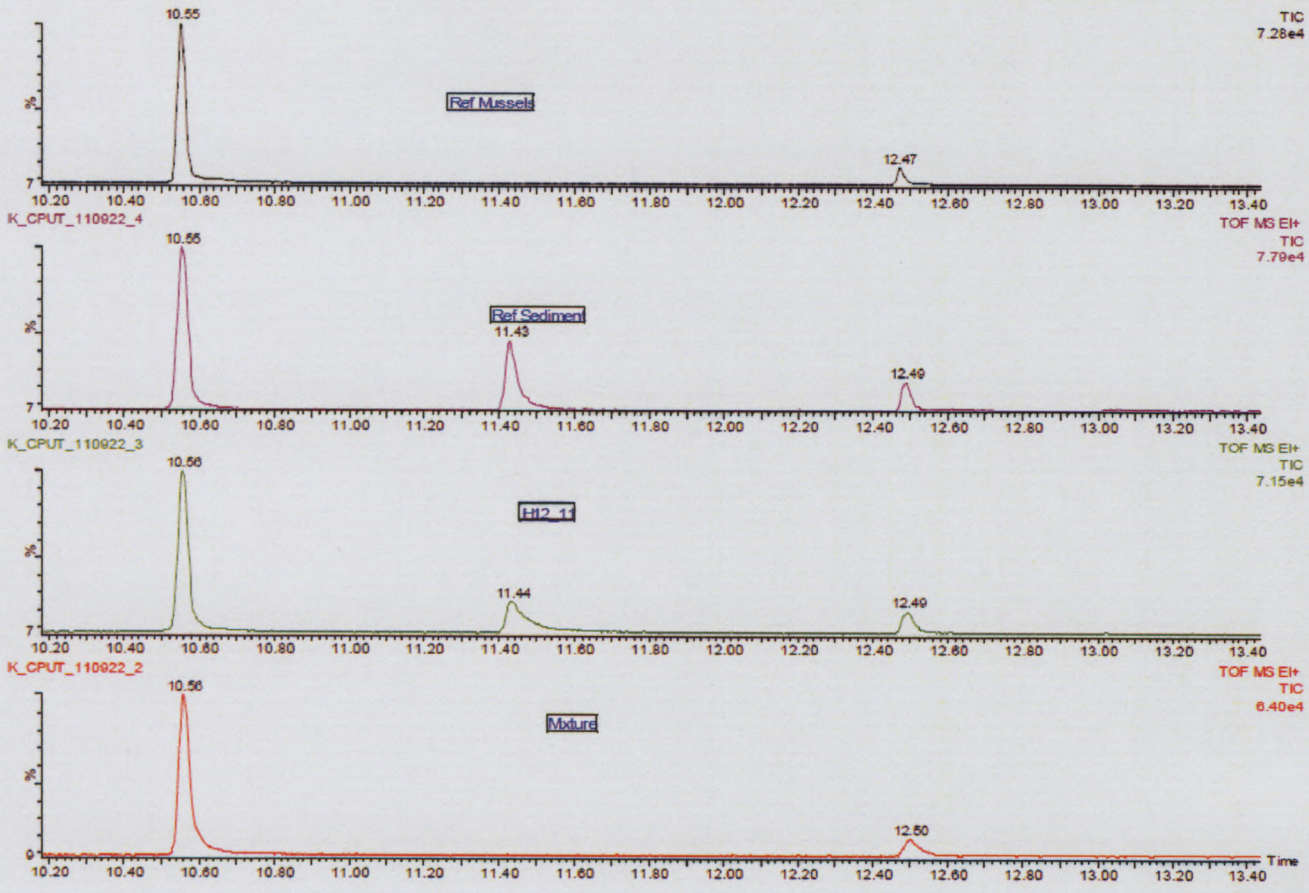


Figure 4.1b: GC-MS TOF chromatograms for the analysis of organotin in reference sediment, sample and mixture

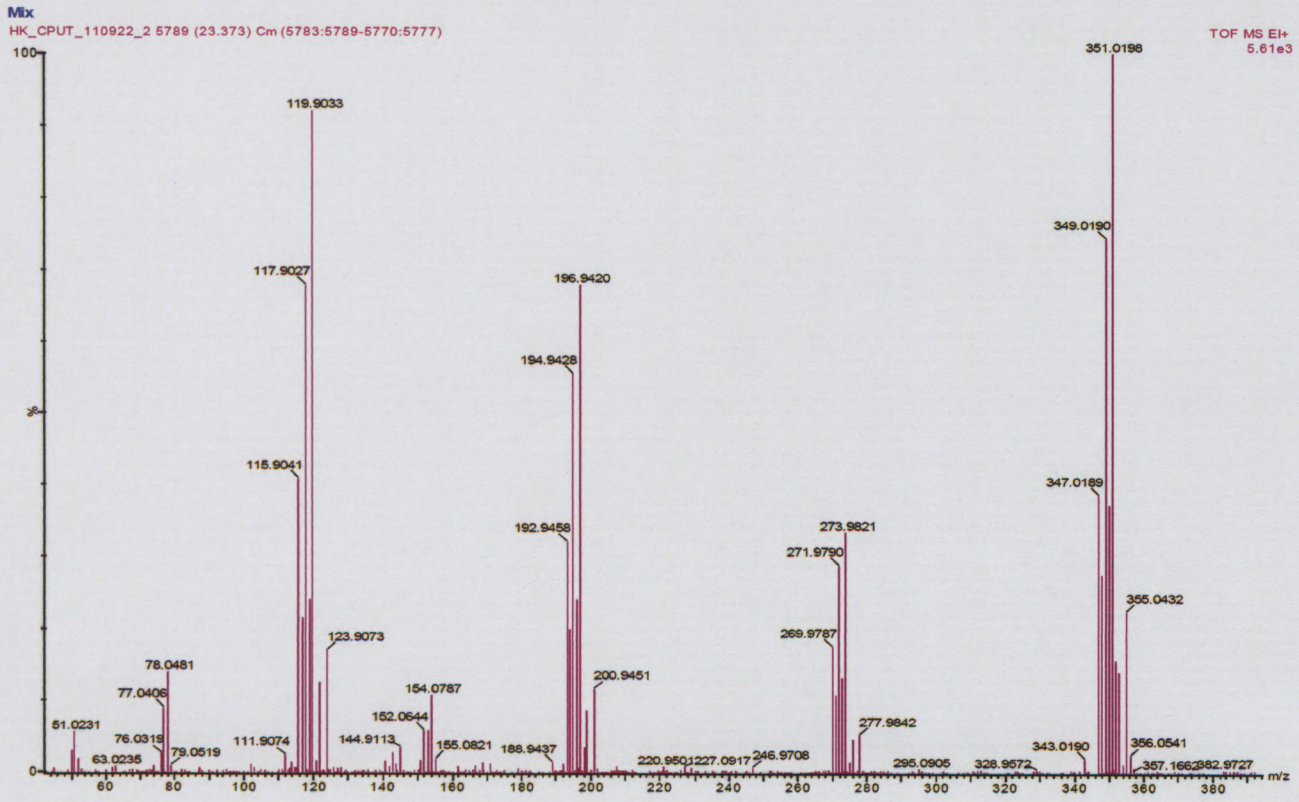


Figure 4.2: TPT mass spectra for the selected molecular clusters in the mix sample

TPT: 351.0198

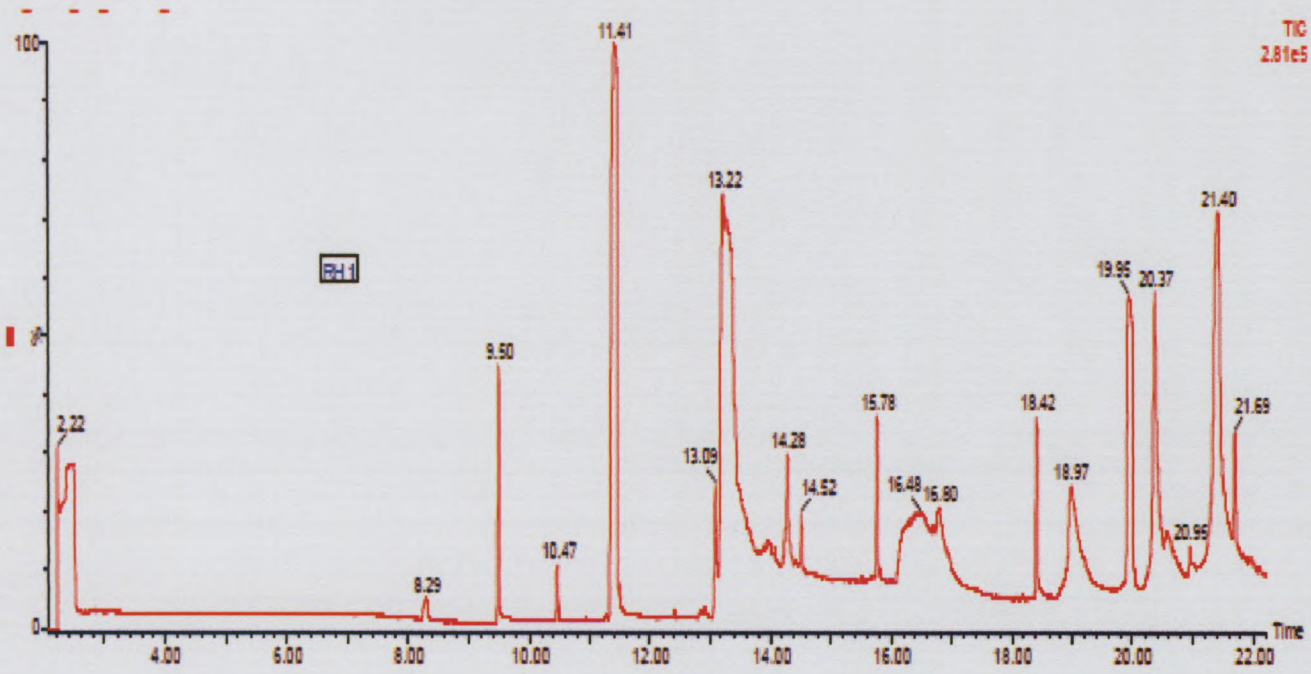


Figure 4.3: GC-MS chromatogramm of organotins in the spiked water sample taken from the laboratory

TBT: 11.41 TPT: 21.40

4.1.2 Analysis of reference materials

Tributyltin was determined in BCR 462 and ERM-CE 477 reference materials. The value found for the TBT was close to the certified values. The GC-MS chromatogram is shown in Figures 4.1b. Results for BCR 462 and ERM-CE 477 showed a good agreement between the certified value and the values obtained from the experimental method. The sonication method showed lower recoveries than the method of mechanical shaking. The same analytical procedure was applied to the real water and sediment samples collected from the Robinson dry dock site of the Cape Town harbour. Replicate samples were analyzed and both TBTs and TPTs were detected. This means that it is imperative to monitor OT compounds at the Cape Town harbour. The GC-MS mass spectra for the organotins in real water samples are shown Figure 4.4. The GC-FPD chromatogram of TBT in real sediment sample is shown in figure 4.5 b, the GC-MS spectra of OTCs derivatives and reference materials for sediment and mussel are shown in Figure 4.5c and 4.7. Table 4.3 represents the characteristics ions for the derivatization products of OTCs.

Table 4.2: Results obtained for the certified reference material

Organotin compound	Reference materials	Certified Value	Obtained value	%Recovery
Tributyltin	BCR 462 reference material for coastal sediment	54±15mg/kg	35±15mg/kg	64.81%
	ERM-CE 477 Reference material for mussel	2.20±0.19mg/kg	1.5±0.19mg/kg	68.18

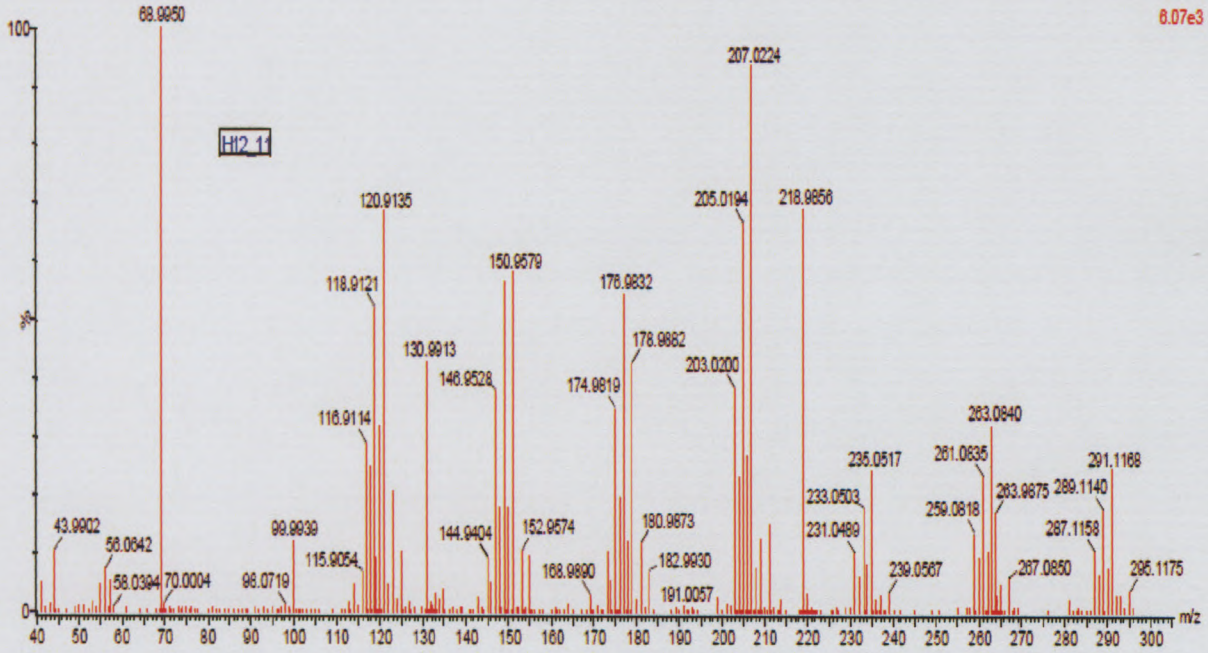


Figure 4.4: Spectra of organotins in the real water samples from Cape Town harbour

TBT: 207.024 9 (Molecular ion)

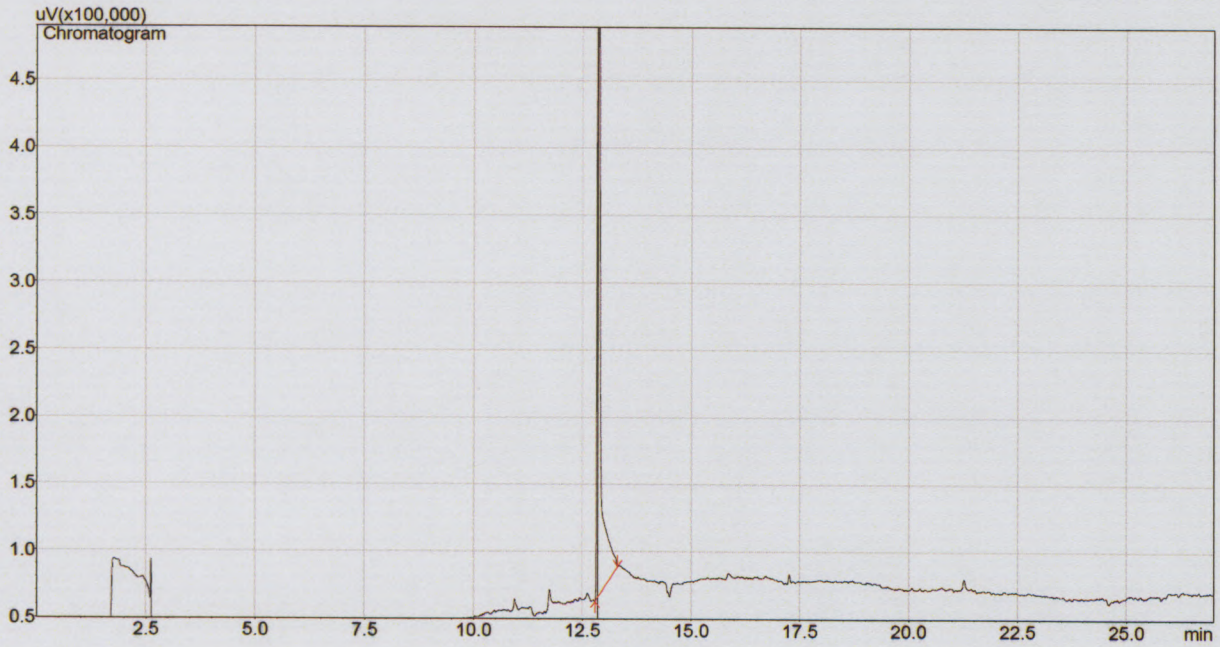


Figure 4.5b: GC-FPD Chromatogram of TBT in the real sediment sample

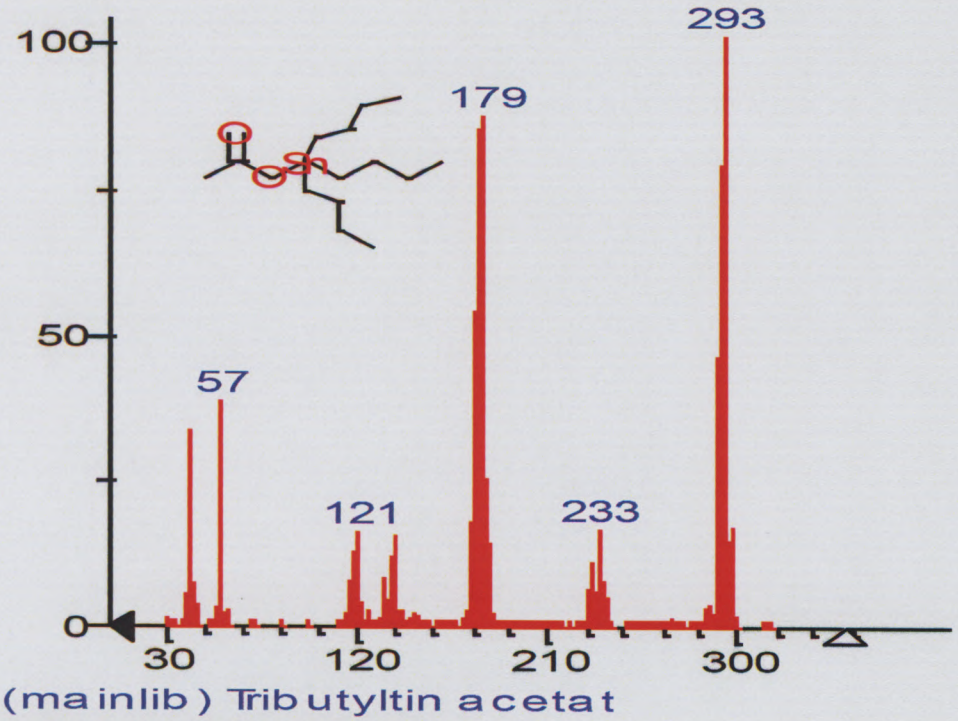


Figure 4.5c: Mass Spectra of organotins derivatives

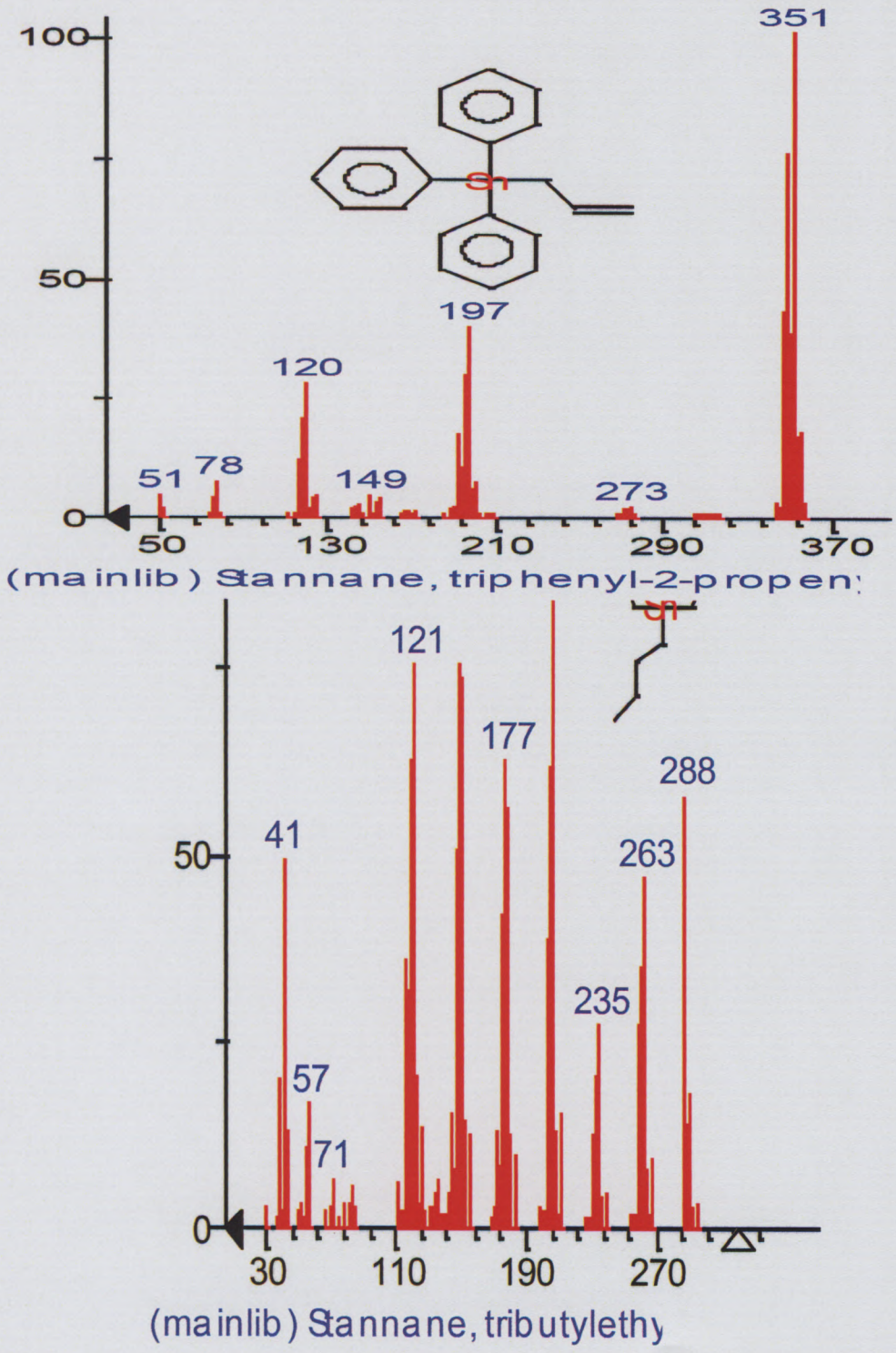


Figure 4.5c: Mass Spectra of organotins derivatives

Table 4.3: Organotin Compounds and characteristics ions for the Derivatization Products

Organotin solute reagent Derivatives	Abbreviation	Molecular ions	Molecular formula
Tributyltin	TBT	291, 289, 207, 205	$C_{12}H_{27}Sn$
Tributyltin acetate	TBT-OAC	57, 121, 179, 233, 293	$C_{14}H_{30}O_2Sn$
Tributylpheny	TBPh	41, 78, 197, 311.	$C_{18}H_{32}Sn$
Tributylethy Stannane	TBE	41, 57, 71, 121, 177, 207, 235, 263, 288.	$C_{14}H_{32}Sn$
Triphenyltin	TPT	51, 78, 120, 149, 197, 273, 351	$C_{18}H_{15}Sn$

4.2 Absorption Characteristics of Organotin compounds using FTIR

An FTIR instrument was used to analyse certified reference material BCR 462 for organotins. The following IR absorption frequencies were recorded as shown in Figure 4.6 693.90, 778.20, 1027.01, 3421.31 (cm^{-1}). Moreover, two certified reference materials for organotins in sediments and that of trace elements were also subjected to XRD investigation. The organotins did not produce any signal during XRD examination and this confirms the absence of mineral phase while the certified reference material for trace element in marine sediment produced characteristics peaks .

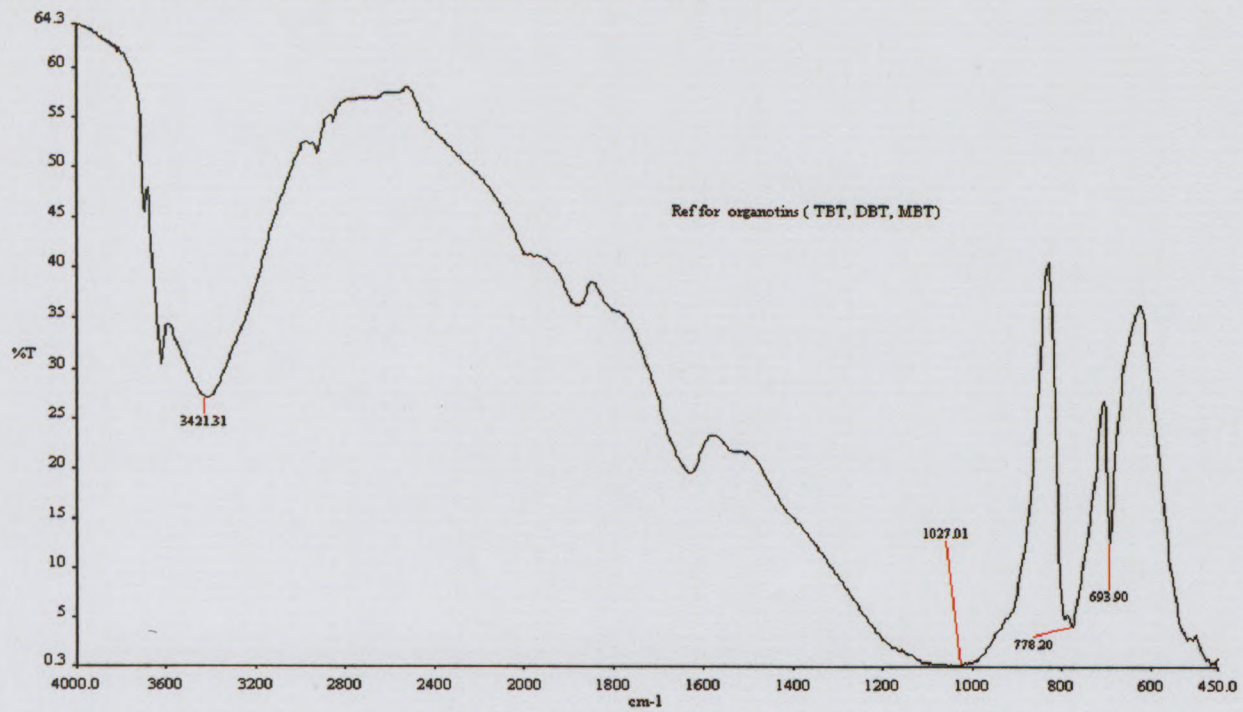


Figure 4.6: FTIR Spectrum of Certified reference sediment sample of organotins compounds

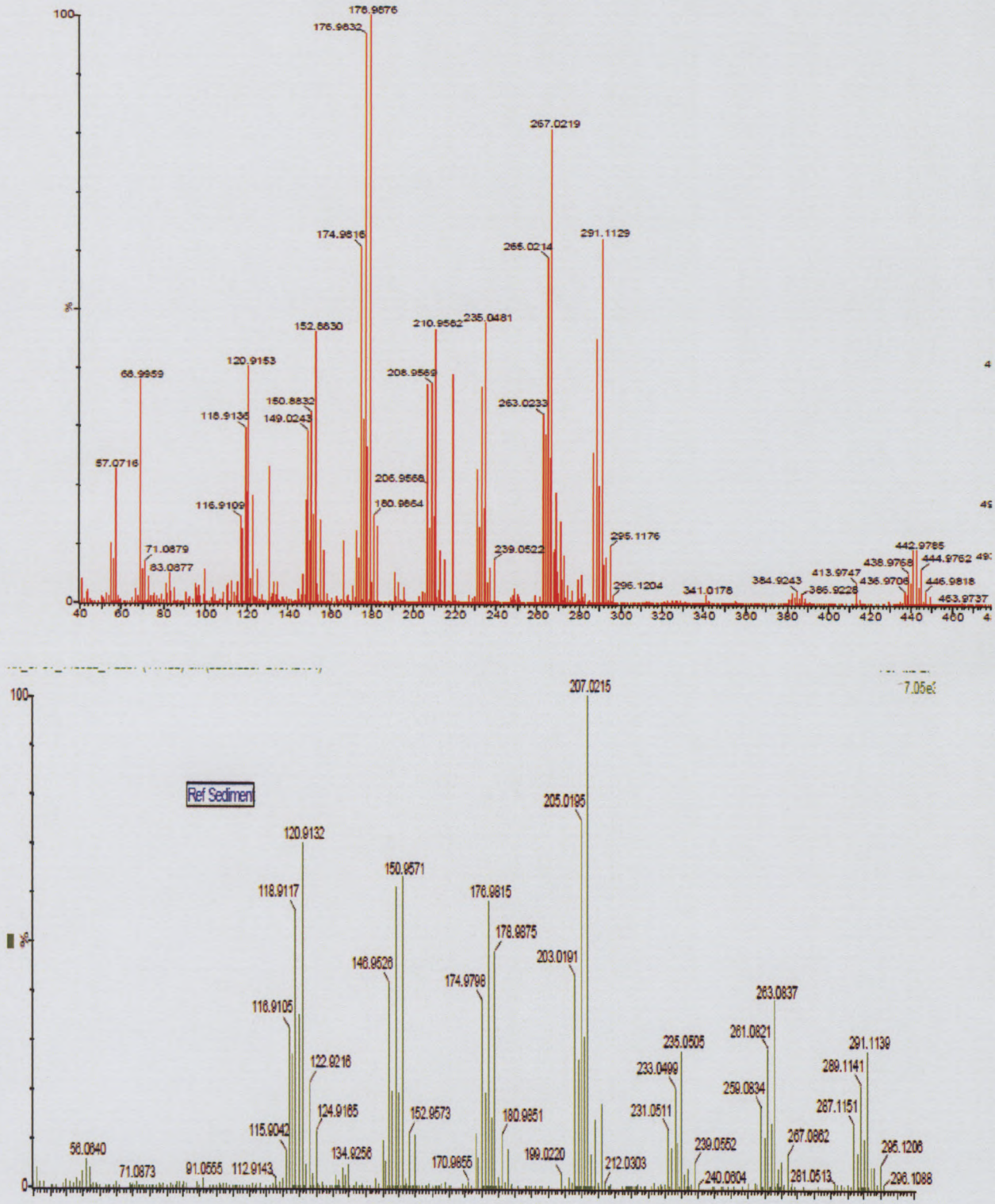


Figure 4.7: Spectra of organotins in the reference materials for sediments and mussels

TBT: 207.0215 (Molecular ion)

4.3: Seasonal variation and annual mean distribution pattern of organotin compounds in seawater from Cape Town harbour

The annual distribution of OTCs in around the harbour during the period of September 2011 to June 2012 is shown in Table 4.4 while the raw datas is presented in Appendix D Table 1.1 a, 1.1b. The concentration of OTCs varies spatially around the harbour. The concentration ranges from 0.067 ± 0.01 to 111.290 ± 32.20 x 10^{-3} µg/l for TBT while that of TPT ranges between ND ± SD to 23008.0 ± 0.03 x 10^{-3} µg/l respectively between locations. Highest concentration of TBT was recorded in location 5 while the least concentration was observed in location 9 (entrance to harbour). TBT was also found in the two control sites in the harbour. A significant variation of $P \leq 0.05$ was observed from statistical analysis between the locations sampled. The seasonal variation of TBT was also investigated, and significant variation of $P \leq 0.05$ was found after statistical analysis. Seasonal variation in TBT and TPT concentrations with a higher level in summer than in winter and spring has been observed. The concentration of TBT varies spatially around harbour. Figure 4.8a represents the annual distributions of OTCs across locations while Figure 4.8b shows representative chromatogram for OTCs in seawater.

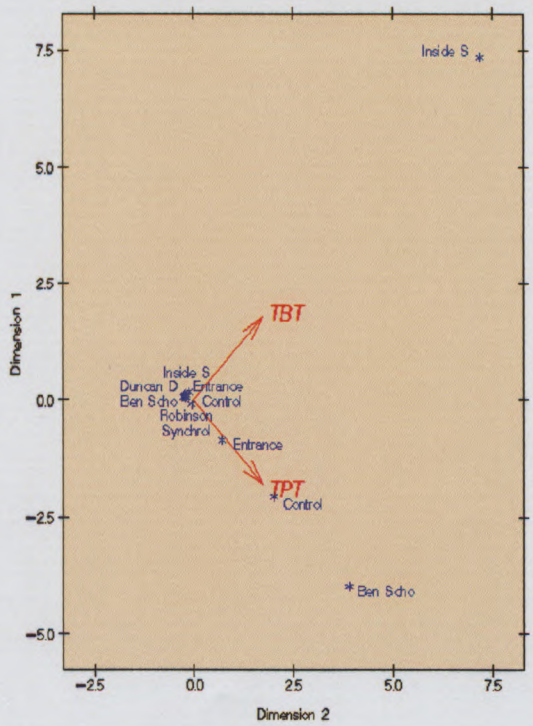


Figure 4.8a : Distribution of TBT in seawater across locations from Cape Town harbour

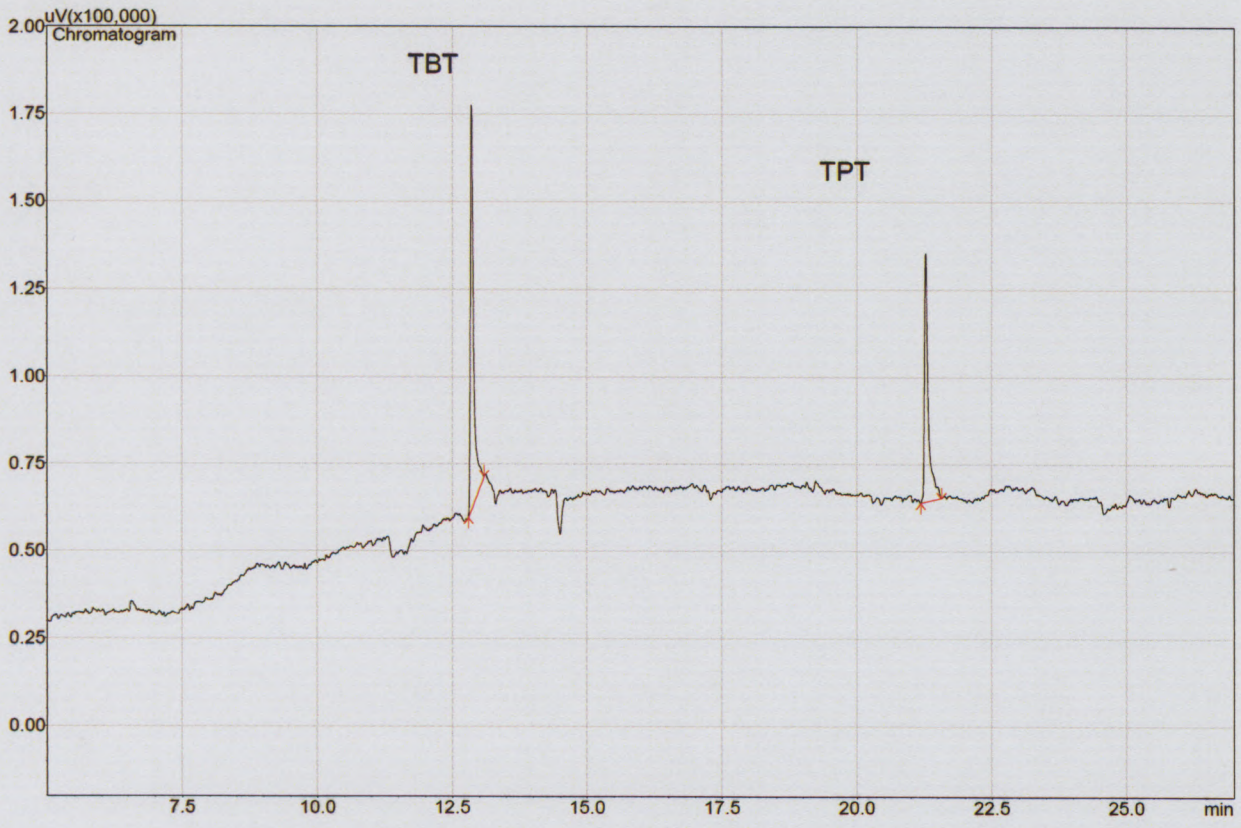


Figure 4.8b: GC-FPD representative chromatogram for organotin compounds in seawater samples from Cape Town harbour,

Table 4.4: Summarised Seasonal and annual mean (±SD) distribution of organotin compounds in Seawater from Cape Town harbour

Item		Seasons			
	Locations	Spring	Summer	Winter	Mean location
TBT µg/L Mean x10^{-6}±SDx10^{-6}	Duncan Dock 1	7.50±2.00	0.723±0.02	0.290±0.01	0.588±0.22
	Duncan Dock 2	1.00±0.00	0.010±0.01	0.303±0.05	0.108±0.15
	Benshoeman Dock	4.54±1.78	0.690±0.38	0.10±0.01	0.385±0.04
	Inside Sea 1	3.40±1.20	1.093±0.60	0.253±0.02	0.562±0.49
	Inside Sea 2	1.02±7.47	323.0±56.00	0.330±0.07	111.290±32.20
	Duncan Dock 3	4.34±1.33	0.010±0.01	0.010±0.00	0.151±0.02
	Robinson Dry Dock	7.05±3.96	1.00±0.00	0.243±0.05	0.320±0.37
	Synchrolift	0.081±0.12	0.590±0.35	0.010±0.01	0.227±0.03
	Entrance to Harbour	0.010±0.01	0.010±0.01	0.180±0.01	0.067±0.01
	Control A	0.150±0.26	3.050±0.32	0.10±0.01	1.070±0.22
	Control B	0.10±0.01	1.353±0.42	0.1931±0.02	0.519±0.67
	Robinson dry dock 2	1.851±0.71	2.427±0.33	0.10±0.01	1.429±0.21
	Mean seasons	1.250±0.33	27.776±16.15	0.154±0.01	
	CV		3.67		
	P≤0.05		***		***
	Interaction P≤0.05		***		
TPT(µg/L Mean x10^{-6}±SDx10^{-6})	Duncan Dock 1	209.0±0.00	383.0±28.00	0.010±0.00	1970.0±1670.00
	Duncan Dock 2	0.010±0.00	181.98±2.69	0.010±0.00	610.0±92.00
	Benshoeman Dock	128.0±0.01	68896.0±1.00	0.0000±0.00	23008.0±0.03
	Inside Sea 1	158.973±27.53	0.010±0.01	0.010±0.00	53.0±15.90
	Inside Sea 2	0.010±0.00	43.273±7.49	92.103±79.92	45.0±6.80
	Duncan Dock 3	0.010±0.00	46.680±8.08	0.010±0.00	16.0±4.70
	Robinson Dry Dock	0.010±0.00	50.870±8.80	0.010±0.00	0.0000±0.00
	Synchrolift	0.010±0.00	45.673±7.91	0.010±0.00	15.0±4.60
	Entrance to Harbour	0.010±0.00	5316.8±897.50	0.010±0.00	1772.0±520.00
	Control A	0.000±0.00	12345.0±0.21	0.0001±0.00	4411.5±0.122.94
	Control B	0.000±0.00	931.0±161.20	0.00±0.00	310.0±93.10
	Robinson dry dock 2	0.010±0.00	0.010±0.00	0.010±0.00	15.0±4.60
	Mean seasons	41.347±9.90	7353.3±1900.00	7.684±3.20	
	CV				
	P≤0.05				
	P≤0.05		***		
	Interaction P≤0.05		***		***

CV: represent coefficient of variation

The concentration of TBT varied seasonally in the following order: summer > spring > winter as observed in Cape Town harbour. This proves that there are some seasonal relationships in the abundance of TBT. Seasonal change has been reported in various studies for water samples and this could be due to seasonal shipping activities in different harbours (Evans and Hugget, 1991; Suzuki *et al.*, 1996; Champ, 2000; Hoch, 2001; Meng *et al.*, 2009). Cape Town harbour is known to have intense shipping activities and is the busiest harbour in Africa. The traffic due to ships and recreational boats is low in winter (June – Sept) and spring in summer (Nov – March). The seasonal variation is represented in Figure 4.9.

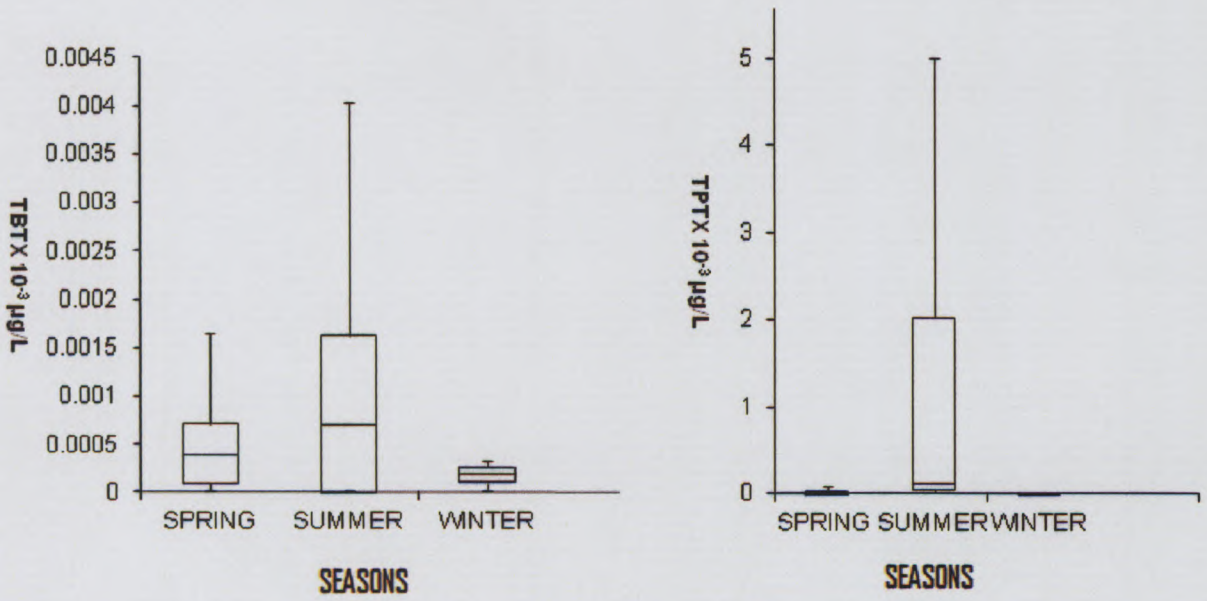
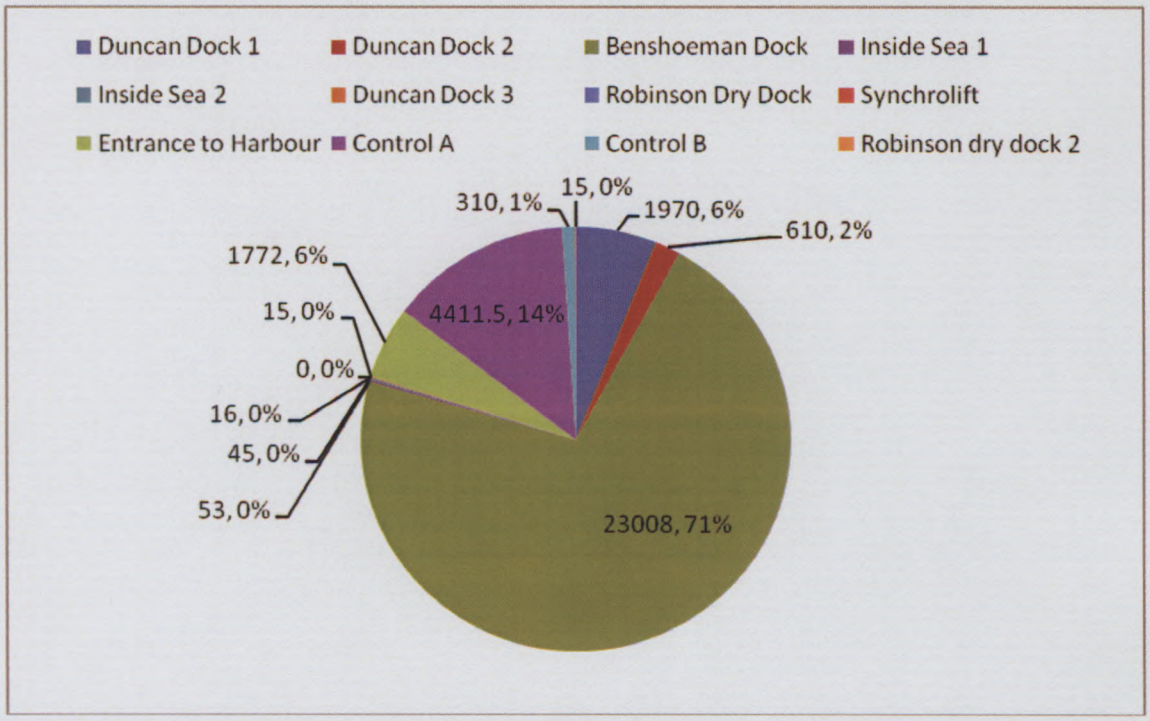


Figure 4.9: Annual Seasonal variation of TBT and TPT in seawater from Cape Town harbour

Apparently, the observed high or low values recorded for OTCs compound in Cape Town harbour could result from an increase or decrease in the traffic of ships and boats. Dilution due to rising sea water levels in the harbour results in a decrease in the concentration of TBT. High values observed for TBT in summer could be associated to ocean currents and tides. In spring, the spring tide effect may cause high or low TBT concentration, but this also depends on the direction of the tide and ocean currents. The observed highest value of TBT recorded in location 4 (95%) (Figure 4.9b) could be as a result of intense ship activities taking place at that location since it is situated inside the harbour, while the least concentration observed for location 9 (entrance to harbour) might be due to less shipping activities taking place at this location. The distribution of TPT varies significantly across locations. Seasonal variation was also observed for TPT. High values were found in BenSchoeman dock (71%) while the least values were found in location 8 (Synchrolift) (Figure 4.9a).

TPT concentration was the highest in summer and lowest in spring. TPT was not detected in winter. High values of TPT recorded in summer could be from various sources along Diep River and wastewater treatment plants that discharges into the harbour. This result is in agreement with a related study carried out by Meng *et al.* (2009). Their findings showed high TPT concentrations in summer than in winter. The concentrations of metals in Diep River, for example, are much higher because of lower water levels and slower currents (Shupping *et al.*, 2011). Thus, the concentrated waters of the river in summer would contribute to the concentrations in the harbour. In general, high TPT concentration in summer is due to its use in agricultural activities and as an antifouling agent in ship painting.

It is therefore suggested that adequate regular monitoring of Cape Town harbour should be put in place. More so, it could be of advantage to investigate the toxic effects of this compound to aquatics life with the use of biomarkers since the concentration of 0.001 µg/l may be toxic to aquatic life, as indicated by (ANZECC, 2000).



TPT

Figure 4.9a annual percentages mean distribution of TPT in seawater

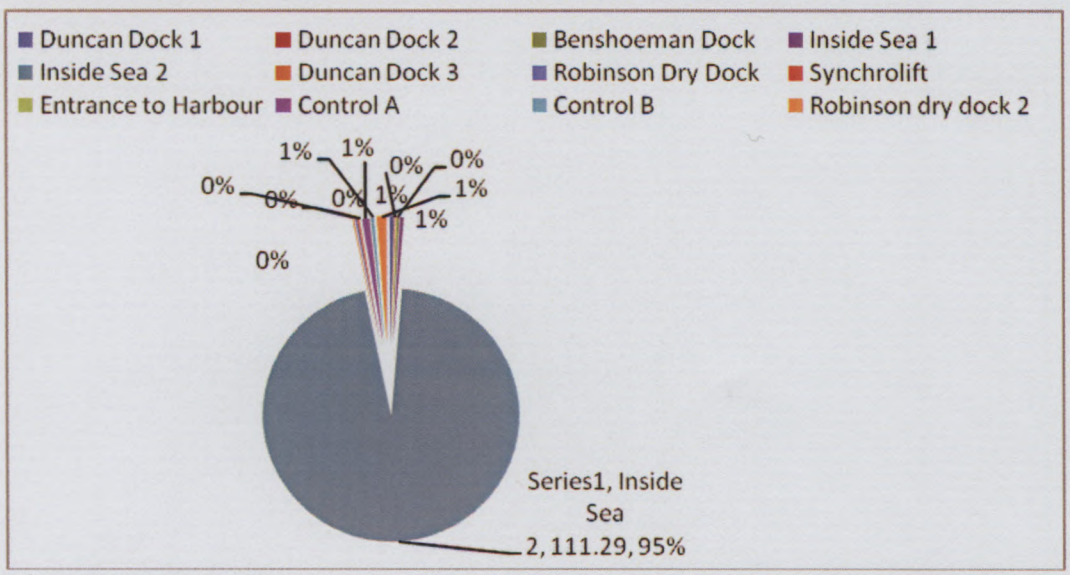


Figure 4.9b: Annual percentages mean distribution of TBT in seawater from collected Cape Town harbour from September 2011 (spring) to June 2012 (winter)

4.4 Seasonal variation and annual mean distribution of Organotin compounds in surface sediment from Cape Town harbour

Sediment samples were collected from September 2011 (spring) to June 2012 (winter) at 11 sites in the harbour. The annual raw datas and summarised mean datas are are shown in (Appendix D Table 2.1a, 2.1b) and (Table 4.5) in that order. The Cape Town harbour is contaminated with antifouling compound TBT and TPT. Concentration of OTCs in the samples ranged from 0.010 to 0.829 µg/g for TBT and zero to 0.691 µg/g for TPT. The highest concentration of TBT was recorded in location 3 (Duncan dock). It appears that Duncan dock 3 is heavily polluted because active recreational boating takes place at this location. The highest concentration of TPT was recorded at location 7 (Robinson dry dock). This might be due to intensive ship reparation activities taking place at this location.TBT was detected in all sediment samples except those collected from location 9 (entrance to harbour, the two control sites (which are located far away from the inner harbour), and location 12 (Robinson dry dock 2). TBT and TPT were not detected from samples collected from the control sites except for TBT that was found in control A during summer. A representative chromatogram showing the OTCs compounds in sediment samples is shown in figure 4.10a. Seasonal variation in the concentration of OTCs in harbour sediments was also investigated. The results show that TBT is present throughout the year, but in this order: summer > winter > spring. High concentration values recorded during summer are due to steady flow of water during summer which enhances deposition of TBT in water into the sediment. Low values recorded for TBT and TPT in winter could be due to erosion as a result of an increase in water flow that removes OTCs from sediments.

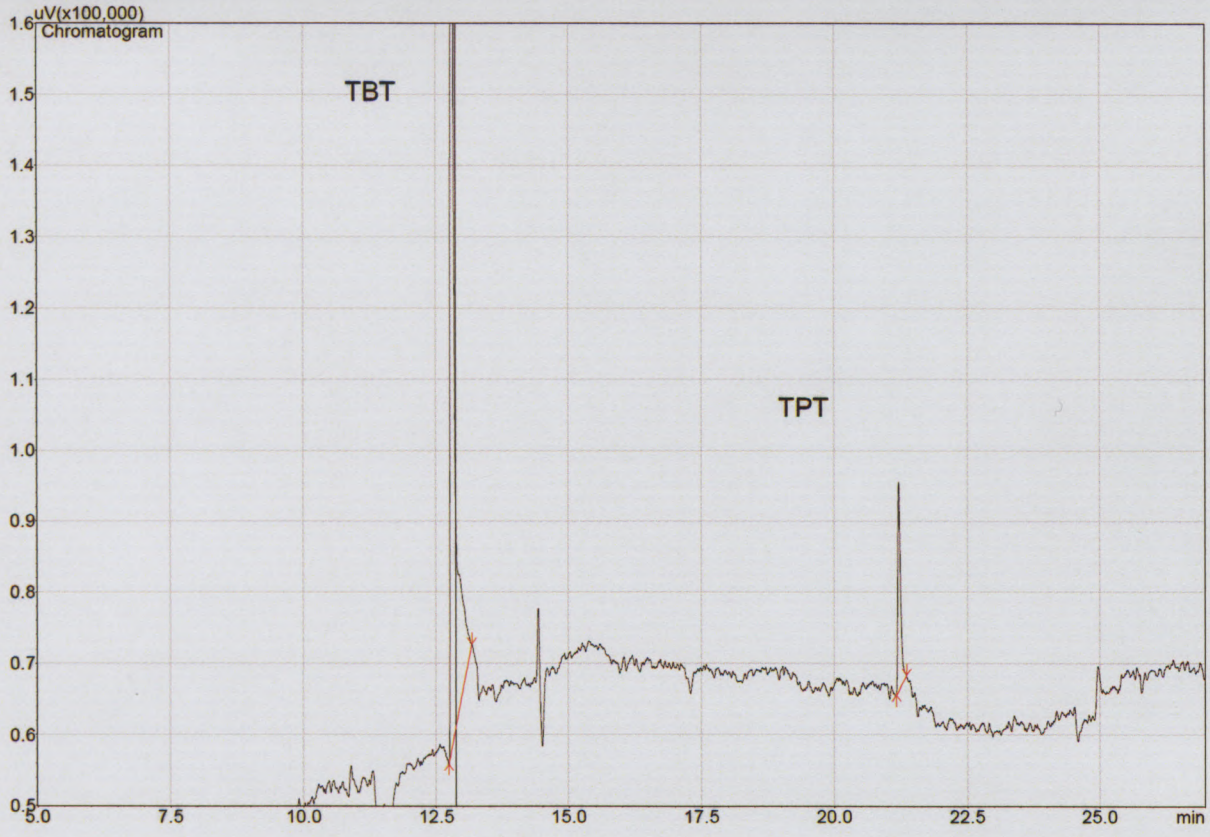


Figure 4. 10a: GC-FPD representative chromatogram for organotin compounds in sediment samples from Cape Town harbour.

Table 4.4: Summarised Seasonal and annual mean (±SD) distribution of organotin compounds in Sediments from Cape Town harbour

Item		Seasons			
	Locations	Spring	Summer	Winter	Mean location
TBT µg/g Mean x10^{-6}±SDx10^{-6}	Duncan Dock 1	0.035±0.0577	0.010±0.00	0.048±0.00	0.017±0.014
	Duncan Dock 2	ND	ND	0.023±0.00	0.014±0.006
	Benshoeman Dock	0.014±0.002	0.01±0.00	ND	0..011±0.002
	Inside Sea 1	NF	ND	0.033±0.001	0.018±0.013
	Inside Sea 2	NF	ND	0.001±0.00	0.010 ±0.00
	Duncan Dock 3	0.001±0.00	0.001±0.00	2.467±0.153	0.829±1.231
	Robinson Dry Dock	0.022±0.002	0.016±0.007	0.001±0.00	0.063±0.071
	Synchrolift	0.044±0.003	0.028±0.001	0.001±0.00	0.027±0.15
	Entrance to Harbour	ND	ND	ND	ND
	Control A	ND	0.087±0.00	ND	0.036±0.038
	Control B	ND	ND	ND	ND
	Robinson dry dock 2	NF	NF	NF	NA
	Mean seasons	0.016 ±0.01	0.030±0.011	0.022±0.069	
	CV%				
	P≤0.05		***		***
	Interaction P≤0.05		***		
TPT(µg/g Mean x10^{-6}±SDx10^{-6})	Duncan Dock 1	0.010±0.00	0.010±0.00	0.010±0.00	0.010±0.00
	Duncan Dock 2	0.010±0.00	0.010±0.00	0.010±0.00	0.010±0.00
	Benshoeman Dock	0.010±0.01	0.010±0.00	0.0000±0.00	0.010±0.00
	Inside Sea 1	NF	0.010±0.00	0.010±0.00	0.010±0.00
	Inside Sea 2	NF	0.010±0.00	0.001±0.00	0.010±0.00
	Duncan Dock 3	0.010±0.00	0.010±0.00	0.010±0.00	0.010±0.00
	Robinson Dry Dock	0.010±0.00	2.052±0.171	0.010±0.00	0.691±0.010
	Synchrolift	0.010±0.00	0.010±0.00	0.010±0.00	0.010±0.00
	Entrance to Harbour	ND	ND	ND	ND
	Control A	ND	ND	ND	0.010 ±0.00
	Control B	ND	ND	ND	0.010±0.00
	Robinson dry dock 2	NF	NF	NF	NA
	Mean seasons	0.010±0.00	0.018±0.574	0.010±000	
	CV%				
	P≤0.05				
	P≤0.05		***		
	Interaction P≤0.05		***		***

Principal component analysis (PCA) was used for data analysis. The tests revealed that TPT concentration was significantly high in summer while TBT was more predominant in winter. Significant variation of P (≤ 0.05) was calculated for the seasonal effects of TPT and TBT. PCA was also used to estimate the effect of locations and seasons on the OTCs investigated (Figure 4.11b, Figure 4.11a; a significant variation of P ≤ 0.05) was obtained while an insignificant correlation was found from the statistical analysis. TBT and TPT were predominantly found in Robinson dry dock and Duncan dock. The highest annual percentage distribution for TPT was recorded in locations 3 (82%) and (7) 6% as shown in Figure 4.10b. The result suggests that the major inputs of TBT and TPT at Robinson dry dock and Duncan dock comes from shipping activities such as ship building and repair activities (Chem *et al.*, 2010). Significant correlation of P (≤ 0.05) was found for all the seasons. Insignificant correlation of P > 0.05 was found for TPT and TBT, respectively. For TBT an average concentration of more than 90% was recorded annually in location 4.

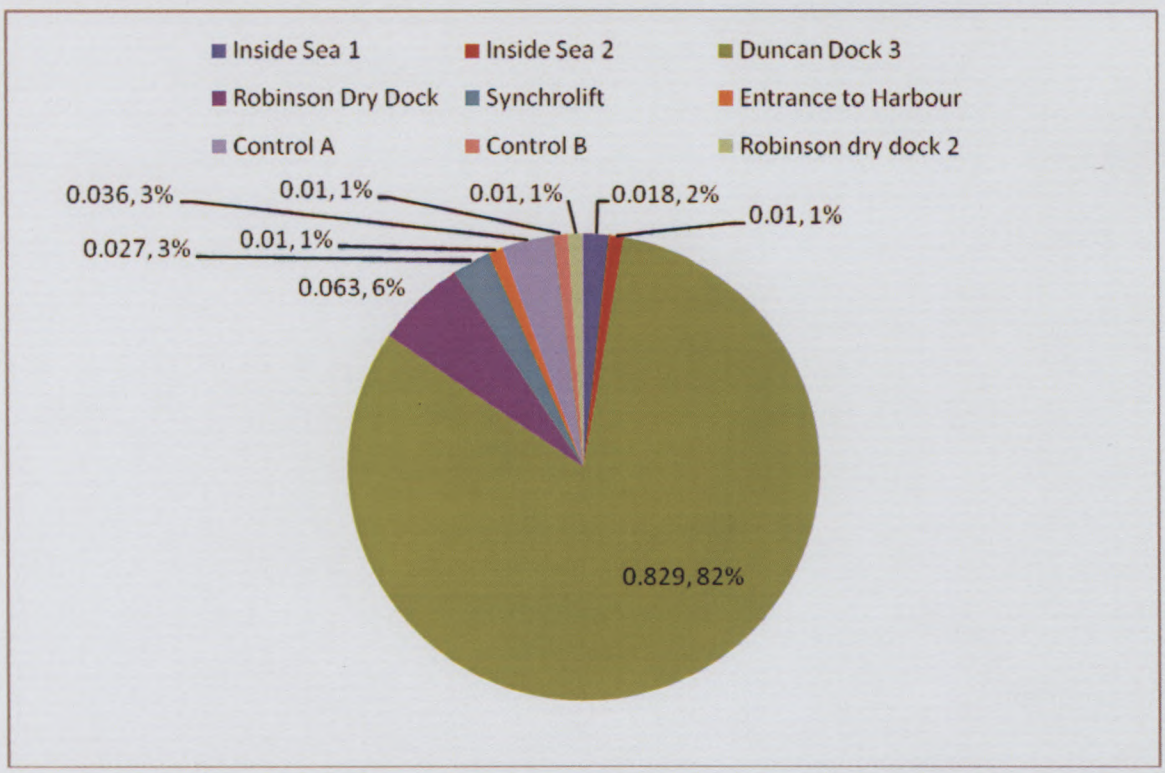


Figure 4.10b: Annual percentages mean distribution of TPT in sediments from collected Cape Town harbour from September 2011 (spring) to June 2012 (winter)

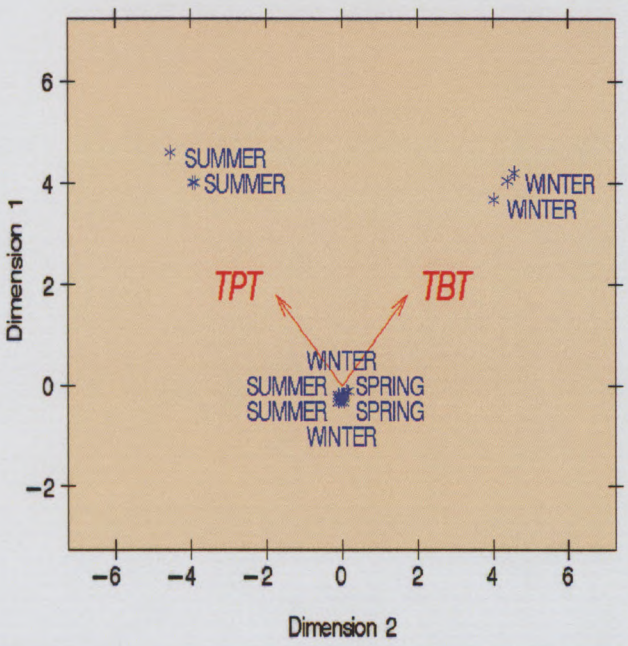


Figure 4.11a: Principal component analysis showing seasonal variation of organotin compound in sediments collected from Cape Town harbour from spring 2011 to winter 2012

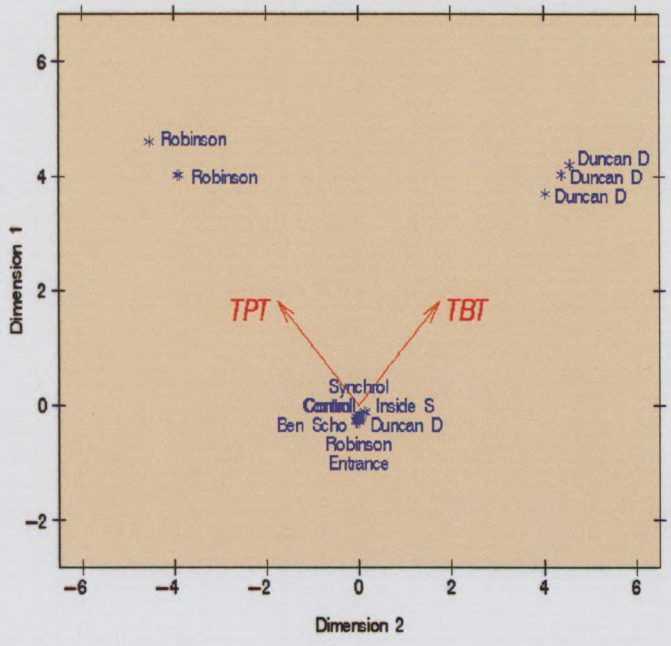


Figure 4.11b: Principal component analysis showing distribution of organotin compounds in sediments collected across locations from Cape Town harbour from spring 2011 to winter 2012

From this study, Triphenyltin, which is a co-toxicant to antifouling compound TBT, was found in some sample but generally lower than that of TBT. TBT was generally found in all locations except in locations 9, 11, and 12 which are further away from the harbour activity. High TPT levels that occurred at location 7 indicates a considerable inputs and persistent in the sediment samples. Previous studies show high concentration levels of OTCs, especially TBT, in marine sediments, and this has also been found for freshwater sediments (Fent and muller, 1991; Fent and Hun, 1995; Langston and pope, 1995; Biselli *et al.,* 2000).

4.5 Results of organotin toxicity effects

4.5.1 Cell viability

Cell viability was calculated at 91.3 ± 3.2 %. It is thus clear that the NNRT procedure did not have a significant effect on cell viability and on the NRRT results.

4.5.2 Neutral Red Retention Time Assay

The mean (±SD) neutral red retention times (NRRT) in minutes were plotted against tributyltin concentration over the experimental period (week 0 – week 4), respectively (Figure 4.12). An insignificant decrease in the NRR times was found over the experimental period for the control group. This can be attributed to handling of the mussels. At the end of week 4 of the experimental period, a significant difference in NRR times ($p < 0.05$) was found between all the three exposure groups, the control group exhibiting the longest mean NRR time of 24.00 ±10.001 minutes, the 0.1 µg/l exposure group a mean NRR time of 14.00 ± 3.005 minutes and the 1.0 g/l exposure group exhibiting the shortest time of 10.00 ± 2.006 minutes (Figure 4.12; Table 4.6). The results found for the exposed group can be associated to stress resulting from TBT exposure. This result is in agreement with Snyman *et al.* (2000). A significant decrease in NRR times observed between week 0 and 1 could be associated to stress. There was however no significant difference ($p > 0.05$) in NRR times between week 0 and week 4 for the control, suggesting that animals adapted to the handling and artificial environment to some extent. In addition, the two groups exposed to 0.1 µg/l of TBT both exhibited significantly shorter ($p < 0.05$) NRR times at the end of week 4, compared to week 0, this result suggests toxic effects due to TBT while a significant shorter retention time observed between week 0 and 1 for the two exposed group can be associated to combined effects caused by handling, artificial environmental stress and TBT. Such a fast response to TBT exposure indicates that TBT is indeed toxic to lysosomal membranes as, measured by the neutral red retention time bioassay, it may possibly be used as an early warning of TBT

induced stress (Ringwood *et al.*, 1998; Snyman *et al.*, 2000; St.Jean *et al.*, 2002; Hagger *et al.*, 2005).

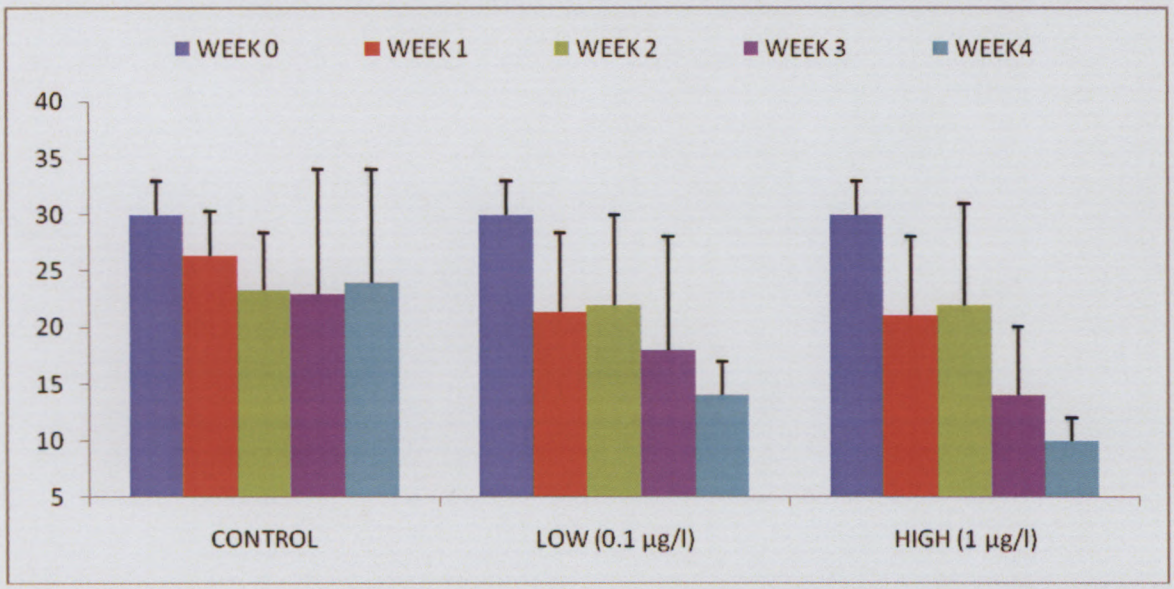


Figure 4.12: Mean (±SD) neutral red retention NRR times (minutes) of hemocytic lysosomes for three groups of *Mytilus galloprovincialis*, before and after exposure to TBT for 4 weeks (n=6 individual per group per week)

Table 4.6: Mean (± SD) neutral red retention (NRR) times (minutes) of hemocytic lysosomes for three groups of *Mytilus galloprovincialis* during four weeks of exposure to TBT (n = 6 individual per group per week).

		WEEKS					
		WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK4	MEAN GROUP
NRRT	CONTROL	30±3.001	26.333±4.005	23.333±5.006	23.000±11.001	24.000±10.001	24.333±11.002
	LOW (0.1 µg/l)	30±3.002	21.333±7.008	22.000±8.005	18.000±10.002	14.000±3.005	18.133±5.007
	HIGH (1 µg/l)	30±3.003	21.000±7.006	22.000±9.001	14.000±6.001	10.000±2.006	16.666± 6.005
	MEAN WEEKS	30±5.006	24.888± 6.09	22.444± 8.98	18.000±6.53	14.000± 7.08	
	CV%			31.8± 6.004			
	P≤0.05			***			***
	INTERACTION			***			****

4.5.3: Body Tributyltin Concentrations

The whole body TBT concentrations in µg/g dry mass, determined from week 0 to 4 are shown in Table 4.7 and Figure 4.13a. A significant increase ($p < 0.05$) was observed for the two exposed groups throughout the experimental period. At the end of week 4 significant differences of ($p< 0.05$) were calculated for the two exposed groups with increased tributyltin concentrations. The control group had no trace of tributyltin their bodies. TBT was clearly accumulated in the bodies of the exposed animals, as also found by other researchers for molluscs exposed to heavy metals (Ringwood *et al.*, 1998; Snyman *et al.*, 2000). Significant differences ($p< 0.05$) were calculated between the exposed and the control group. Tables and dose-response curves are shown in Appendix B. Figure 4.13b represents the chromatogramm of TBT in *Mytilus galloprovincialis*.

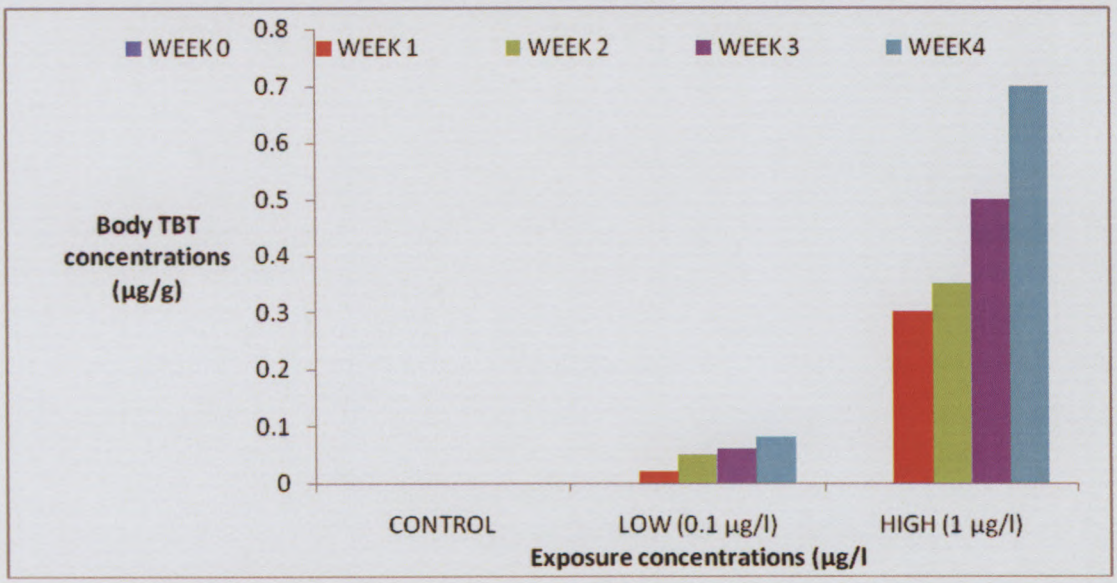


Figure 4.13a: Mean (±SD) whole body tributyltin concentrations (µg/g dry mass), for three groups *Mytilus galloprovincialis* before and after exposure to TBT for 6 weeks (n=6 individual per group per week)

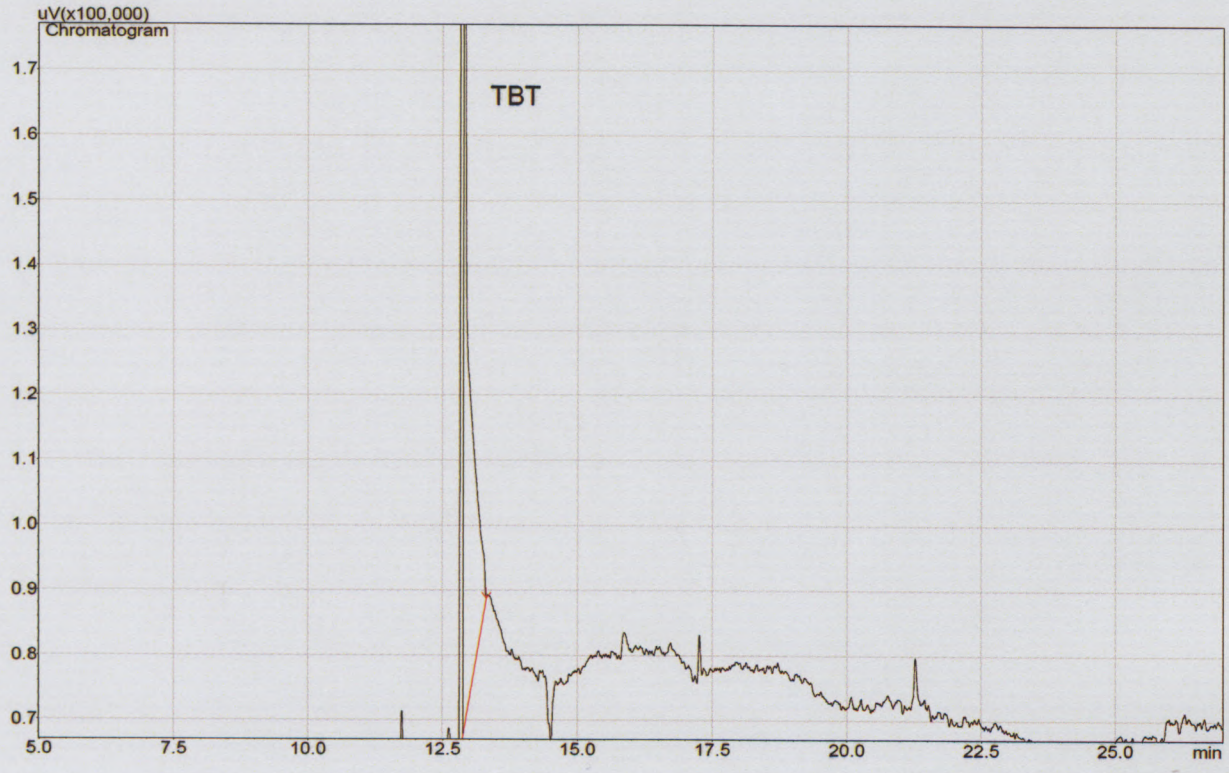


Figure 4.13b: GC-FPD representative chromatogram for TBT in *Mytilus galloprovincialis*

Table 4.7: Mean (±SD) whole body tributyltin concentrations for week 0 - week 4 for three groups *Mytilus galloprovincialis* after exposure to TBT for 6 weeks (n=6 individual per group per week) in µg/g

	Week 0	Week 1	Week2	Week 3	Week 4
CONTROL	ND±SD	ND±SD	ND±SD	ND±SD	ND±SD
LOW	ND±SD	0.020±0.00017	0.050±0.00012	0.06±0.00011	0.080±0.00010
HIGH	ND±SD	0.30±0.00013	0.35±0.00018	0.50±0.00010	0.70±0.00030

4.5.4: Changes in NRR Times over time

The NRR times for the three groups are plotted against the time at which these data were taken. The NRR times obtained after the four weeks of exposure experiment for the control group was found to be insignificant ($P > 0.05$) compared to the start of the experiment, while for both exposed groups a trend of shortened NRR times with longer exposure times to TBT was observed (Figure 4.14). The results could be associated to toxic effects of TBT, as discussed by Snyman *et al.*, 2000; Bekri and Pelletier, 2004 and Gopalakrishnan *et al.*, 2011 for their respective species and pollutants.

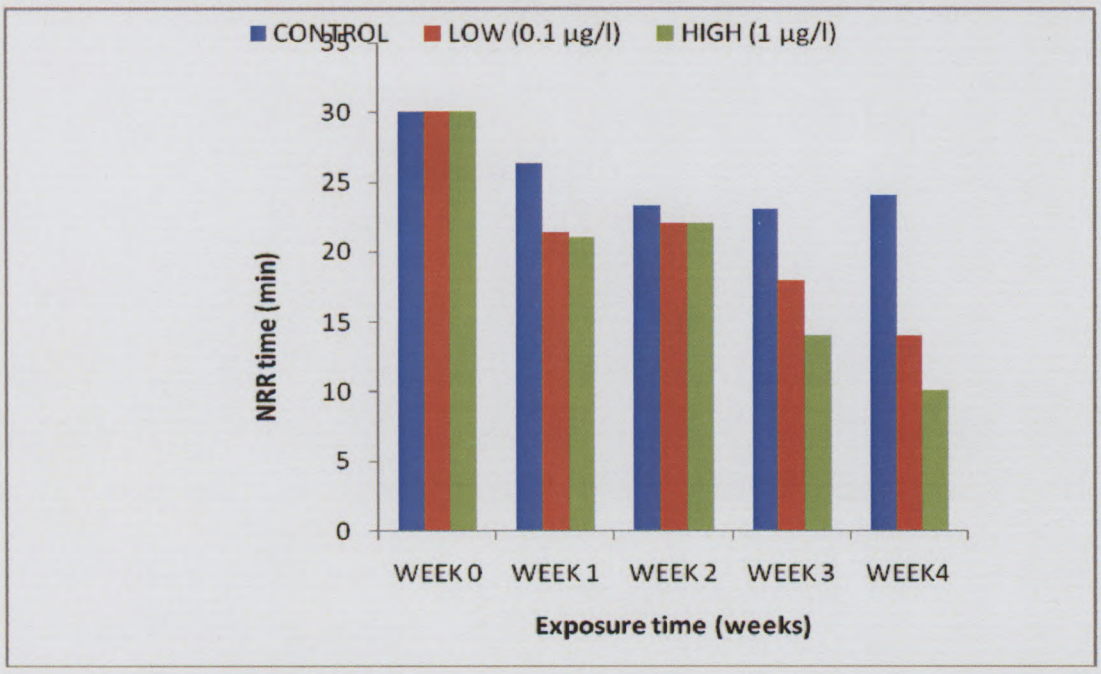


Figure 4.14: Mean (± SD) neutral red retention (NRR) times (minutes) of hemocytic lysosomes for three groups of *Mytilus galloprovincialis* during four weeks of exposure to TBT (n = 6 individual per group per week).

4.5.5 NRR Times versus body tributyltin concentrations

The two exposed groups showed a remarkable change after 1 week of exposure except the control group. A strong negative correlation was observed for the entire experimental period between increasing body TBT loads and shortening neutral red retention times. Regression analysis was performed on the three groups. The two exposed groups showed a decrease in NRR time with increasing concentration of TBT in their bodies, as also found by Snyman *et al.*, 2000; Bekri and Pelletier, 2004 and Gopalakrishnan *et al.*, 2011 for their respective species and pollutants. The linear plots had R^2 values of 0.850 and 0.971 for 0.1 µg/l and 1.0 µg/l TBT concentration, respectively. Figure 4.15 shows the relationship between mean NRR times and mean body tributyltin concentrations for each of the treatment group for a period of four weeks.

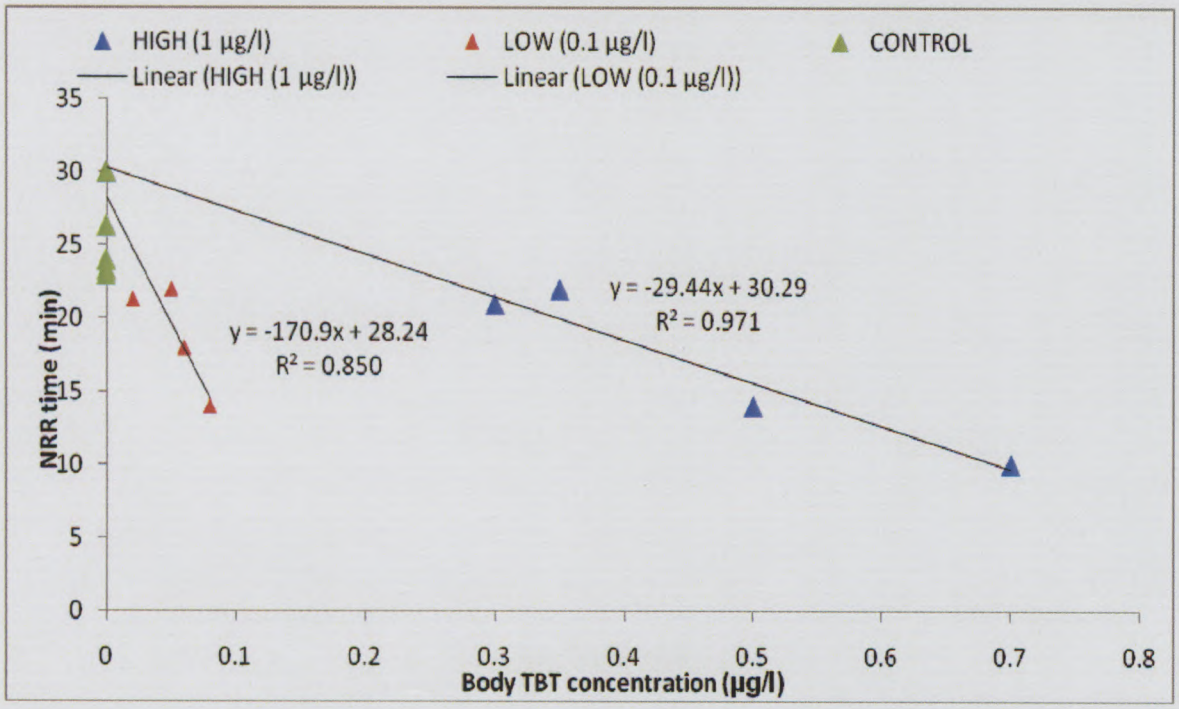


Figure 4.15: The relationship between neutral red retention (NRR) time (minutes) and whole body tributyltin concentrations (µg/g dry mass) in three groups of *Mytilus galloprovincialis* exposed to 0 µg/l , 0.1 µg/l and 1.0 µg/l TBT respectively, over the duration of the experimental period of 4 weeks (n= 6 individuals per week).

4.6: Environmental assessment of heavy metals in Cape Town harbour

Trace metals are among the most common contaminants bound to estuarine sediments. The bioavailability and toxicity of these metals to aquatic organisms depend on the physical and chemical forms of the metal as well as several physicochemical parameters such as temperature, pH, salinity, dissolved oxygen and particulate matter composition. In fresh water, pH is the controlling factor while salinity is stated as one of the controlling factors affecting the partitioning of contaminants between sediments and water in marine or estuarine environment due to the great variability of this parameter (Chapman and Wang, 2001). Several studies relating to the effects of salinity and pH on heavy metal mobility in estuarine and marsh sediments are reported (Liang and Wong, 2003; Riba *et al.*, 2003; Riba *et al.*, 2010). A decrease in the salinity of dredged harbour sediments may lead to different partitioning coefficients (ratio between metal in sediment and the interstitial water, K_d) of heavy metals. Salinity decrease depends on several predominant processes such as

heavy metals. Salinity decrease depends on several predominant processes such as mobilisation of metals through complexation with seawater anions (Cl^- and SO_4^{2-}) (Chapman and Wang, 2001). Changes in salinity play a major role in metal distribution in dredged harbour sediments, especially when washing procedure is applied as a remediation technique or when dredged harbour sediments are deposited in the open air.

In related studies, Guevara-Riba *et al.*, 2005, investigated the influence of pH and salinity on the toxicity of heavy metals in estuarine sediments. They found out that heavy metals tend to be more bioavailable at lower salinity than at higher salinity value and this may be more toxic to the exposed organisms. They were able to establish that the effect of the salinity varies from metal to metal, and that it depends on two counteracting processes, i.e., desorption from sediments to water or coagulation, flocculation and precipitation. From their results, sediments collected in area affected by chronic heavy metal contamination tend to be more efficient in trapping Zn, Cu and Pb at low salinity values. They found out that Cd tends to be more mobile as salinity increases.

In another study Guevara- Riba *et al., 2005,* worked on the effect of chloride on heavy metal mobility of harbour sediments. They modified the BCR- SEP extraction technique in order to assess the extent trace element mobility (Cd, Cr, Cu, Ni, Pb and Zn). Their mobility could be influenced by chloride content in sediments. Washed and non-washed sediment were compared. The relative mobility order found for the six trace metals was not seen to be influenced by the presence of chloride in the sediments. An increase in mobility was observed for Cd and Zn (the most mobile metals) when chloride was present in the sediments. This was in agreement with findings of Riba *et al* (2004).

Therefore, further studies on the combined effects of pH and salinity on heavy metal mobility in marine harbour are recommended in order to be able to compare smaller differences in salinity values and ascertain the major influence of chloride on heavy metal mobility.

4.6.1: Heavy metal speciation in sediment from Cape Town harbour

4.6.1.1: Heavy metals fractionation and distribution

The results of total metal concentration levels showed that aluminium has the highest average concentration while mercury has the least concentration. In general, locations 2, 7 and 8 recorded significantly high concentrations for most heavy metals studied. This indicates that these sites were the most polluted of all the eleven locations sampled. Location 8 is particularly the most polluted area within the harbour. Coincidentally, location 8 is the synchrolift dry dock, a maintenance facility where ships are regularly lifted up for repair. The results showing the concentrations of metal in each fraction and locations is summarised in Table 4.8 – 4.11 and Figure 4.16a -4.16i in that order.

Table 4.8: Concentrations of Al and Si in each fraction and locations

		FRACTIONS					
Item	LOCATIONS	Carbonate	Mn&Fe Oxides	Organic	Oxidisable	Residual	Mean location
Al(mg/kg)	Ben Schoeman	288.85±30.49	3770.55±895.24	4.88±0.06	2.00±0.00	201.74±14.73	853.60±419.24
	Control A	244.45±39.96	3045.81±177.75	3.15±0.15	29.74±5.21	56.01±1.59	675.83±319.00
	Control B	66.80±10.14	1153.25±70.03	3.68±0.79	11.20±9.20	40.08±2.00	255.00±120.78
	Duncan Dock1	414.55±14.96	8888.09±687.57	6.70±1.43	2.00±0.00	307.78±30.86	1923.82±938.90
	Duncan Dock2	438.51±130.26	10414.47±392.95	7.94±2.88	2.00±0.00	215.01±31.32	2215.58±1098.70
	Duncan Dock3	593.85±84.64	8318.41±813.92	8.77±3.20	17.71±8.31	235.22±21.77	1834±1940
	Entrance	172.57±20.79	1092.28±50.23	3.41±0.21	2.00±0.00	14.31±1.111	256.92±113.32
	Inside sea point 4	265.05±53.07	2609.50±166.56	2.72±0.10	9.39±7.39	58.67±6.40	589.07±272.81
	Inside sea point 5	230.81±1.56	2012.97±140.97	5.13±0.79	2.00±0.00	42.82±2.08	458.75±210.26
	Robinson Dry dock	784.29±359.99	17929.50±1369.11	15.57±2.64	28.75±16.23	237.06±2.01	3799.03±1904.82
	Synchrolift	813.01±111.44	20693.52±1884.22	23.76±0.56	120.16±118.16	262.584±24.79	4382±2204
	Mean fractions	392.07±51.88	7266.21±1164.89	7.79±1.16	20.63±10.78	151.93±18.85	
	P≤0.05			****			
	Interaction			***			***
Si(mg/kg)	Ben Schoeman	1.5±0.00	1662.88±615.84	4.51±0.04	1.50±0.00	2.08±0.04	334.49±205.78
	Control A	1.50±0.00	44.26±42.76	1.98±0.13	1.50±0.00	7.14±0.86	11.28±8.49
	Control B	1.5±0.00	1.50±0.00	1.08±0.14	1.5±0.00	9.03±1.22	2.92±0.84
	Duncan Dock1	38.52±37.03	12544.27±1183.30	7.75±1.86	1.50±0.00	2.17±0.14	2518.84±1354.57
	Duncan Dock2	1.50±0.00	13513.50±1809.09	9.43±3.45	1.5±0.00	1.79±0.017	2705.54±1476.29
	Duncan Dock3	1.50±0.00	9686.81±1298.56	9.76±4.14	1.50±0.00	1.99±0.19	1940.31±1058.19
	Entrance	1.50±0.00	1.50±0.00	0.77±0.05	1.5±0.00	5.75±0.42	2.20±0.48
	Inside sea point 4	1.50±0.00	415.04±0.00	1.53±413.54	1.50±0.38	4.00±0.00	84.71±82.67
	Inside sea point 5	1.50±0.89	95.26±0.00	0.86±93.75	1.50±0.39	3.86±0.00	20.59±18.73
	Robinson Dry dock	120.16±0.27	22317.42± 118.66	14.83±1280.72	1.50±2.34	2.43±0.00	4491.27±2392.05
	Synchrolift	320.43±0.43	18878.70±166.69	18.51±2713.84	1.50±0.77	2.46±0.00	3844.32±2061.21
	Mean fractions	44.64±22.81	7196.47±1462.99	6.45±1.14	1.5±0.00	3.88±0.43	
	P≤0.05			***			***
	Interaction			***			

Table 4.9: Concentrations of Fe and Cu in each fraction and locations

		FRACTIONS					
Item	LOCATIONS	Carbonate	Mn&Fe Oxides	Organic	Oxidisable	Residual	Mean locations
Fe(mg/kg)	Ben Schoeman	2425.03±959.46	27849.05±9657.11	3.26±0.08	32.11±18.888	210.96±4.34	6104.08±3345.70
	Control A	557.39±112.69	12680.07±1005.04	0.55±0.08	90.88±31.30	177.69±4.10	2701.31±1345.34
	Control B	284.25±82.56	7392.87±804.63	0.99±0.21	36.86±11.94	71.70±7.99	1557.34±792.14
	Duncan Dock1	633.74±125.35	33908.78±2414.75	4.47±0.58	2.00±0.00	254.34±5.12	6960.67±3624.74
	Duncan Dock2	1943.91±662.61	40889.36±2252.48	29.11±19.03	31.34±15.56	216.24±19.32	8621.99±4334.41
	Duncan Dock3	2805.94±397.66	50002.93±5382.99	27.20±9.34	30.13±6.73	198.66±9.34	10612±5349.64
	Entrance	538.14±50.61	7284.03±817.82	1.10±0.22	26.09±5.67	28.24±2.19	1575.52±777.17
	Inside sea point 4	963.85±125.21	14476.11±1234.41	1.27±0.16	60.12±39.69	75.02±0.20	3115.28±1535.55
	Inside sea point 4	800.17±78.87	10006.03±671.52	1.46±0.28	35.82±24.43	51.07±1.02	2178.91±1055.20
	Robinson Dry dock	2622.60±686.08	132470.80±4642.92	66.93±11.67	75.68±51.01	375.78±22.89	27122.36±14102.43
	Synchrolift	3768.14±236.52	124807.26±15033.31	82.88±11.40	401.41±368.81	372.21±12.36	25886.38±13465.95
	Mean fractions	1576.65±226.92	41978.85±7758.19	19.92±5.34	74.77±34.07	184.72±20.51	
	P≤0.05			***			***
	Interaction			****			
Cu(mg/kg)	Ben Schoeman	17.48±10.79	101.09±24.00	360.93±0.09	1.00±0.00	183.98±3.77	132.89±35.40
	Control A	140.03±16.65	325.49±102.19	147.43±5.60	60.79±8.27	438.74±36.82	222.49±41.45
	Control B	6.59±5.59	81.88±16.62	172.90±12.10	5.66±4.66	172.39±20.39	87.88±20.54
	Duncan Dock1	31.43±5.42	388.50±103.11	1413.83±681.48	1.00±0.00	1507.65±57.34	668.48±212.00
	Duncan Dock2	151.06±43.31	683.02±12.93	2712.54±1067.26	24.05±14.57	2796.62±408.01	1273.45±381.27
	Duncan Dock3	114.37±20.30	379.48±97.72	1802.56±751.34	5.87±4.87	713.12±36.80	603.08±215.40
	Entrance	37.62±10.74	80.49±26.05	54.46±10.79	21.38±12.62	80.07±13.83	54.808±8.64
	Inside sea point 4	55.29±54.29	23.76±2.82	116.24±9.84	96.47±85.66	105.82±14.85	79.52±19.73
	Inside sea point 5	22.07±10.68	61.86±17.86	135.07±17.21	50.77±49.77	64.36±2.26	66.82±13.82
	Robinson Dry dock	642.07±284.68	2011.29±172.91	50.12±10.56	504.89±68.85	20.77±1.82	645.83±202.18
	Synchrolift	562.20±3.57	1554.33±63.10	63.87±8.28	948.15±379.82	18.42±1.55	629.39±166.96
	Mean fractions	161.84±43.82	517.39±113.63	639.08±189.75	156.36±58.87	554.72±149.27	
	P≤0.05			***			***
	Interaction			***			

Table 4.10: Concentrations of Zn and Cd in each fraction and locations

		FRACTIONS					
Item	LOCATIONS	Carbonate	Mn&Fe Oxides	Organic	Exchangeable	Residual	Mean locations
Zn(mg/kg)	Ben Schoeman	390.16±73.73	975.79±132.43	0.55±0.008	99.46±38.52	0.80±0.021	293.35±102.32
	Control A	510.45±93.33	1215.93±75.33	0.50±0.02	251.69±31.19	1.83±0.23	396.08±122.40
	Control B	148.75±7.44	872.44±176.6	0.41±0.06	111.40±49.73	0.47±0.014	226.70±93.06
	Duncan Dock1	572.97±68.23	2401.85±210.14	2.40±0.59	15.19±2.36	1.76±0.10	598.84±250.77
	Duncan Dock2	1724.60±317.87	5070.27±566.06	3.53±1.13	92.54±24.68	2.61±0.19	1378.71±534.93
	Duncan Dock3	1542.46±258.51	3083.76±547.28	1.89±0.76	166.79±91.82	1.15±0.08	959.21±339.45
	Entrance	298.76±44.68	817.38±165.09	0.28±0.07	128.00±33.63	0.35±0.07	248.96±86.57
	Inside sea point 4	370.41±85.57	687.03±52.19	0.43±0.06	205.58±60.32	0.35±0.02	252.76±71.65
	Inside sea point 5	591.27±83.23	967.18±67.15	0.66±0.04	120.26±47.28	0.31±0.04	335.94±104.37
	Robinson Dry dock	7231.96±4216.19	19053±299.77	20.43±2.72	985.13±20.86	9.81±1.206	5460.09±2080.40
	Synchrolift	6287.93±892.67	29360.9034±4216.83	43.00±6.87	1444.72±438.63	11.63±1.14	7429.64±3082.75
	Mean fractions	1788.16±535.43	5864.15±1625.71	6.74±2.33	329.16±84.08	2.83±0.68	
	P≤0.05			***			***
	Interaction			***			
Cd(mg/kg)	Ben Schoeman	2.303±0.28	5.21±1.40	4.20±0.00	0.80±0.12	1.13±0.09	2.73±0.52
	Control A	1.97±0.94	1.98±0.25	0.68±0.05	1.20±0.57	1.33±0.23	1.43±0.24
	Control B	1.68±0.44	5.34±0.84	1.80±0.22	0.80±0.43	2.07±0.35	2.34±0.46
	Duncan Dock1	8.24±0.49	13.10±3.12	11.78±3.46	0.45±0.10	3.144±0.22	7.34±1.52
	Duncan Dock2	16.42±5.57	24.74±1.77	14.74±4.17	1.45±0.19	4.98±0.58	12.47±2.54
	Duncan Dock3	6.55±1.83	11.86±1.64	15.98±9.68	1.30±0.45	3.72±1.75	7.88±2.24
	Entrance	1.51±0.29	3.28±0.40	0.96±0.03	0.80±0.14	0.70±0.14	1.44±0.27
	Inside sea point 4	1.68±0.09	5.22±0.24	3.47±0.59	1.34±0.51	4.24±2.50	3.19±0.59
	Inside sea point 5	2.77±0.22	5.79±0.51	6.05±0.65	0.59±0.06	1.46±0.16	3.33±0.61
	Robinson Dry dock	10.67±4.96	19.87±1.58	19.71±4.55	3.18±0.30	5.60±0.87	11.81±2.20
	Synchrolift	10.18±1.86	34.91±6.02	66.44±1.45	4.07±1.24	13.17±2.04	25.75±6.21
	Mean fractions	5.81±1.04	11.94±1.86	13.26±3.31	1.45±0.23	3.78±0.66	
	P≤0.05			***			***
	Interaction			***			

Table 4.11: Concentrations of Sn and Hg in each fraction and locations

		FRACTIONS					
Item	LOCATIONS	Carbonate	Mn&Fe Oxides	Organic	Oxidisable	Residual	Mean locations
Sn(mg/kg)	Ben Schoeman	0.53±0.036	8.34±2.33	0.30±0.00	0.59±0.08	87.67±48.41	19.49±12.28
	Control A	8.84±0.41	10.04±0.69	3.92±0.88	12.17±0.27	119.57±10.28	30.91±11.99
	Control B	5.64±0.32	15.78±7.32	15.24±3.37	5.94±0.40	76.06±8.43	23.73±7.36
	Duncan Dock1	0.29±0.24	13.83±5.38	4.16±1.99	0.05±0.00	105.62±3.18	24.79±10.93
	Duncan Dock2	7.55±1.09	18.08±3.02	10.67±8.88	0.80±0.20	134.38±6.93	34.29±13.60
	Duncan Dock3	1.82±1.08	11.81±5.48	7.94±6.95	0.90±0.16	95.92±3.88	23.67±9.85
	Entrance	16.49±0.82	18.36±2.63	20.93±3.58	24.64±5.87	23.72±1.91	20.82±1.53
	Inside sea point 4	0.57±0.08	5.26±2.93	13.28±6.38	0.91±0.04	25.71±1.26	9.15±2.80
	Inside sea point 5	1.32±0.94	9.12±4.10	13.13±5.06	0.49±0.05	32.06±9.08	11.22±3.59
	Robinson Dry dock	41.60±3.97	76.70±5.73	14.45±1.70	71.41±10.08	614.92±24.93	163.82±60.75
	Synchrolift	23.93±0.49	124.77±10.34	53.63±0.64	28.65±3.68	1143.33±55.93	274.86±116.85
	Mean fractions	9.87±2.20	28.37±6.47	14.33±2.67	13.32±3.79	223.54±58.79	
	P≤0.05			***			***
	Interaction			***			
Hg(mg/kg)	Ben Schoeman	0.09±0.05	0.18±0.00	0.40±0.00	0.23±0.04	1.96±0.20	0.57±0.19
	Control A	0.05±0.00	0.05±0.00	0.28±0.05	0.05±0.00	1.99±0.75	0.48±0.24
	Control B	0.05±0.00	0.05±0.00	0.21±0.13	0.08±0.03	2.11±0.40	0.50±0.23
	Duncan Dock1	0.64±0.07	0.35±0.02	0.05±0.00	0.91±0.13	6.30±0.42	1.65±0.63
	Duncan Dock2	0.26±0.05	0.28±0.02	2.49±1.14	0.33±0.01	5.68±0.21	1.81±0.60
	Duncan Dock3	0.08±0.02	0.05±0.00	0.69±0.48	0.05±0.00	2.86±0.34	0.75±0.31
	Entrance	0.05±0.00	0.05±0.00	0.07±0.02	0.05±0.00	1.34±0.15	0.31±0.14
	Inside sea point 4	0.10±0.05	0.05±0.00	0.13±0.09	0.13±0.04	2.38±0.23	0.56±0.24
	Inside sea point 4	0.08±0.03	0.05±0.00	0.05±0.00	0.38±0.34	1.81±0.27	0.48±0.19
	Robinson Dry dock	0.40±0.06	0.14±0.06	1.74±0.79	0.40±0.14	18.23±2.19	4.18±1.92
	Synchrolift	0.19±0.07	0.16±0.06	1.07±0.30	0.05±0.00	20.62±3.63	4.42±1.25
	Mean fractions	0.18±0.03	0.13±0.02	0.65±0.18	0.24±0.05	5.93±1.21	
	P≤0.05			***			***
	Interaction			***			

Table 4.12: Concentrations of Pb in each fraction and locations

		FRACTIONS					
Item	LOCATIONS	Carbonate	Mn&Fe Oxides	Organic	Oxidisable	Residual	Mean location
Pb	Ben Schoeman	98.83±31.31	305.69±67.82	14.16±0.01	6.04±0.99	197.65±15.09	124.48
	Control A	87.49±5.35	430.76±37.55	17.76±1.09	19.71±1.18	423.37±13.32	195.82±
	Control B	42.17±13.59	250.28±15.28	15.14±6.82	17.33±1.01	204.22±18.51	105.83±
	Duncan Dock1	125.98±8.69	634.45±50.03	28.49±10.08	2.21±0.11	855.98±41.6	329.43±
	Duncan Dock2	171.09±33.11	978.64±64.09	22.29±9.26	9.59±2.11	1007.08±17.76	437.74±
	Duncan Dock3	309.11±57.61	968.38±136.28	47.89±16.76	4.69±0.88	605.21±41.39	387.06±
	Entrance	34.24±2.58	146.93±2.52	15.93±0.86	14.34±2.62	116.68±34.15	65.62±
	Inside sea point 4	66.85±17.18	189.95±15.07	4.63±0.96	6.71±0.43	123.29±8.04	78.29±
	Inside sea point 4	94.11±16.38	195.20±9.12	12.95±2.64	9.68±2.45	121.80±12.07	86.75±
	Robinson Dry dock	282.02±181.64	1608.61±92.30	140.79±44.27	12.29±0.40	2734.25±176.27	955.60±
	Synchrolift	309.66±45.94	2286.18±260.21	237.90±0.81	29.26±13.73	4390.34±351.87	1450.67±
	Mean fractions	147.41±23.45	726.83±118.64	50.72±12.82	11.98±1.72	979.99±231.87	
	P≤0.05			***			***
	Interaction			***			

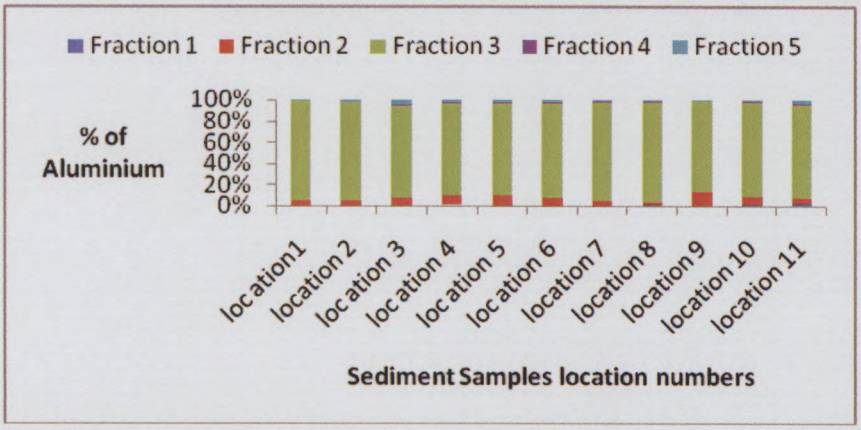


Figure 4.16a: Percentage distribution of Al in sediments

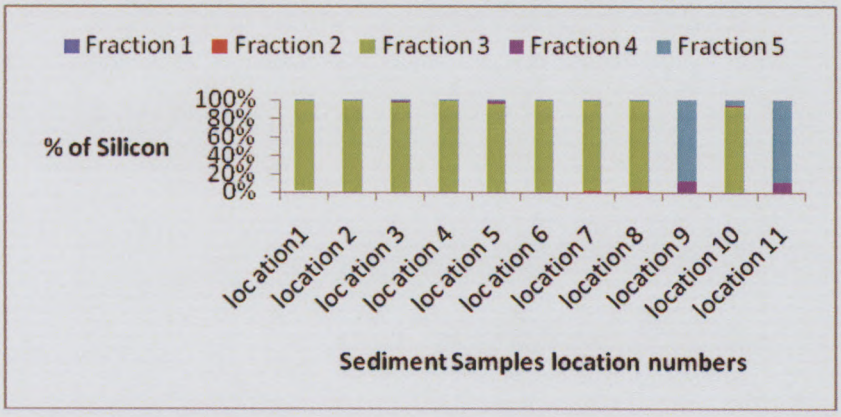


Figure 4.16b: Percentage distribution of Si in sediments

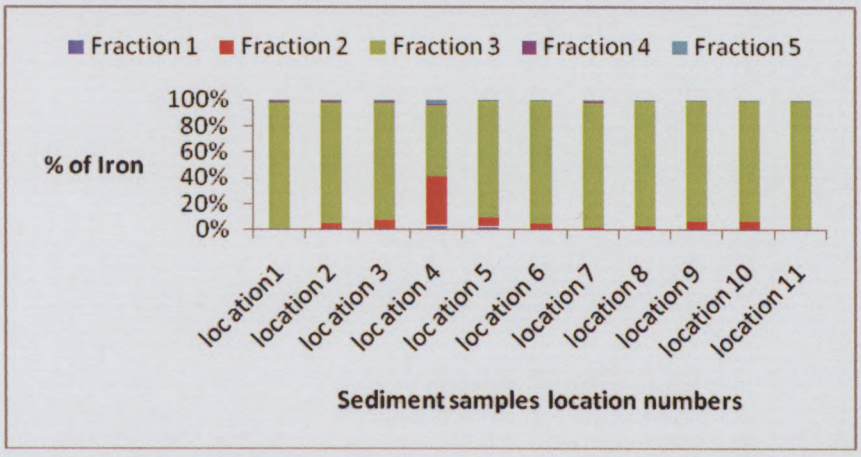


Figure 4.16c: Percentage distribution of Fe in sediments

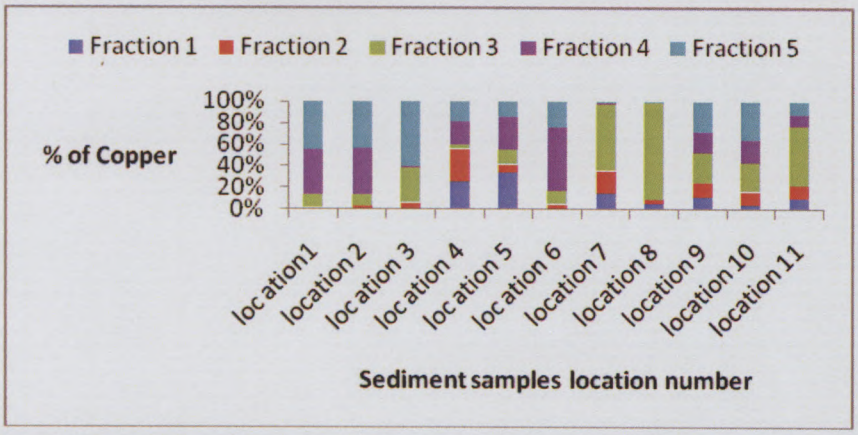


Figure 4.16d: Percentage distribution of Cu in sediments

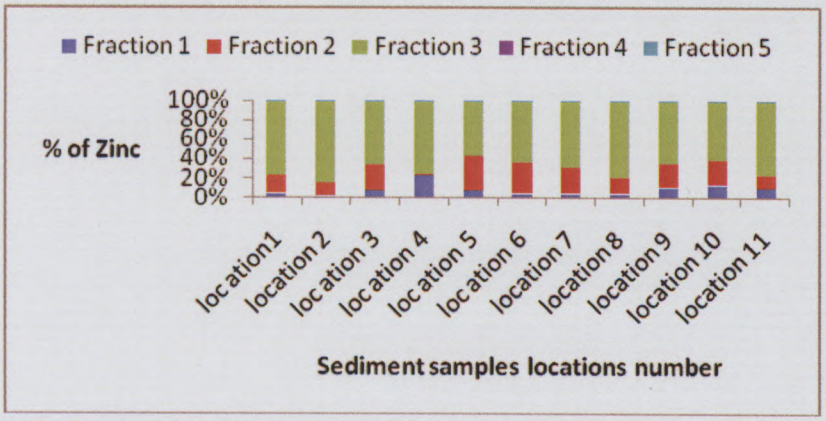


Figure 4.16e: Percentage distribution of Zn in sediments

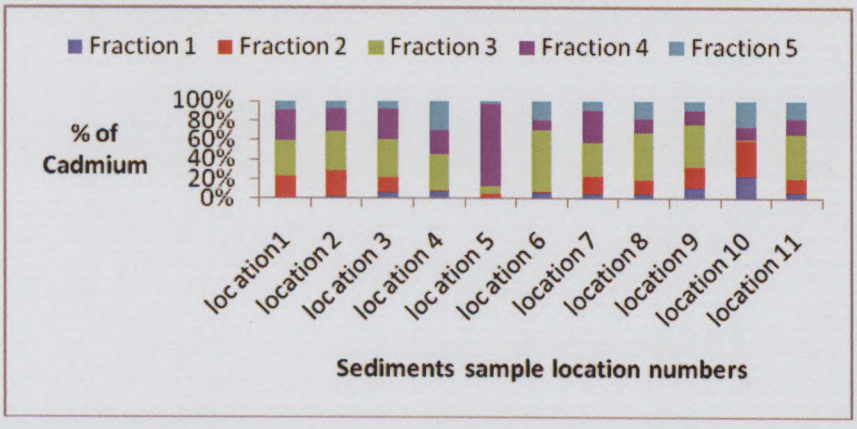


Figure 4.16f: Percentage distribution of Cd in sediments

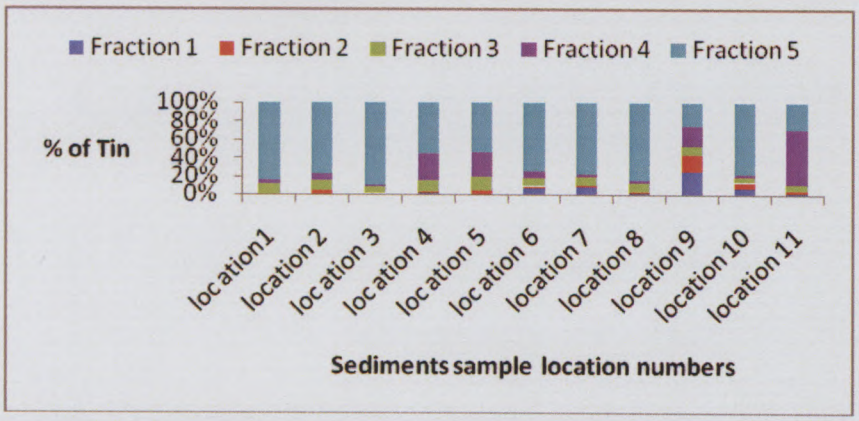


Figure 4.16g: Percentage distribution of Sn in sediments

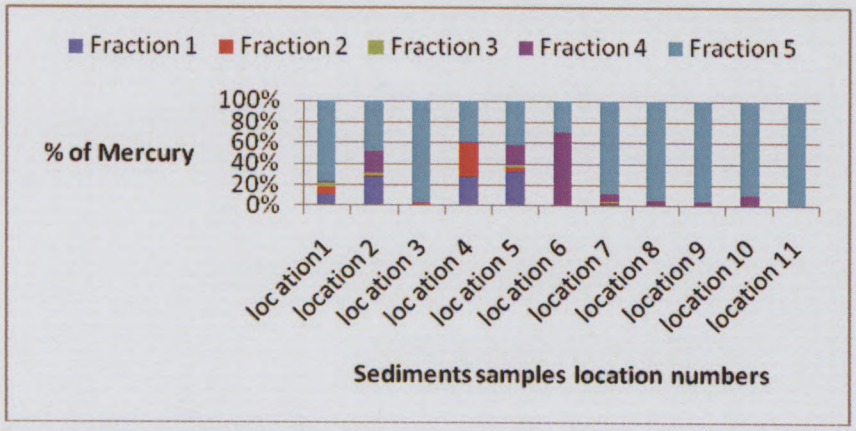


Figure 4.16h: Percentage distribution of Hg in sediments

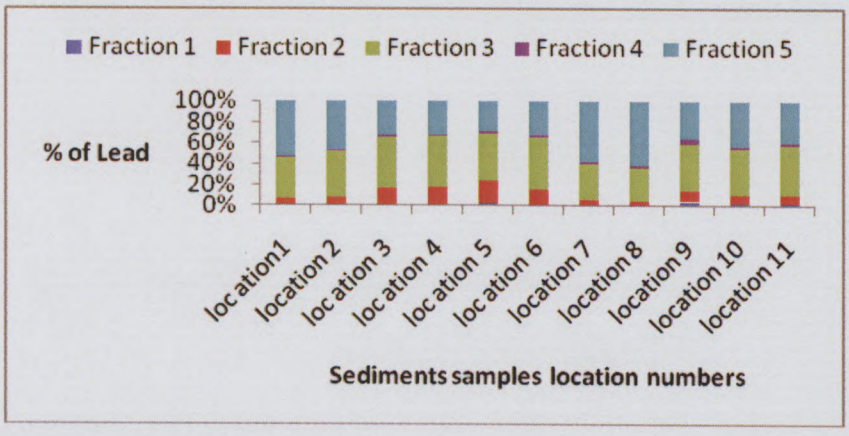


Figure 4.16i: Percentage distribution of Pb in sediments

The analytical results obtained for the concentration of heavy metals in the five constituent fractions of sediment samples collected at the eleven sampling sites are shown in Figures 4.16a-4.16i while the distribution of heavy metals in the various fractions is presented in Table 4.8-4.12. The proportions of bioavailability and non- bioavailability of heavy metals are summarised in Table 4.14.

4.6.1.1.1: Silicon

The percent distribution of silicon in the five fractions is shown in Figure 4.16b. Si concentration was generally low in all the chemical forms except in residual fractions at locations 9 and 11, whereas for other locations, silicon was mainly bound to Mn/Fe oxides with concentrations ranging from 12.979 mg/kg at location 10 to 22317.42 mg/kg at location 7 (Table, 4.8). The waste discharges from ship maintenance facility could be responsible for the high values recorded at locations 7 and 8. It may also be due to the specific type of sediment at the sites, as clay particles contain high amounts of silicon as part of the clay mineral structure (Alessandro Delle Site, 2001).

4. 6.1.1.2: Aluminium

The distribution of aluminium in the five fractions of sediment samples is presented in Figure 4.16a. The highest aluminium content was in the fractions bound to Mn/Fe oxides. The second highest concentration was in the carbonate fraction. The exchangeable fraction contained the least concentration of aluminium. The high concentration values recorded for aluminium at location 8 could be due to routine repair and painting of ships at this location but it may also be due to the specific sediment type at this location, which may contain a high percentage of clay particles. Clay particles in soils often contain aluminium as part of the clay mineral structure (SNMMC, 2002).

4. 6.1.1.3: Iron

The distribution of iron in all the sediment fractions presented in Figure 4.16c followed the same pattern as for aluminium. The concentration of iron in the Mn/Fe oxide extract ranged from 14476.11 mg /kg at location 4 to 132470.80 mg /kg at location 7 (Table, 4.9). Relatively higher values were also recorded at location 8 for the same sediment fraction. Fe is mostly present in bioavailable form (>95%). High values recorded at locations 7 and 8 could be attributed to wastewater from domestic activities around the Victoria &

Albert Waterfront in addition to contribution from industrial activities. It may also be due to the specific sediment type at this location, which may contain a high percentage of clay particles. Clay particles in soils often contain iron as part of the clay mineral structure. (Dumbleton and West, 1966).

4. 6.1.1.4: Copper

The percent distribution of Cu in the five fractions of sediment samples is presented in Figure 4.16d. Copper exhibited highest concentration in the fraction bound to Mn/Fe oxides. This was followed closely by carbonate and exchangeable fractions. Locations 8 again recorded the highest concentration for copper (Table 4.9, Figure 4.16d). The high percentage recorded for Cu in the bioavailable forms at locations 7 and 8 could be attributed to the use of antifouling paint on ships in addition to other industrial and domestic activities around the harbour (Idris *et al.*, 2007).

4. 6.1.1.5: Zinc

The concentration of zinc was generally higher than that of copper at all locations. In terms of distributions, zinc was mainly present in the fraction bound to Mn/ Fe oxides, followed by carbonate and the exchangeable fractions, in that order (Figure 4.16e). The highest total concentration was recorded at location 8 (Table, 4.10). In terms of mobility, Zn was mostly present in bioavailable forms across all locations (> 99.20%). This is in agreement with literatures as several authors had previously reported high concentrations for zinc, especially in the Mediterranean and European harbours (Van den Hurk *et al.*, 1997, Schintu and Degetto, 1999 and Guevara *et al.*, 2004).

4. 6.1.1.6: Cadmium

The percent distribution of Cadmium in the five fractions of sediment samples is presented in Figure 4.16f. Cadmium was mainly present in the fraction bound to Fe/Mn oxides with concentrations of 34.9 and 19.9 mg /kg at locations 7 and 8, respectively (Table 4.10). In terms of mobility, Cd was mostly present in bioavailable forms (> 60%) across all locations except locations 1, 4, 5 and 8. The predominance of cadmium in location 8 (Figure 4.16f) could be attributed to waste discharges from industrial activity around the harbour. Some authors had previously linked the presence of Cd at harbours to oil refining (Idris *et al.*, 2007). Oil sheen was indeed observed on the surface of the marine water near the dockyard area at the time of sampling. In a related study, high values of Cd had been

attributed to run-offs from nearby mining zones as well as industrial activities around an Italian harbour (Schintu and Degetto, 1999).

4. 6.1.1.7: Tin

The percent distribution of tin in the five fractions of sediment samples is shown in Figure 4.16g. Tin was present in the residual phase with concentrations of 1143.33 mg /kg and 614.92 mg /kg at locations 7 and 8, respectively (Figure 4.16g and Table 4.11). Tin was mostly present in non-bioavailable forms across all locations (>80%) except at locations 7 and 9. The exceptionally high value of tin observed at locations 7 and 8 was due to repair and painting of ships that take place in these locations. Organic tin is the major active ingredient in the antifouling paints.

4. 6.1.1.8: Lead

The distribution of lead in all the sediment fractions follows a similar pattern of tin. The percent distribution of lead in the five fractions of sediment samples is presented in Figure 4.16i. Comparably high values of Pb were also recorded at location 8, especially in fraction 3 (bound to Mn/Fe oxides), with the concentrations of 146.93 mg/kg and 2286.18 mg/kg at locations 8 and 9, respectively (Table 4.12). The high sorption potential of Mn/Fe oxides for Pb could explain the binding behaviour observed for this heavy metal (Morillo *et al.*, 2004). In terms of mobility, Pb was more in the bioavailable forms (55- 77%) across all locations except at locations 1, 8 and 7 where it was present in non-bioavailable forms. The relatively higher levels recorded in the bioavailable forms has important implications in public and ecosystem health.

4. 6.1.1.9: Mercury

There appears to be a diverse variation in the concentration of mercury across all locations. The percent distribution of mercury in the five fractions of sediment samples is presented in Figure 4.16h its concentration was significantly high in fraction 5 (residual fraction). The highest concentrations recorded were 20.62 mg/kg and 2.11 mg/kg (Table 4.11) at locations 8 and 11, and this corresponds to 89.57% and 98.59% of the total concentration of Hg in the sediment, respectively (Figure 4.16h). The results indicate that Hg is locked-up in the sediments. As observed previously for other metals, location 8 recorded highest total concentration of mercury. Locations 7 and 8 (dry-dock area) contained the highest concentration of metals, which might also be attributed to the various ship

maintenance activities including welding, stripping and painting. Hg was mostly present in non-bioavailable forms (> 60%) across all locations except location 4 where it was present in bioavailable forms (60%). In summary, high concentrations recorded for Cd, Cu, Pb and Zn at locations 8 and 9 (entrance to harbour) could be attributed to urban discharges in the area, run-offs from ship maintenance and corrosion of metallic materials (Idris *et al.*, 2007)

.

Table 4.13: Comparison of mean metal concentrations in marine sediments from selected African countries in mg/kg

Locations	Year	Si	Al	Cu	Fe	Zn	Cd	Sn	Pb	Hg	Ref
Cape Town Harbour		1.5-7196	7.79-7266	161-639	19-41978	2.83-5864	1.45-13.26	9.87-223	11.98-979	0.13-5.93	Current Study
Richards Bay Harbour	1998	NE	ND	10-25.8	ND	14-193	NE	NE	NE	NE	Archibald and Parson, 1998.
Richards Bay Harbour	1996 - 1997	NE	8125-75086	1.82- 53.5	11934-57310	48.1-181.1	NE	NE	NE	NE	Wepener and Vermeulen, 1998
Abu-kir Bay Egypt		NE	NE	12	4500	102	2.02	NE	-	NE	Saad *et al*., 1981
Port Said Egypt				14	2500	50	3.2		-		Saad *et al*., 1981
Ebrie Lagoon Cote d – Ivore				37.0	52400	187			57.6		Kouadio and Trefry, 1987
Lagos Lagoon Nigeria			15		36380	147	4.1		178.9		Okoye et al., 1991
SA guidelines				50-500		150-750	1.5-10		100-500		SA Guidelines (Fatoki and Mathabata, 2001
EPA guidelines				18.7		124	0.7		30.2	0.13	EPA, 2002

EPA Guidelines (Canadian Environmental Quality Guidelines. Updated 2002)

Proposed SA Guidelines for metal concentrations in marine sediments (Fatoki and Mathabata, 2001).

Cadmium concentration levels in sediments in the harbour are below the South African guidelines across all locations except location 8 which this study has identified as the most polluted site. Cd concentration levels were found to be above EPA guidelines (CEQG, 2002) (Table 4.13), especially at location 8 where docking and painting activities are taking place. Cu concentration levels have been found to be higher than maximum values stipulated by the South African guidelines except at the control site and locations 9 and 11, which are the entrances to the harbour. Pb concentration values were lower than the maximum value stipulated in the South African guidelines except at location 9. On the other hand, Pb levels were higher than EPA maximum values in the guidelines across all sampling sites. Zn concentrations at locations 9, 10, 11, 6, 5, 4, 3 and 1 were lower than the South Africa guidelines while it was higher at locations 2, 7and 8. Zn levels were higher than EPA guidelines at all locations except location 11 which served as the control, and Hg concentration levels were generally above the EPA standards across all locations (Table 4.13).

Table 4.14: Percent of bioavailable and non-bioavailable proportions of heavy metals in the sediments

Locations	Fraction	Al	Si	Fe	Cu	Zn	Cd	Sn	Hg	Pb
1	Bioavailable	96.7	99.89	99.24	12.57	99.86	58.93	11.74	22.93	46.3
	Non-bioavailable	3.3	2.5	0.04	7.75	0.14	40.28	88.26	76.83	53.7
2	Bioavailable	97.98	99.9	99.38	13.63	99.9	68.38	15.4	31.67	52.95
	Non-bioavailable	2.02	0.08	0.57	86.36	0.05	31.62	84.6	68.33	47.01
3	Bioavailable	95.16	98.5	99.08	46.79	99.88	60.14	9.74	2.97	77.06
	Non-bioavailable	4.84	1.5	0.92	53.21	0.09	38.4	90.26	97.07	22.94
4	Bioavailable	30.5	99.56	97.06	59.92	99.94	51.57	14.66	60.32	67.34
	Non-bioavailable	69.5	0.44	2.96	40.08	0.06	48.43	85.34	39.68	32.66
5	Bioavailable	97.9	98.37	99.53	55.11	99.96	12.8	19.37	40	68.93
	Non-bioavailable	2.1	1.63	0.47	44.89	0.04	87.2	80.63	60	31.07
6	Bioavailable	97.34	99.88	99.57	16.84	99.93	50	12.25	1.41	66.25
	Non-bioavailable	2.66	0.12	0.43	83.16	0.07	50	87.75	98.99	33.75
7	Bioavailable	98.67	99.93	99.57	97.8	99.88	57.12	23.16	4.33	39.83
	Non-bioavailable	1.33	0.07	0.43	2.2	0.11	42.88	76.84	95.67	60.17
8	Bioavailable	98.68	99.89	99.65	99.54	99.87	37.63	12.93	6.09	36.03
	Non-bioavailable	1.32	0.1	0.35	0.46	0.13	62.37	87.06	93.91	63.96
9	Bioavailable	98.62	0	99.63	52.67	99.94	76.71	57.1	0	59.59
	Non-bioavailable	1.38	100	0.37	47.32	0.051	23.29	42.9	100	40.41
10	Bioavailable	98.25	93.59	98.68	42.63	99.87	72.22	20.08	0	54.97
	Non-bioavailable	1.75	7.01	1.28	57.41	0.12	27.78	79.94	100	45.03
11	Bioavailable	96.61	0	99.9	76.86	99.26	66.67	10.71	0.68	58.16
	Non-bioavailable	3.39	100	0.1	23.14	0.74	33.33	89.29	99.32	41.84

The percentage of bioavailable and non bioavailable proportions of heavy metals with respect to the sum of fractions 1, 2 and 3 (bioavailable) and fractions 4 and 5 (non-bioavailable) in the marine sediments from each extraction step are summarised in Table 4.14. These metals are classified as bioavailable and non-bioavailable groups on the basis of their relative mobility and toxicity to the aquatic environment. All the metals except Hg and Sn exhibited higher percentages in the Mn / Fe oxide phase, which means that Zn, Fe, Si, Al, Cd, Cu and Pb are more mobile and hence, bioavailable in sediments from Cape Town harbour. The experimental data shows that the mobility of heavy metals decreases in the following order: Zn > Fe > Si > Al > Cd > Cu> Cu > Pb > Hg > Sn.

The present study has demonstrated that Sn and Hg are the least bioavailable metals in the sediments across all locations. High percentage of metals recorded in fractions 1 to 3 was due to the greater mobility, as well as the effluent coming from the Diep River (Shuping *et al.*, 2011). A similar observation had been reported in a related study (Usero *et al.*, 1998). Many factors could be responsible for the accumulation and bioavailability of metals in sediments. Concentrations and variability observed may be due to other abiotic factors such as the physico-chemical properties of the overlying water and/or variability in sediment composition (Magnusson *et al.*, 1996). Total metal concentrations do not necessarily reflect bioavailability (Thompson *et al.*, 1984; Coetzee, 1993). The present results indicate that even though the total concentrations of most of the highly toxic metals are above the guideline values, a greater percentage of these metals exist in a non-bioavailable form.

Standard two-dimensional PCA was carried out and plotted on the axes for nine heavy metals (Al, Si, Fe, Cu, Zn, Cd, Sn, Hg and Pb) measured at the 11 sampling locations in Cape Town harbour sediments (Figure, 4.17c). The distribution of Pb, Sn and Hg components were highly concentrated at the Robinson dry dock, which showed that they were from anthropogenic sources, while Al, Fe, Si and Zn were highly concentrated in Duncan dock, and this confirms that theses metals are of natural origin. Cu and Cd were also concentrated in Robinson, BenSchoeman, and Synchrolift and Duncan docks. Significantly low concentration levels of metals were recorded at the control sites 10 and 11. PCA was also used to show the distribution of all the heavy metals analyzed in different fractions (Figures 4.17a and 4.17b). The results obtained with the PCA method are comparable with Pearson correlation analysis.

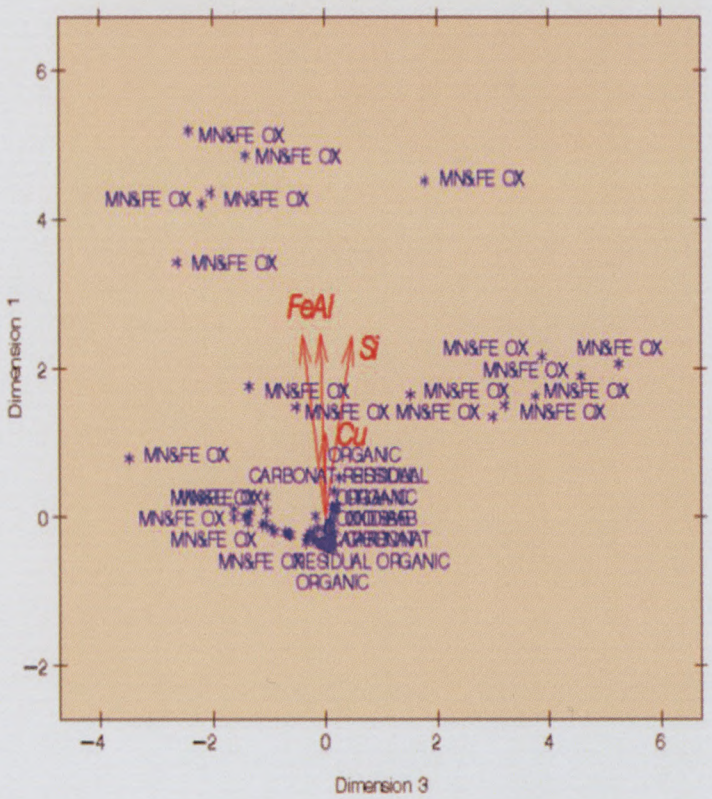


Figure 4.17a: Principal Component Analysis (PCA) of some metals in different fractions in in Cape Town harbour sediment

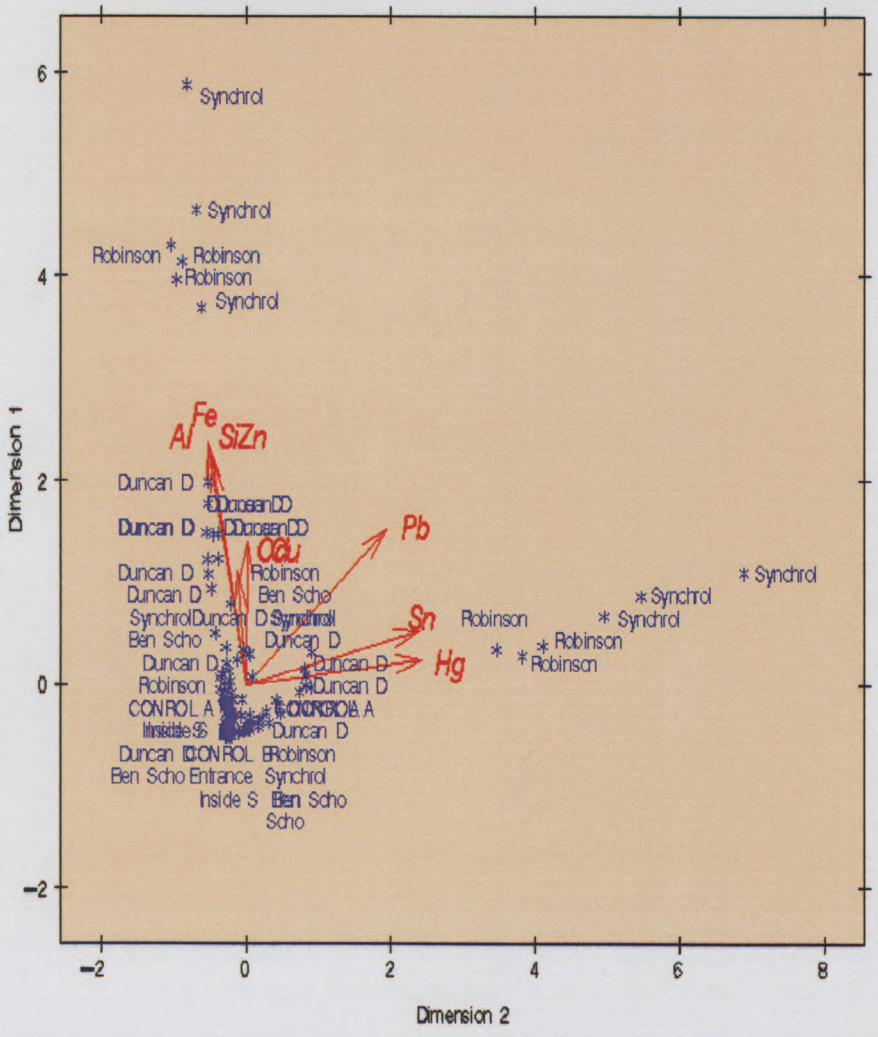


Figure 4.17b: Principal Component Analysis (PCA) of heavy metals with fractions in Cape Town harbour

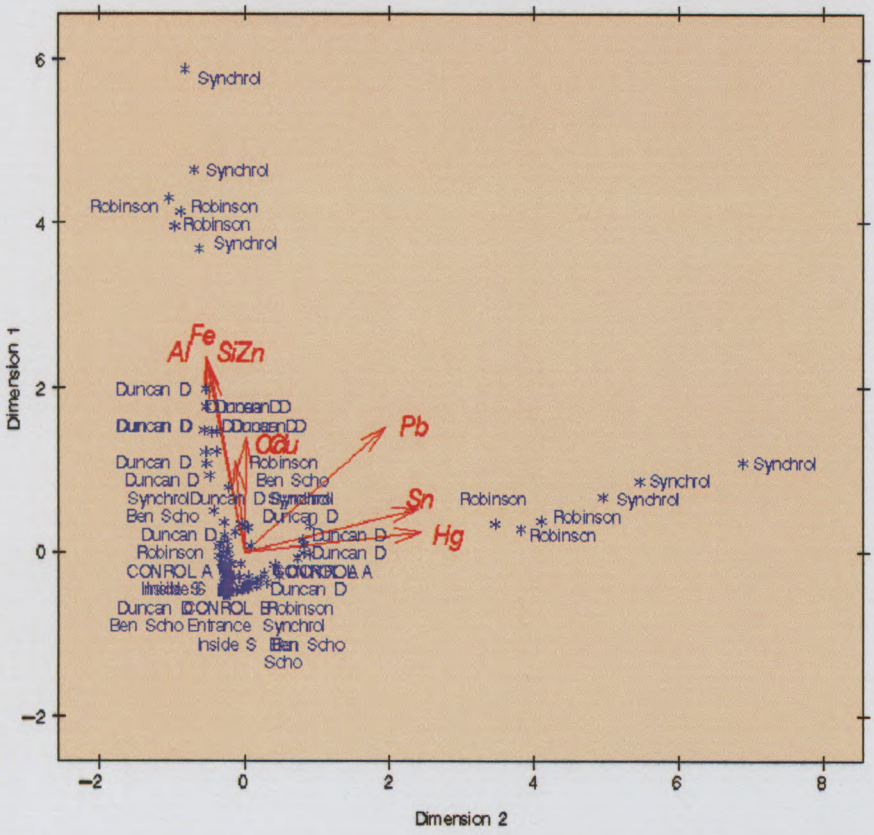


Figure 4.17c: Principal Component Analysis (PCA) of heavy metals with locations in Cape Town harbour

Table 4.15: Eigen value of the metals from the correlation matrix

Metals	Eigen values	Difference	Proportion	Cumulative
Zn	4.39	1.76	0.49	0.49
Cd	2.64	1.73	0.29	0.78
Sn	0.91	0.21	0.10	0.88
Hg	0.69	0.48	0.08	0.96
Pb	0.21	0.14	0.02	0.98
Al	0.07	0.02	0.01	0.99
Si	0.04	0.02	0.01	0.99
Fe	0.03	0.02	0.01	0.99
Cu	0.01	0.00	0.00	1.00

Component variations in the heavy metals from the Eigen values are shown in Table 4.15. The Eigen values decrease in the order: Zn > Cd > Sn> Hg >Pb> Al> Si> Fe> Cu. Al, Si, Fe and Cu have very low Eigen values and they could be said to be of natural origin while

Zn, Cd, Sn, Hg and Pb were of anthropogenic sources due to their high level of component variations.

Table 4.16: Pearson correlation coefficients, of metals N= 165

	Al	Si	Fe	Cu	Zn	Cd	Sn	Hg	Pb
Al	1.0000	0.9645	0.9751	0.9645	0.8693	0.4255	0.0053	0.1085	0.4352
		<.0001	<.0001	<.0001	<.0001	<.0001	0.9462	0.1652	<.0001
Si		1.00000	0.9364	0.3210	0.8027	0.4176	0.0015	0.0993	0.4103
			<.0001	<.0001	<.0001	<.0001	0.9851	0.2046	<.0001
Fe			1.0000	0.3315	0.8890	0.4029	0.0072	-0.1094	0.4242
				<.0001	<.0001	<.0001	0.9269	0.1620	<.0001
Cu				1.0000	0.3462	0.2875	0.0020	0.0519	0.1801
					<.0001	0.0002	0.9793	0.5081	0.0206
Zn					1.0000	0.4348	0.0216	-0.1055	0.4074
						<.0001	0.7835	0.1776	<.0001
Cd						1.0000	0.1005	0.0460	0.2894
							0.1988	0.5573	<.0002
Sn							1.0000	0.9182	0.8795
								<.0001	<.0001
Hg								1.0000	0.8010
									<.0001
Pb									1.00000

KEY: Upper value=correlation coefficient r

Lower value=Significance level P≤ 0.05; <.0001

Pearson correlation coefficients for heavy metals in Cape Town harbour sediment are presented in Table 4.16. Strong correlation coefficients (r > 0.7) were calculated between (Al and Si, Fe, Zn,); (Si and Fe, Zn) and (Fe and Zn), and this indicates a strong association between the various metal pairs. Al, Si, Fe and Zn would, therefore most likely be of natural origin. High correlation coefficients (r > 0.8) were also calculated for the (Sn and Hg, Pb) group, which indicates strong association and hence would most probably be of anthropogenic origin. These findings are in agreement with the results obtained from the PCA method. The total metal content was derived from total acid digestion method using microwave acid-leaching assisted method. The levels of heavy metal contamination were varied across all locations. Table 4.17 shows the range values and mean of heavy metal concentrations using ICP-MS. There appears to be diverse variations in the concentration of silicon across all locations. The concentration of Si was generally low. The highest mean concentration recorded for silicon was 0.368 ± 0.110 mg/kg for location 9 (entrance to harbour), followed by 0.351 mg/kg recorded for location 8 (Synchrolift) and lowest concentration of 0.023 ± 0.012 mg/kg recorded for location 1 (Duncan dock).

The high values recorded could be due to intensive reparation and painting activities being carried out at location 8 (Synchrolift). For Iron, the distribution of iron in all the sediments varied across locations. Iron was principally present in form of iron oxide with the concentration ranging from 28.753 ± 1.850 mg/kg for location 7 and 30.013 ± 2.041 mg/kg

for location 8 respectively, the exceptionally high values of iron at location 7 and 8 could be connected with the intensive industrial operations that were previously mentioned above. The lowest concentration for Fe was recorded at location 9 (entrance to harbour). The lower value could be connected to less activity taking place at this location.

Copper exhibited highest mean concentration at location 2 while it recorded lowest mean concentration at location 7. Industrial activities taking place at location 2 could be responsible for this observation. The concentration of zinc was generally lower than that of copper at all locations. The highest concentration was recorded at location 8 again while the lowest concentration was recorded at location at location 9. The highest concentration was due to industrial activity and anthropogenic inputs. The concentration of Al varied across all locations. The highest values recorded were 19.922 ± 2.236 mg/kg for location 6 and lower concentration of 1.247 ± 0.13 mg/kg for location 9. This follows the same pattern with zinc and iron, the high concentration values recorded for Al in location 8 could be as a result of routine painting and reparation of ships being undertaken at this location and from anthropogenic sources. Cadmium was generally present at lower concentration across all locations.

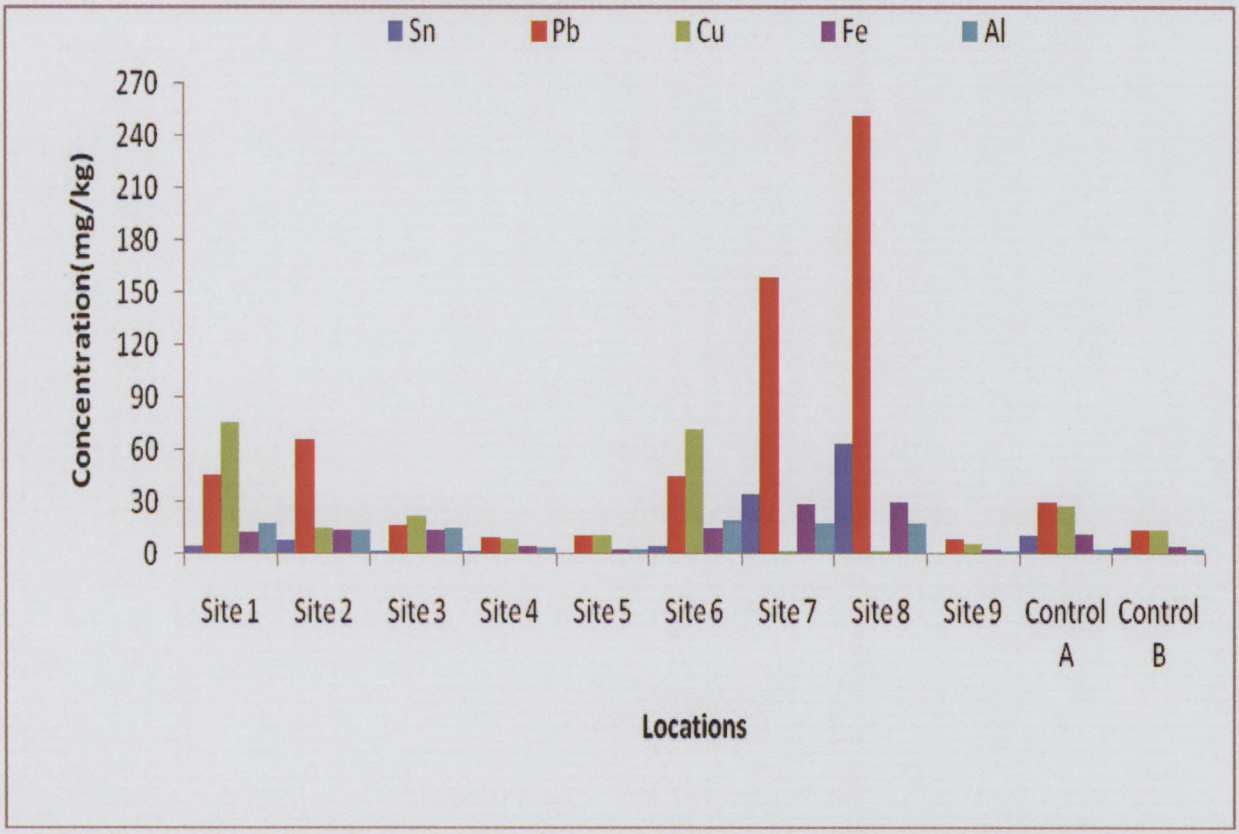
The concentration of cadmium was generally lower than that of Al, the highest concentration of cadmium was recorded at location 8 (1.693 ± 0.075 mg/kg) while the minimum concentration was recorded was 0.080 ± 0.002 mg/kg for location 9. Tin was principally present across all locations. Copper exhibited highest concentration followed by tin across all the locations. The highest value recorded was 63.977± 4.97 mg/kg for location 8 and minimum concentration of 1.145 ± 0.382 mg/kg for location 9. The high values recorded for tin at this location were in no doubt as result of reparation and painting of ships being undertaken and in which organic tin was the major active ingredients used as antifouling agents therefore polluting the harbour. These effects have adverse effect on the aquatic life in the sea (Fatoki and Mathabata, 2001; Wepener and Vermeulen, 2005). Therefore adequate proper control must take pace to ban the use of this antifouling agent (organotins) in ship painting and more the government must ban the importation of ships whose hulls are coated with antifouling agents as it was banned in European country and other developed countries.

The distribution of lead across all locations follows the same pattern as for tin. The highest concentration recorded across location was 251.713 ± 29.023 mg/kg at location 8 while the lowest concentration was 8.728 ± 1.065 mg/kg respectively. The nature of the industry which is located near the harbour (explosive etc) is also the reason why one could expect effluents to be a relatively high source of lead in the sediment of Cape Town harbour. Mean concentration recorded was generally low across all location for Hg. There appear to be a diverse variation in the concentration of Hg across all locations. The concentration

ranges between 1.002 ± 0.168 mg/kg for location 8 and 0.049 ± 0.005 mg/kg for location 4 respectively. The high value recorded at location 8 could be as a result of run-off from large motor assembly plant which lies to the south part of the port, apart from the fact that this location is closer to motor scrap points. This finding were well documented in a related studies carried out by (Fatoki and Mathabata, 2001; Wepener and Vermeulen, 2005).

4.7: Geochemical assessment of sediment in Cape Town harbour

The concentration levels of nine elements determined by ICP-MS instrument are shown in Table 4.17. Tin was generally present across all locations. The highest value recorded for Sn was 63.977 ± 4.97 mg/kg for location 8 and a minimum concentration of 1.145 ± 0.382 mg/kg for location 9. The high values recorded for tin at this location was certainly due to repair and painting of ships. Tributyltin (TBT) is the major antifouling agent added in ship paint. Organotin compounds have adverse effect on the aquatic life in the sea. Therefore adequate proper control must be undertaken to ban the use of this antifouling agent (organotins) in ship painting. The distribution of lead (Pb) across all locations follows the same pattern as tin. The highest concentration recorded was 251.713 ± 29.023 mg/kg at location 8 while the lowest concentration was 8.728 ± 1.065 mg/kg at location 9. The nature of industrial activities such as explosive manufacturing industry near the harbour could explain the presence of high concentration of lead in the sediment of Cape Town harbour. Copper exhibited the highest mean concentration at location 2 while it recorded lowest mean concentration at location 7. Copper (Cu) had the highest concentration followed by tin (Sn) across all the locations. Industrial activities taking place at location 2 could be responsible for this observation.



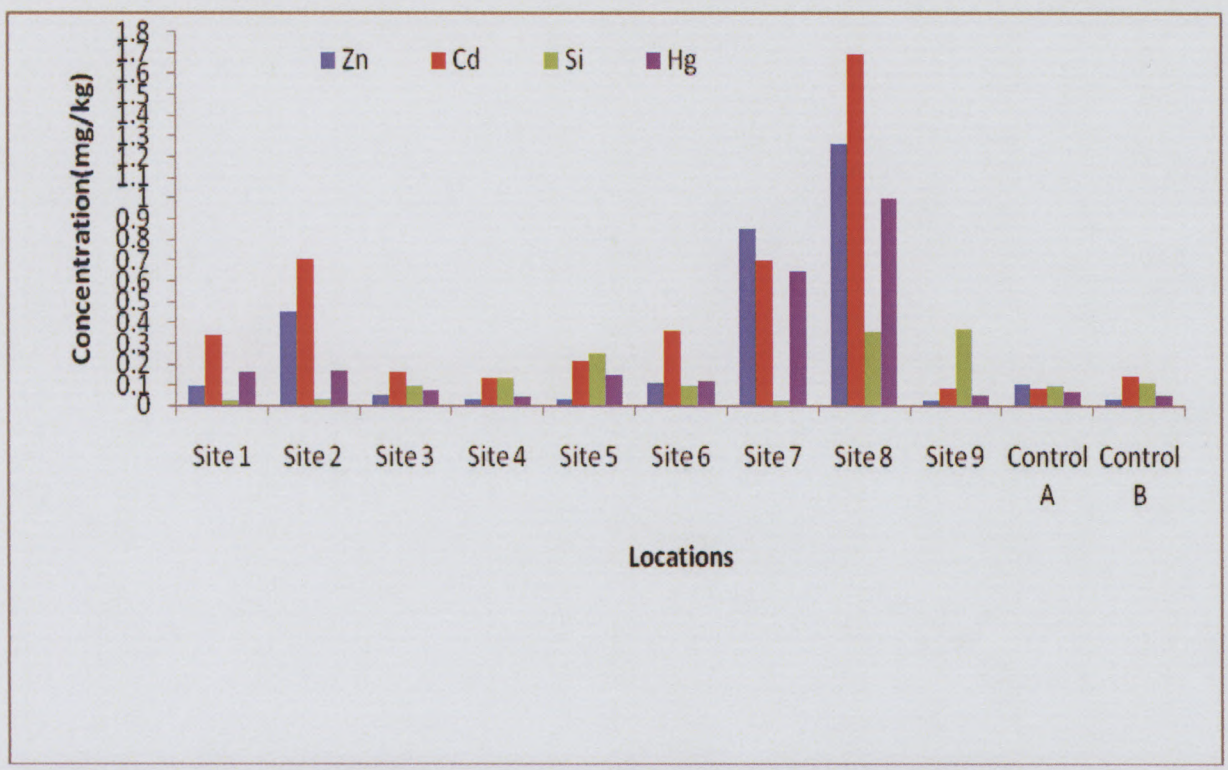


Figure 4.18a and b: Concentration levels of metals in the eleven sampled sites

Table 4.17: Results of Total Metal analysis in mg/kg in sediment sample from Cape Town Harbour

Locations	Sn	Pb	Cu	Zn	Fe	Cd	Hg	Al	Si
1	4.971±1.042	45.355±7.612	75.679±11.13	0.100±0.01	13.154±2.568	0.340±0.05	0.161±0.051	18.027±2.312	0.023±0.012
2	7.689±2.790	65.886±24.23	1462.34±39.0	0.446±0.31	13.268±3.122	0.704±0.231	0.172±0.059	13.703±4.334	0.035±0.013
3	2.051±0.218	16.587±2.740	21.937±2.946	0.057±0.00	13.480±0.829	0.164±0.026	0.079±0.012	14.781±1.631	0.094±0.011
4	1.180±0.303	9.220±0.292	8.316±0.410	0.031±0.003	4.716±0.218	0.137±0.015	0.049±0.005	3.793±0.356	0.131±0.018
5	1.150±0.154	10.31±0.284	10.468±3.755	0.029±0.00	3.042±0.120	0.218±0.010	0.146±0.119	2.513±0.075	0.250±0.120
6	5.021±0.071	44.403±0.872	71.175±1.187	0.114±0.17	15.090±1.204	0.362±0.040	0.122±0.099	19.922±2.236	0.094±0.019
7	34.336±2.707	158.519±12.715	1.511±0.112	0.854±0.04	28.753±1.850	0.696±0.047	0.650±0.096	17.625±0.970	0.024±0.003
8	63.977±4.947	251.713±29.023	1.783±0.278	1.260±0.07	30.013±2.041	1.693±0.075	1.002±0.168	17.272±0.680	0.351±0.337
9	1.145±0.382	8.728±1.065	6.149±0.613	0.022±0.00	2.234±0.119	0.080±0.002	0.056±0.007	1.247±0.131	0.368±0.110
10	10.638±4.92	30.045±3.927	27.665±1.644	0.102±0.00	12.026±0.185	0.086±0.009	0.0693±0.037	2.978±0.310	0.097±0.031
11	3.699±1.36	13.650±3.055	13.536±1.732	0.035±0.00	4.365±0.133	0.138±0.142	0.056±0.008	2.321±0.184	0.110±0.027

The concentration of zinc was generally lower than that of copper at all locations. The highest concentration was recorded at location 8 again while the lowest concentration was recorded at location 9. The highest concentration would most certainly be due to the intense industrial activities and this is anthropogenic in nature .The distribution of iron in all the sediments varied across locations. Iron was mainly present as iron oxide with the concentration ranging from 28.753 ± 1.850 mg/kg for location 7 and 30.013 ± 2.041 mg/kg for location 8, respectively. The exceptionally high values of iron at location 7 and 8 could be connected to the intensive industrial operations that were previously mentioned above. The lowest concentration for Iron (Fe) was recorded at location 9 (entrance to harbour). This is because the area is sandy and therefore has naturally low iron content in the sediment.

Cadmium (Cd) was generally present at lower concentration across all locations. The highest concentration of cadmium was recorded at location 8 while the minimum concentration was recorded at location 9. Mercury concentration was low across all levels. The concentration ranged between 1.002± 0.168 mg/kg for location 8 and 0.049 ± 0.005 mg /kg for location 4. The high value recorded at location 8 could be as a result of run-off from motor scrap collection points. The concentration of Aluminium (Al) varied across all locations. The highest values recorded were 19.922 ± 2.236 mg/kg for location 6 and lower concentration of 1.247 ± 0.13 mg/kg for location 9. This follows the same pattern with zinc (Zn) and iron. The high concentration values recorded for Al in location 8 could be due to routine painting and reparation of ships being undertaken at this location and from anthropogenic sources. There was variation in the concentration of silica between the sites sampled. The concentration of Si was generally low. The highest concentration recorded for silicon was 0.368 ± 0.110 mg/kg for location 9 (entrance to harbour), followed by 0.351 mg/kg for location 8 (Synchrolift), and the lowest concentration of 0.023 ± 0.012 mg/kg was in location 1 (Duncan dock). The high silicon concentrations recorded in location 8 and 9 could be as a result of intensive reparation and painting activities at location 8 (Synchrolift) as well as from sand, diatom frustules and runoffs at location 9.

Enrichment factor (EF) is widely employed to identify the anthropogenic source of metallic element (Fang *et al.* 2006). The value of enrichment coefficient is however used to evaluate the degree of enrichment of some chemical elements when they are compared with their natural sources (Muller, 1969). The EF may be due to either natural or anthropogenic causes. The EF has been calculated using the expression below.

$$EF = \frac{(X / RE)_{Sample}}{(X / RE)_{Crust}}$$

Where EF represent = Enrichment factor

RE = Reference element

X_{sample}= measured element

$(RE)_{control}$= Control value for reference element

$(X)_{Control}$= Control value for measured element

In this study Si was chosen as the reference element due to its steady chemical characteristics. The sources of these heavy metals have been confirmed by calculating the EF for all the metals using Si as a reference element and also location 11 as control site due to the reduced level of metal contamination recorded at this location. The EF for all the metals are summarised in Table 4.18. EF values of between 0.5 and 2 are considered to be indicative of natural factors while values greater than 2 are attributed to anthropogenic causes (Ding *and* He, 2010). The EF values of locations 4 and 5 (inside sea), 10 (Remote location) and 9 (entrance to harbour) ranged from 1.00 to 3.26, and this is due to both anthropogenic and natural contribution.

Table 4.18: Metals Enrichment Factors in sediments from harbour

Sampling sites	Metals								
	Sn	Pb	Cu	Zn	Fe	Cd	Al	Si	Hg
1	6.34	15.89	26.74	13.67	14.41	11.78	37.15	1.00	13.73
2	6.53	15.17	339.55	40.07	0.33	16.03	18.56	1.00	9.64
3	0.65	1.42	1.90	1.91	3.61	1.39	7.45	1.00	1.68
4	0.27	0.57	0.52	0.74	0.91	0.83	1.37	1.00	0.73
5	0.14	0.33	0.34	0.36	0.31	0.70	0.48	1.00	1.15
6	1.59	3.81	6.15	3.81	4.05	3.07	10.04	1.00	2.54
7	42.55	53.23	0.51	111.90	30.19	23.12	34.80	1.00	53.10.
8	5.44	5.78	0.04	11.29	2.15	3.84	2.33	1.00	53.60
9	0.09	0.19	0.14	0.19	0.15	0.17	0.16	1.00	0.30
10	3.26	2.50	2.32	3.31	3.12	0.71	1.46	1.00	1.40

The EF of all the metals in location 1 and 2 (Duncan Dock) are generally greater than those in location 3 (Ben Schoeman Dock), The EF values for location 3 were between 0.65 to 3.65 for all the metals except Al which is higher than 3 (7.45), the enrichment of Al at this location is due to anthropogenic effects while others were due to natural changes. At locations 6, Pb, Cu, Zn, Fe, Cd, Al are highly enriched while the EF values for Hg and Sn

were below 3. For locations 7 and 8 (Robinson dry-dock and Synchrolift), the EF values recorded for Sn, Pb, Zn, Fe, Cd, Al and Hg were much higher. Metal contamination in these two locations is due to anthropogenic activities such as ship repairs, runoff from domestic, industrial and storm water into the harbour. The EF values for Copper in these locations were below 1, and this signifies that its presence is due to natural changes. Higher level of EF recorded for Iron across the locations was an indication of intensive industrial operations. The highest EF for copper was observed in location 2, which confirms the predominant presence of calcite in this location. Industrial activities taking place at this location could also be responsible for these observations.

FTIR was used to investigate the mineral composition of the harbour sediments. The absorption frequencies of the peaks in the spectra of each location in wave number units (cm^{-1}) are recorded in Table 4.19. The following minerals were confirmed in the sediment samples across all locations when their observed frequencies were compared with that of literature: quartz, calcite, pyrites, and carrolite (Table 4.20). The following minerals were identified from the analysis of two certified reference materials: quartz, feldspar, kaolinite and calcite (Table 4.20). The presence of Fe was confirmed across all locations. The literature values of the absorption frequencies of most minerals are summarised in Table (4.20) and the typical FTIR spectra for all samples is shown in Figure 4.19 while that of certified reference material for trace element is shown in Figure 4.20.

Table 4:I9: IR Frequencies of Cape Town Harbour sediments and their assignments

Locations	Observed IR absorption frequencies (cm^{-1})
1 (Duncan Dock)	458.23, 502.12, 685.92, 828.58, 1034.33, 1336.10, 1465.10, 1640.61, 2928.57, 3434.75, 3697.80
2 (Duncan Dock)	460.97, 532.30, 691.41, 823.09, 1333.36, 1455.77, 1599.46, 1766.81, 2516.48, 2934.06, 3434.91
3 (Ben Schoeman Dock)	496.63, 688.67, 823.09, 1341.59, 1452.07, 1602.21, 2521.97, 3406.59, 3619.89, 3697.33
4 (Inside Sea)	463.71, 617.34, 778.24, 823.09, 1308.67, 1466.24, 1585.75, 2510.98, 2923.07, 3420.02
5 (Inside Sea)	469.20, 707.87, 825.84, 866.99, 1253.80, 1602.21, 2516.48, 3016.48, 3427.05
6 (Duncan Dock)	499.38, 685.92, 823.09, 1330.61, 1455.80, 1585.75, 2516.48, 2934.06, 3431.38
7 (Robinson DRY Dock0	535.04, 685.92, 825.84, 908.14, 1455.89, 2516.48, 2912.08, 3420.81, 3703.29
8 (Synchrolift)	535.04, 688.67, 831.32, 919.11, 1325.13, 1455.74, 1583.00, 1794.24, 2928.57, 3434.35
9 (Entrance to harbour)	669.46, 1190.70, 1473.27, 3445.05
10 (Control A)	707.87, 820.35, 869.73, 1465.04, 1748.88
11 (Control B)	589.91, 820.35, 2923.07, 3450.54

Table 4.20: IR absorption frequencies cm^{-1} of quartz, Pyrite*, Calcite, Carolite***, Kaolinite**** present in the**

1	2	3	4	5	6	7	8	9	10	11	BCR-277R (for trace element)	BCR -462 (for butyltins)
458	460.97, 691.41	689	778.24	825.84	685.92	825.84	688.67	669.46	820.35	820.35	797.6	NF
502*	532.30*, 691.41*	688.67*	617.34*		685.92*	535* 685*	535* 685*	669.5*		589*		NF
685.92, 828.58**, 3434*****	691.4** 823.1** 3434***	688.7** 823.1**	617.3** 778.2** 823.1** 3420***	707** 825** 3427***	685.9** 823.1** 3420***	685** 825**	688** 831** 3434***	669** 3445***	.707** 820** 870**	820** 3450***	797.62** 3622****	NF

Numbers 1-11: sampling locations acrross the harbour

BCR - 277R: Certified reference materials for trace elements

BCR - 462: Certified reference materials for butyltins

Table 4.21: Band assignments for different minerals in sediment samples and reference materials compared with those reported elsewhere.

Minerals	Frequency	References
Quartz	458 & 455 798 695	Hiavay etal.,(1978);Coates (1977). Wenshi (1983)
Feldspar	540-533 589-586 644-640	Coates (1977) Coates (1977) Hiavay et al. (1977); Coates (1977)
Kaolinite	908 1035-1030 3620 3696-3695	Russell(1987); Summer (1995)
Calcite	1420-1380 1300-1180 1017- 980 900-600	Manoharan et al. (1990)
Pyrite	500-700	Dun et al. (1992)
Carrolite	Around 3500	(Current study)
BCR- 277R (for trace element) Certified Refference Materials	797.62, 1637.87, 1799.73, 2516.48, 3622.25	(Current study)
BCR -462 (for butyltins) Certified Refference Materials	693.90, 778.20, 1027.01, 3421.31	(Current study)

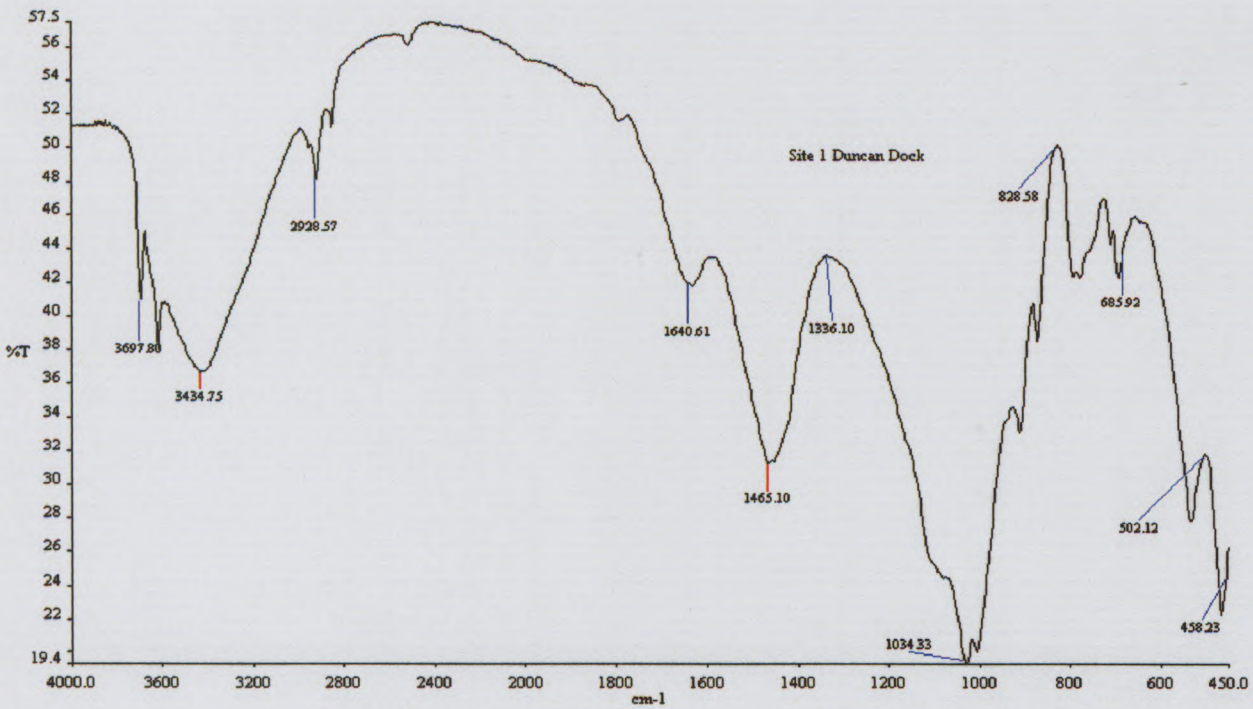


Fig 4.19: A typical FTIR spectrum of sediment samples collected from locations 1-9

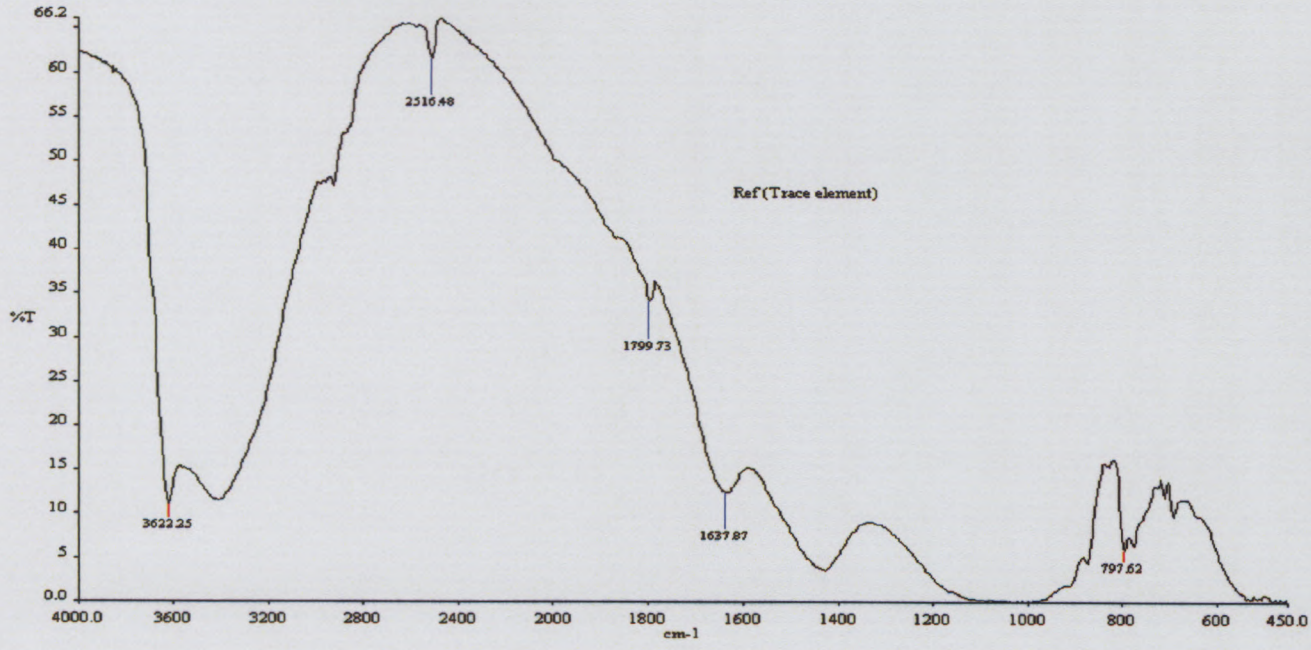


Figure 4.20: Typical Spectrum of Certified reference sediment sample for trace elements

All the mineral phases observed from the XRD data are summarised in Table 4.22. XRD data was used for qualitative mineralogical determination of marine sediments. The qualitative mineralogy of the marine sediment of Cape Town harbour were determined with the standard interpretation procedures of XRD. The minerals phases were explored from the XRD data. The representative diffractograms of all samples is shown in Figure 4.21a-c.

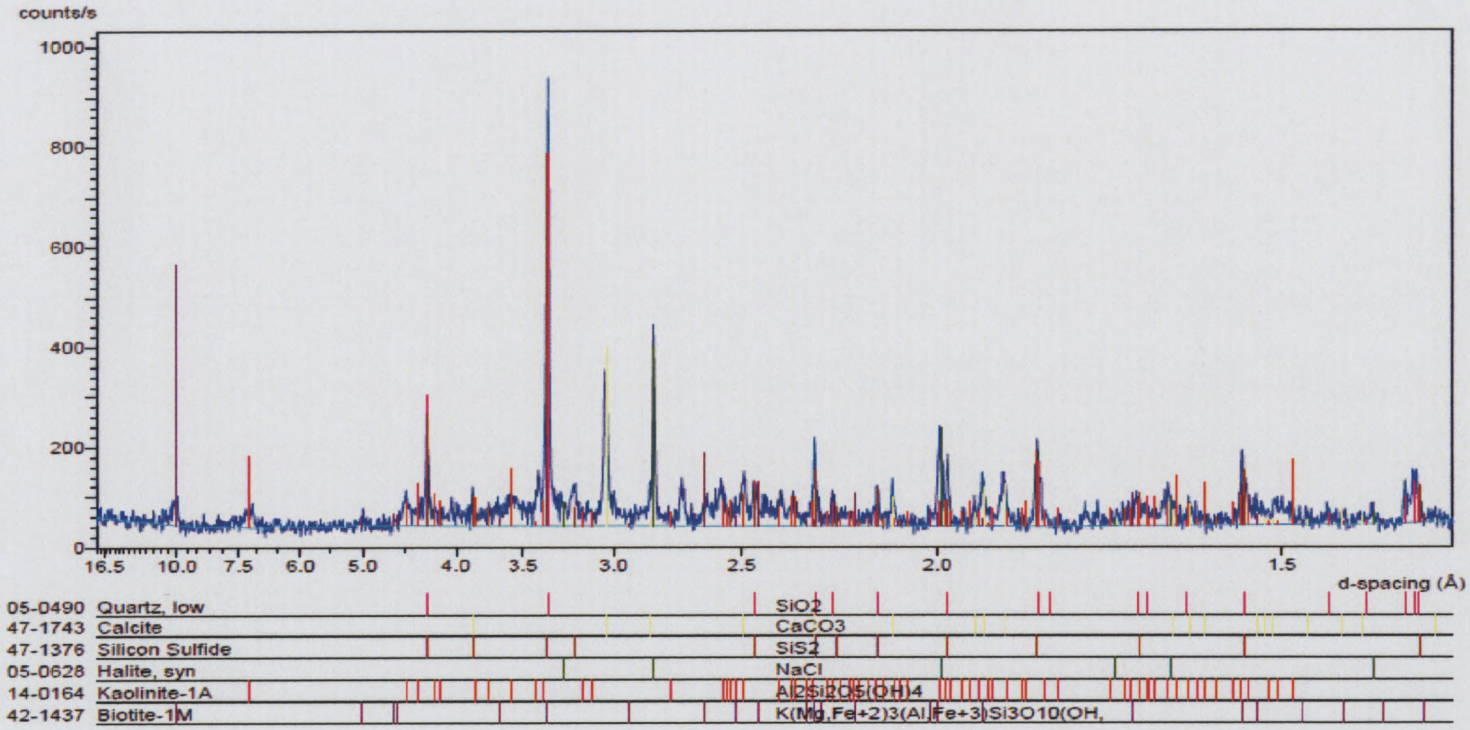


Figure 4.21a: A typical XRD diffractogramme for all samples characterized from inner harbour (Snychrolift site) during summer season

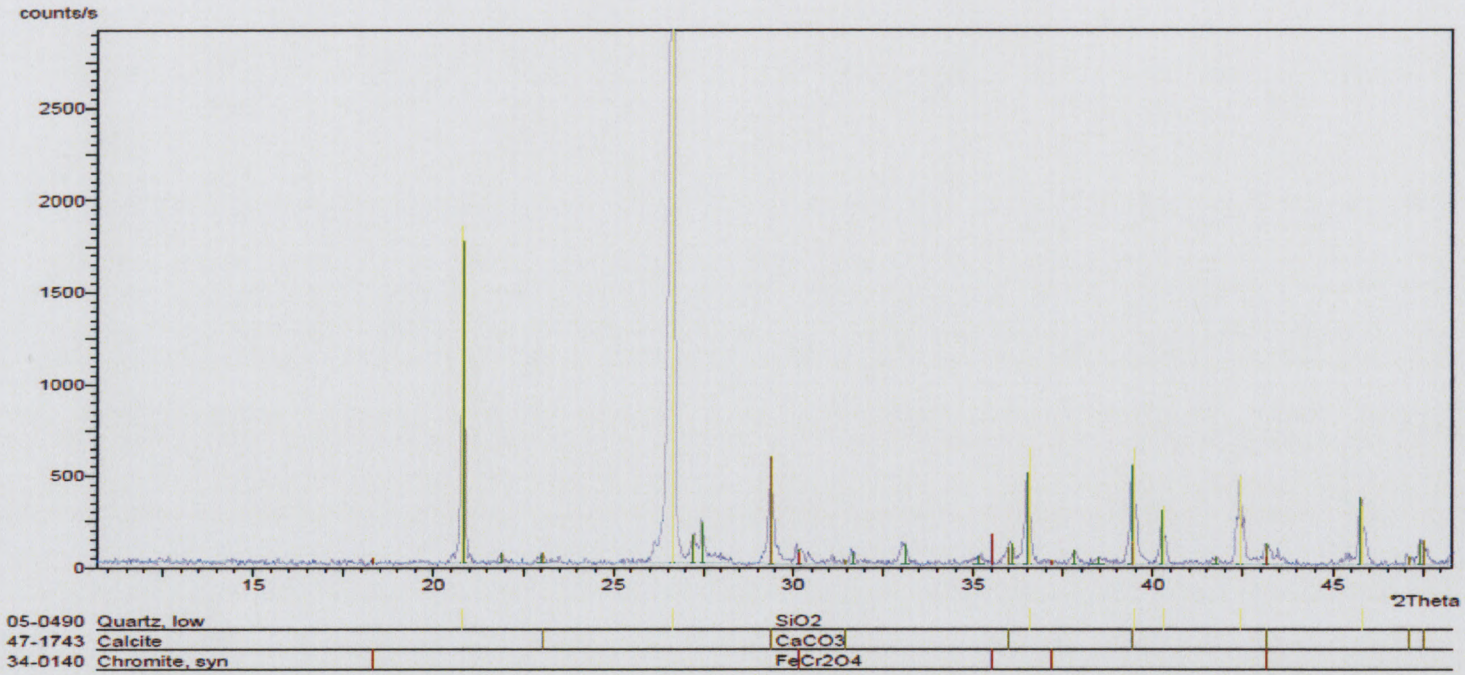


Figure 4.21b: A typical XRD diffractogramme for all samples characterized from control site during winter season

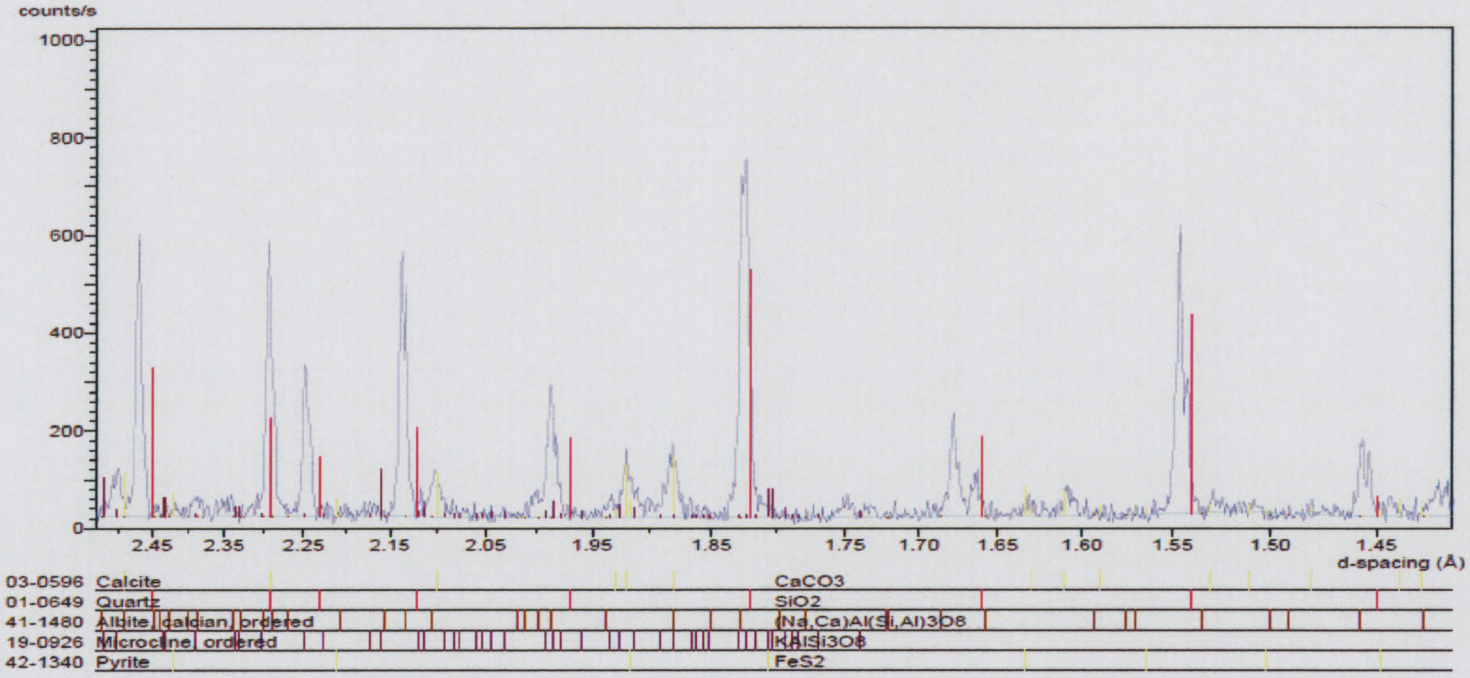


Figure 4.21c: A typical XRD diffractogramme for all samples characterized from location 4 (inside sea) 500m away from inner harbour during winter season

Quartz is the most common mineral in the geosphere, and it is normally found in most geological environments. Calcite and carrolite are also found in most of the sampled sites. Calcite is a carbonate mineral of calcium. Pyrites are present in locations 2, 6, 8 and 10. Carbonates are commonly found in marine sediments when the shells of dead planktonik life settle and accumulate on the sea floor (Ravisankar *et al.* 2010). Carrolite is a grey metallic sulphide mineral containing a rich mixture of copper, cobalt, and nickel. It is composed of approximately 40% cobalt and 20% copper. The clay mineral phase consists of biotites, kaolinites and halites. Iron, nickel and sulphur are also found in this mineral. It has chemical formula. Cu $(Co, Ni)_2S_4$, Carrolite is a rare mineral recently found in Africa. The presence of this mineral in these locations leads to high concentration levels of copper and iron observed in location 1, 2, 4, 5,6, 7, 8, 9 and 11. Kaolinite and feldspar were only confirmed in the BCR -277 certified reference material as shown in Table 4.22 . Two certified reference materials were also subjected to XRD investigation. The organotins did not produce any signal during XRD examination and this confirms the absence of mineral phase while the second reference material produced characteristic peaks . Figure 4.21 represents typical XRD spectra for all the samples while the remain chromatogramm were shown in Appendix A.

Table 4.22: Mineral Phases in Cape Town Harbour sediment (extracted from XRD)

Locations	Quartz	Feldspar	Chromite	Pyrite	Kaolinite	Calcite	Carrolite
1(Duncan Dock)	•					•	•
2(Duncan Dock)	•			•		•	•
3(Ben Schoeman Dock)	•					•	
4(Inside Sea1	•						•
5(Inside Sea)	•						•
6(Duncan Dock)	•			•		•	•
7(Robinson Dry Dock)	•					•	•
8(Synchrolift)	•			•		•	•
9 (Entrance to harbour)	•					•	•
10 (Control A)	•						•
11(Control B)	•			•		•	
BCR- 277R (for trace element)	•	•			•	•	
BCR -462 (for butyltins)	NF	NF	NF	NF	NF	NF	NF

NF – Not found
BCR - Certified Refference Materials
• - Mineral presence in each location

Apparently, the present study showed qualitative mineral identification of Cape Town harbour sediment using FTIR and XRD techniques respectively. The study reveals the levels of contamination of Cape Town harbour. The results are an indication of the contributions of heavy metals which carry runoff from the waste water treatment plant that discharges into the Diep River, as well as domestic, ocean current, and storm water inflow into the harbour. Ship reparation is a major suspected factor responsible for the higher metal contamination in dockyard areas of the harbour. The enrichment factor recorded for Sn, Pb, Zn, Fe, Cd, Al and Hg in locations 7 and 8 were higher, metal contamination in these locations is due to activities previously mentioned in this section. Copper concentration was below 1, meaning that its presence is due to natural changes mentioned above. Since EF for Sn is high, there should be legislation to monitor the docking of ships coated with organotins as antifouling. The ban on the use of these antifouling agents has been put under control in European and other developed countries but in Africa the ban has not been applied. The FTIR and XRD analyses indicate the presence of quartz, pyrite, calcite and carrolite in the harbour sediments. The combination of the two techniques showed that they are useful techniques for mineral analysis.

4.8: Heavy metals monitoring in seawater from Cape Town harbour

In the present study, Cape Town harbour water was monitored seasonally for a period of one year. There are few published data on heavy metals in and around Cape Town harbour. It is thus necessary to investigate heavy metals concentration and distribution in the harbour and compare the effects of seasonal variation on heavy metal concentration. The result of heavy metal concentration in this study is summarised in table 4.23 and 4.24.

Table 4.23: Seasonal mean of As, Cd, and Hg concentration in Cape Town Harbour in µg/l.

	Locations	Spring	Summer	Winter	Mean location
As µg/L	Duncan Dock 1	2.33±0.18	2.03±0.15	8.15±1.23	4.17±3.05
	Duncan Dock 2	1.16±0.12	2.69±0.23	4.92±1.19	2.92±1.74
	Benshoeman Dock	1.60±0.09	2.25±0.09	1.91±0.62	1.92±0.42
	Inside Sea 1	1.90±0.20	3.01±0.24	7.98±4.00	4.30±3.44
	Inside Sea 2	2.13±0.26	1.94±0.10	4.58±1.67	2.88±1.53
	Duncan Dock 3	2.59±0.05	1.24±0.04	5.44±0.62	3.09±1.88
	Robinson Dry Dock1	2.20±0.13	2.27±0.29	5.31±0.35	3.26±1.56
	Synchrolift	2.36±0.09	1.21±0.19	4.32±0.40	2.63±1.38
	Entrance to Harbour	2.41±0.17	1.85±0.24	2.57±0.12	2.27±0.37
	Control A	2.07±0.22	2.45±0.08	4.67±1.75	3.07±1.50
	Control B	2.27±0.14	2.91±0.09	6.10±1.35	3.76±1.90
	Robinson Dry Dock 2	2.13±0.07	1.86±0.11	4.33±0.47	2.78±1.20
	Mean seasons	2.10±0.40	2.15±0.58	5.02±2.18	
	CV%		28.880%		
	P≤0.05		***		***
	Interaction P≤0.05		***		
Cd µg/L	Duncan Dock 1	1.43±2.07	2.67±0.11	34.11±2.21	12.74±16.11
	Duncan Dock 2	0.20±0.02	0.91±0.08	0.97±0.31	0.69±0.40
	Benshoeman Dock	0.18±0.03	0.37±0.02	0.76±0.01	0.44±0.25
	Inside Sea 1	1.69±0.09	0.91±0.04	1.38±0.16	1.33±0.35
	Inside Sea 2	0.20±0.03	0.30±0.09	1.29±0.16	0.60±0.53
	Duncan Dock 3	0.49±0.05	0.07±0.03	1.77±0.38	0.78±0.79
	Robinson Dry Dock1	0.34±0.03	0.13±0.02	1.76±0.18	0.74±0.77
	Synchrolift	0.34±0.02	0.07±0.02	0.41±0.35	0.28±0.23
	Entrance to Harbour	0.53±0.20	0.44±0.07	1.04±0.07	0.67±0.30
	Control A	0.23±0.03	3.92±0.28	0.88±0.19	1.67±1.71
	Control B	0.67±0.06	0.24±0.06	0.82±0.18	0.58±0.28
	Robinson Dry Dock2	0.31±0.04	0.11±0.03	0.78±0.07	0.40±0.30
	Mean seasons	0.55±0.69	0.84±1.17	3.83±9.28	
	CV%		30.055%		
	P≤0.05		***		***
	Interaction P≤0.05		***		
Hg µg/L	Duncan Dock 1	3.49±0.10	0.82±1.01	4.71±1.10	3.01±1.87
	Duncan Dock 2	2.56±2.52	0.53±0.41	1.93±0.77	1.67±1.61
	Benshoeman Dock	1.26±0.78	0.61±0.01	11.32±1.57	4.40±5.27
	Inside Sea 1	0.67±0.05	0.59±0.51	13.70±0.79	4.99±6.55
	Inside Sea 2	0.46±0.06	0.26±0.30	2.06±0.21	0.92±0.87
	Duncan Dock 3	2.17±0.12	0.43±0.30	150.38±8.55	50.99±74.66
	Robinson Dry Dock1	0.29±0.07	1.07±0.42	2.91±1.43	1.42±1.39
	Synchrolift	2.31±0.45	0.10±0.02	3.08±0.30	1.84±1.37
	Entrance to Harbour	2.12±0.08	0.18±0.07	5.26±0.51	2.52±2.23
	Control A	0.75±0.41	0.06±0.01	2.92±0.22	1.24±1.31
	Control B	0.32±0.04	0.06±0.01	1.12±0.06	0.50±0.48
	Robinson Dry Dock 2	0.64±0.08	0.06±0.01	0.88±0.07	0.53±0.37
	Mean seasons	1.42±1.22	0.40±0.45	16.69±41.11	
	CV%		25.60%		
	P≤0.05		***		***
	Interaction P≤0.05		***		

Table 4.24: Seasonal mean of Pb and Sn concentration in Cape Town Harbour in µg/l

Metals		Seasons			
	Locations	Spring	Summer	Winter	Mean location
Pb µg/L	Duncan Dock 1	12.45±5.10	5.59±0.77	16.58±2.58	11.54±5.61
	Duncan Dock 2	4.26±0.96	61.47±5.56	4.95±1.12	23.57±28.57
	Benshoeman Dock	3.85±0.29	6.30±1.67	5.39±1.37	5.18±1.53
	Inside Sea 1	21.70±2.93	15.53±1.47	22.19±23.14	19.8±12.12
	Inside Sea 2	0.46±0.06	0.26±0.30	8.05±0.94	5.91±1.90
	Duncan Dock 3	11.51±0.89	1.54±0.56	54.79±1.58	22.61±24.53
	Robinson Dry Dock 1	12.06±4.41	5.51±2.22	25.47±3.70	14.35±9.34
	Synchrolift	8.02±2.47	1.62±0.30	4.75±0.33	4.80±3.05
	Entrance to Harbour	13.35±3.53	8.24±0.83	17.97±1.07	13.19±4.62
	Control A	12.96±2.23	5.47±0.67	7.70±0.74	8.71±3.55
	Control B	19.93±1.45	3.69±0.58	7.12±1.31	10.25±7.49
	Robinson Dry Dock 2	9.80±0.32	1.27±0.38	4.87±0.31	5.32±3.72
	Mean seasons	11.21±5.92	10.11±16.22	14.99±15.21	
	CV%		36.282%		
	P≤0.05		***		***
	Interaction P≤0.05		***		
Sn µg/L	Duncan Dock 1	16.80±0.08	14.51±0.56	9.10±0.41	13.47±3.44
	Duncan Dock 2	8.40±1.60	141.08±24.62	0.62±0.01	50.03±69.47
	Benshoeman Dock	7.28±0.57	85.42±13.34	1.78±1.35	31.49±41.07
	Inside Sea 1	37.94±0.46	670.67±205.84	20.38±34.22	243.00±337.38
	Inside Sea 2	11.08±0.38	133.93±41.64	0.62±0.01	48.55±67.49
	Duncan Dock 3	12.00±0.39	86.17±25.25	3.15±4.38	33.78±41.51
	Robinson Dry Dock 1	9.25±0.71	63.65±7.00	0.62±0.01	24.51±29.80
	Synchrolift	3730.96±50.97	360.02±61.05	0.62±0.01	1363.87±1782.57
	Entrance to Harbour	14.07±4.08	82.46±29.45	1.83±1.17	32.79±40.47
	Control A	12.14±0.89	138.88±16.64	0.62±0.01	50.55±66.96
	Control B	8.41±0.30	63.10±8.29	0.62±0.01	24.04±29.78
	Robinson Dry Dock 2	9.23±0.23	86.44±14.13	2.20±1.27	32.62±41.09
	Mean seasons	323.13±1042.18	160.53±184.78	3.514±10.02	
	CV%		23.97		***
	P≤0.05		***		
	Interaction P≤0.05		***		

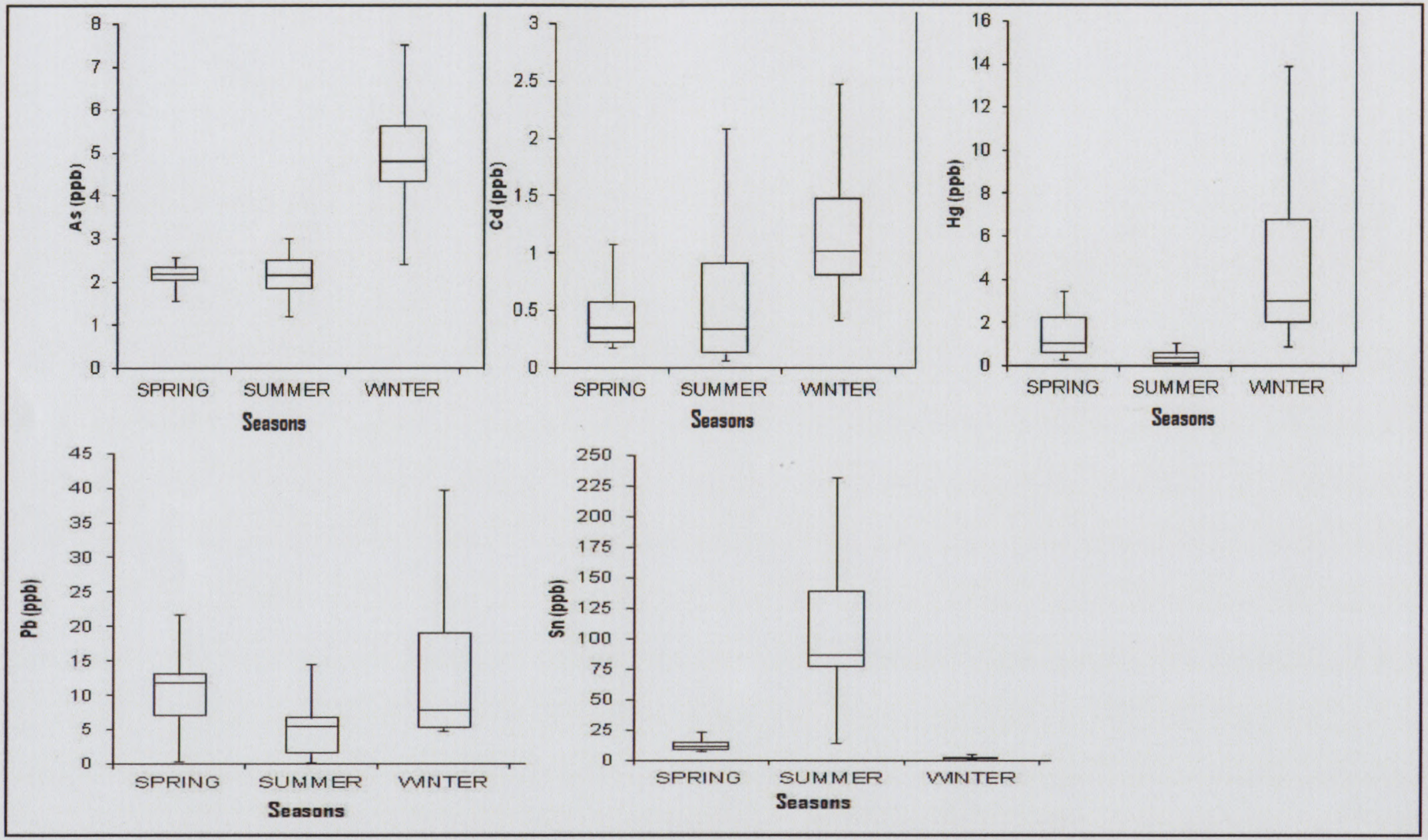


Figure 4.22: Box and whisker plot for the annual spread of metal concentrations in Cape Town harbour water

4.8.1: Arsenic

Arsenic is a metalloid which occurs in the elemental state to a small extent. As concentration at all locations ranges from 1.92 ± 0.42 to 4.30 ± 3.44 $\mu g/l^{-1}$. Location 5 recorded the highest concentration of 4.30 ± 3.44 $\mu g/l^{-1}$ while the lowest concentration of 1.92 ± 0.42 $\mu g/l^{-1}$ was recorded in location 3 (Ben Schoeman dock). The highest concentration value for arsenic was recorded in winter. The order of increasing seasonal values is spring < summer< winter as summarised in Table 4.23 and Figure 4.22. A significant variation of P (≤ 0.05) was found. The highest concentration value recorded for arsenic at location 5 could be as a result of biological transformations taking place at this location, since $A3^+$ and $A5^+$ are subject to series of biological transformations. The predominant natural occurrence of arsenic recorded in this study is due to volcanic eruptions and biological activities. Contribution from volcanic eruptions and biological activities is very small compared to the anthropogenic inputs such as agricultural chemicals, smelting of ores. Arsenic readily enters the atmosphere easily because of its relative volatility (Arsenic emissions inevitably yield relatively high residues during the rain season (Riedel, 1993). This is in agreement with the data collected in this study. Arsenic concentration was prevalently higher in winter than other season and the Western Cape of South Africa is a winter rainfall region.

4.8.2: Cadmium

Generally, a significant variation (P ≤ 0.05) was calculated for the distribution of Cd across all locations. Seasonal variation of Cd was such that it is more prevalent during summer because of less dilution. The decreasing order of concentration is summer > winter > spring. The concentration of Cd across locations ranged from 0.28 ± 0.23 to 12.74 ± 16.11 $\mu g/l^{-1}$. The highest concentration was recorded at location 1 (inside the inner harbour) while the lowest value was recorded at location 8. A similar study was conducted in the Port Elizabeth harbour in South Africa by Fatoki and Mathabata (2001). In their study, the concentration of Cd ranged from 0.0002 – 0.072 $\mu g/l^{-1}$ which showed that Cape Town is more polluted by Cd than the Port Elizabeth harbour. Moreover, the significant high value recorded for location 1 could be as a result of discharges of sewage, industrial spoils, ship repair and other activities in the harbour (Yilmaz and Sadikolglu; 2011). The major anthropogenic sources of Cd are: atmospheric deposition from smelting and refining of non ferrous metals and domestic waste. Cd is relatively mobile in aquatic systems. Its most frequent chemical species are Cd^{2+}, $Cd(OH)_3$, $Cd(OH)_4$, $CdCO_3$ and various organic and inorganic complexes .

4.8.3: Lead

A significant variation of P (≤ 0.05) was found for lead across all locations. The highest concentration values were found in location 2 (Duncan Dock 2) followed by Duncan dock 3 54.79 ± 1.58 $\mu g/l^{-1}$. The lowest concentration value of 5.18 ± 1.53$\mu g/l^{-1}$ was found in Ben Schoeman dock (Table 4.24 and Figure 4.22). In another related study; it was recorded at concentration range from 0.0006 to 0.0163 $\mu g/l^{-1}$ (Port Elizabeth harbour) (Fatoki and Mathabata, 2001). This shows that our finding is still within the range. Significantly high concentration levels of heavy metals were recorded in most of the locations. This could be the result of discharges from the Diep River. The Diep River is a major freshwater ecosystem in the Western Cape. It passes through various metal pollution sites such as agricultural land, landfill sites, and sewage treatment plants before it joins the harbour (Shuping *et al.,* 2011). Waste water treatment plants could be another source (Mee, 1992; Turkoghu 1992; Surent *et al.,* 2001; Yilmaz and Sadikoghu, 2011). Perez-Lopez *et al.* (2003) in Spain studied Pb contamination in seawater. The concentration values they recorded were from 0.33 ± 0.05 to 2.05 ± 0.20$\mu g/l^{-1}$. Pb and Cd concentration in seawater samples were quite similar to the values recorded in different parts of the world. In Turkey the concentration levels of Pb and Cd were found to be 0.0738 $\mu g/l^{-1}$ and 0.00939 $\mu g/l^{-1}$, respectively.

4.8.4: Mercury

The concentration values recorded for Hg varied significantly across all locations. A very high significant variation was found between the seasons. The mean seasonal value was 16.69 ± 41.11$\mu g/l^{-1}$ for winter, 0.40 ± 0.45 $\mu g/l^{-1}$ (summer) and 1.42 ± 1.22 $\mu g/l^{-1}$ for spring (Table 4.23 and Figure 4.22). The highest mean concentration value was recorded at location 3 (50.99± 74.66 $\mu g/l^{-1}$) followed by location 4 (open sea), 4.99 ± 6.55 $\mu g/l^{-1}$ while the least concentration value was recorded at control site (location 11). The high value recorded in location 3 could be as a result of industrial activities taking place such as combustion of fossil fuels which is the major source of anthropogenic inputs of mercury emissions on a global scale. The high value recorded for Hg during winter would be as a result of rainfall which transports pollutants, by products of waste incineration and smelting of ores to the harbour.

4.8.5: Tin

Concentration of tin varied significantly across all locations P (≤ 0.05). A very high significant value of P (≤ 0.05) was found when the samples from different seasons were compared with each other as shown in Table 4.24 and Figure 4.22. Sn was mainly present in summer and its winter and spring concentration levels were relatively low. The highest concentration was recorded at location 8, followed by location 5. The lowest value of 13.47± 3.44 μgl^{-1} was found in location 1 (Duncan dock). High value recorded at location 8 could be a result of ship repair activities, painting and other industrial activities taking place at Synchrolift. Ships are being painted with antifouling paints (tributyltin), and this might contribute to high concentration values of Sn at this location.

Table 4.25a: Comparison of International Guidelines for metals in seawater

Metals	EU (2001)($\mu g/l^{-1}$)	EPA ($\mu g/l^{-1}$)	WHO 1993($\mu g/l^{-1}$)	TSE 266 (1988) ($\mu g/l^{-1}$)
As	10			
Pb	10	10	10	10
Cd	5	10	10	10
Hg	1			
Sn	-	-	-	-

EU acceptable limits for Hg in seawater are 1.0 $\mu g/l^{-1}$. According to WHO, EPA, TSE 266 guidelines for Pb in seawater, the concentration values recorded in locations 1, 3, 5, 6, 9 and 10 were more than the permissible value (10 µg/l) as shown in Table 4.25a. According to EEC and ANZECC guidelines, the values recorded for Cd across all locations were within the permissible limit for aquatic life. With reference to EU directive for Cd limits in drinking water, Cd concentration levels in all other locations were found within the limit range except in location 1 (12.74 ± 16.11 $\mu g/l^{-1}$) which was more than 5$\mu g/l^{-1}$ above the permissible limit. The seasonal results for metals investigated were also compared with other results obtained in a related study carried out in South Africa and internationally as represented in Table 4.25b.

Table 4.25b: Comparison of data of heavy metals in seawater worldwide in µg/l^{-1}

Locations	Year	Cd	Pb	As	Sn	Hg	References
Cape Town Harbour	2012	0.28 – 12.74	4.80 – 23.57	2.27- 4.30	13.47 – 1363.87	0.50- 50.99	Current study
Port Elizabeth Harbour	2001	0.0002 – 0.072	0.0006 – 0.0163	-	-	-	Fatoki and Mathabata, 2001
Kepez Harbour, Turkey	2011	0.0738	0.00939	-	-	-	Yilmaz and Sadikoglu, 2011
Seawater, Galicia NW Spain	2003	0.01-0.05	0.17-2.05	-	-	-	Lopez et al. 2003

Standard two dimensional PCA for five heavy metals (Cd, Sn, Hg, As and Pb) measured at the 12 locations in Cape Town harbour water (Figure 4.23) are plotted. The distribution of Pb, Sn and Hg components are highly concentrated in Robinson dry dock, Synchrolift, Ben Schoeman dry dock, and the harbour entrance). This indicates that they were from anthropogenic sources. PCA was also used to show seasonal variation in the concentration of heavy metals in all locations as shown in Figure 4.24. Primer- version 6 software was used to plot the cluster analysis graph and PCA (Figure 4.24 and 4.24c). The results obtained with the MSD plot (Figure 4.2b) confirmed that the seasonal effect is greater than the location effects as shown on the cluster analysis graph. The metals that are grouped together on the same axis indicate that these metals have similar effects on the samples.

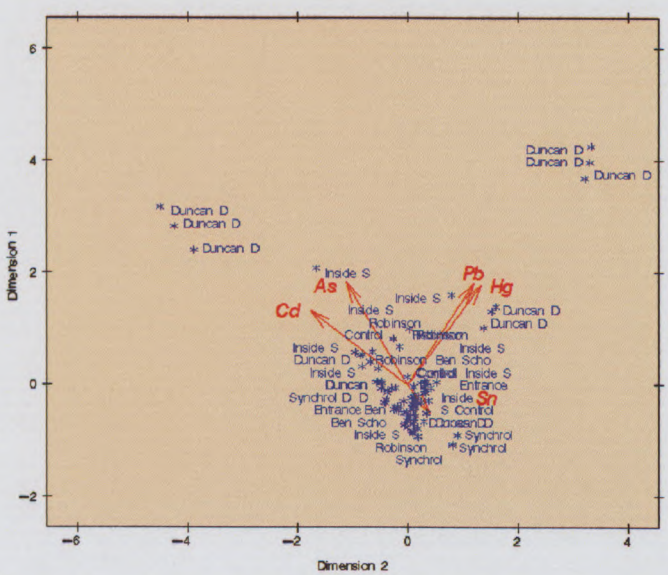


Figure 4.23, PCA showing annual seasonal distribution of heavy metals across locations in Cape Town harbour

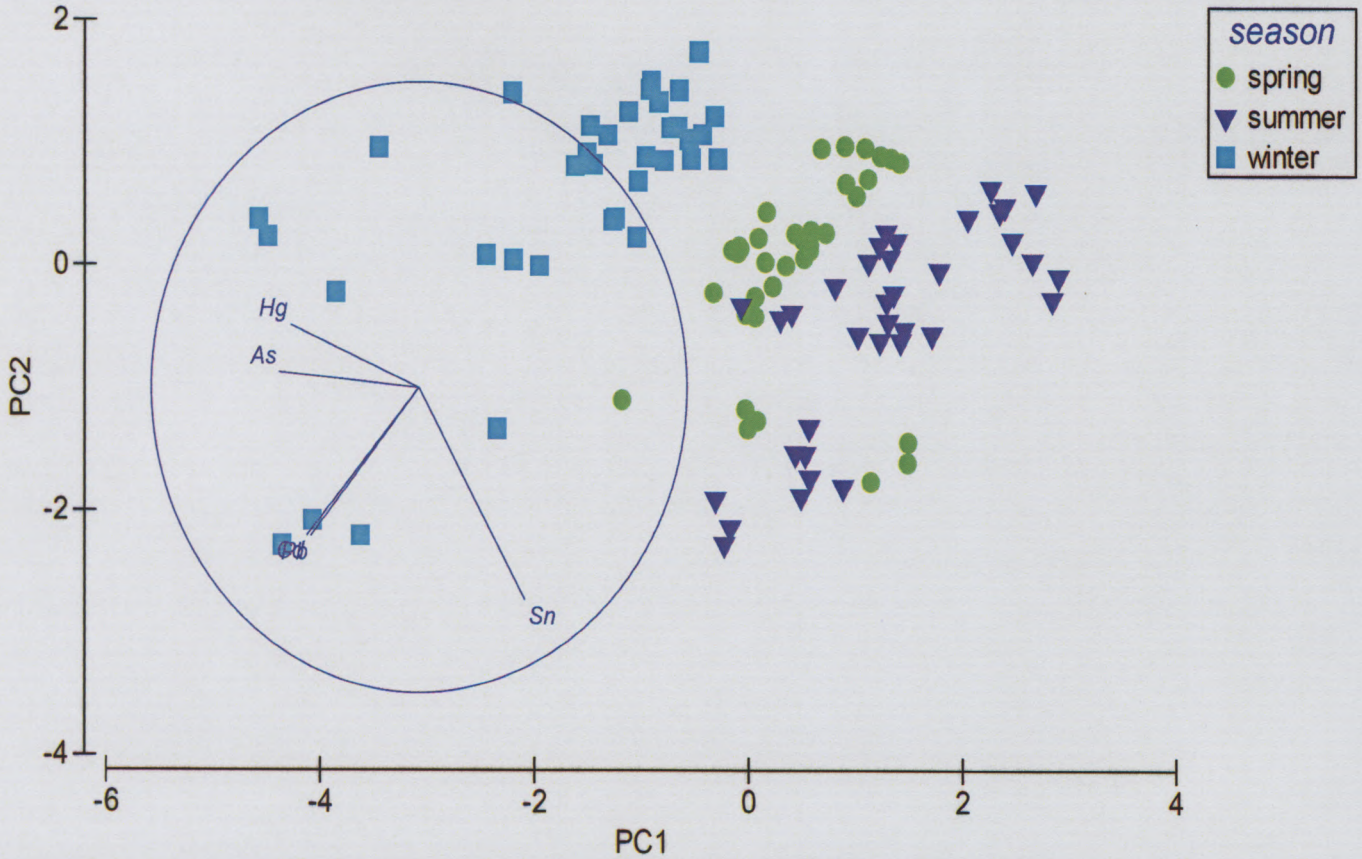
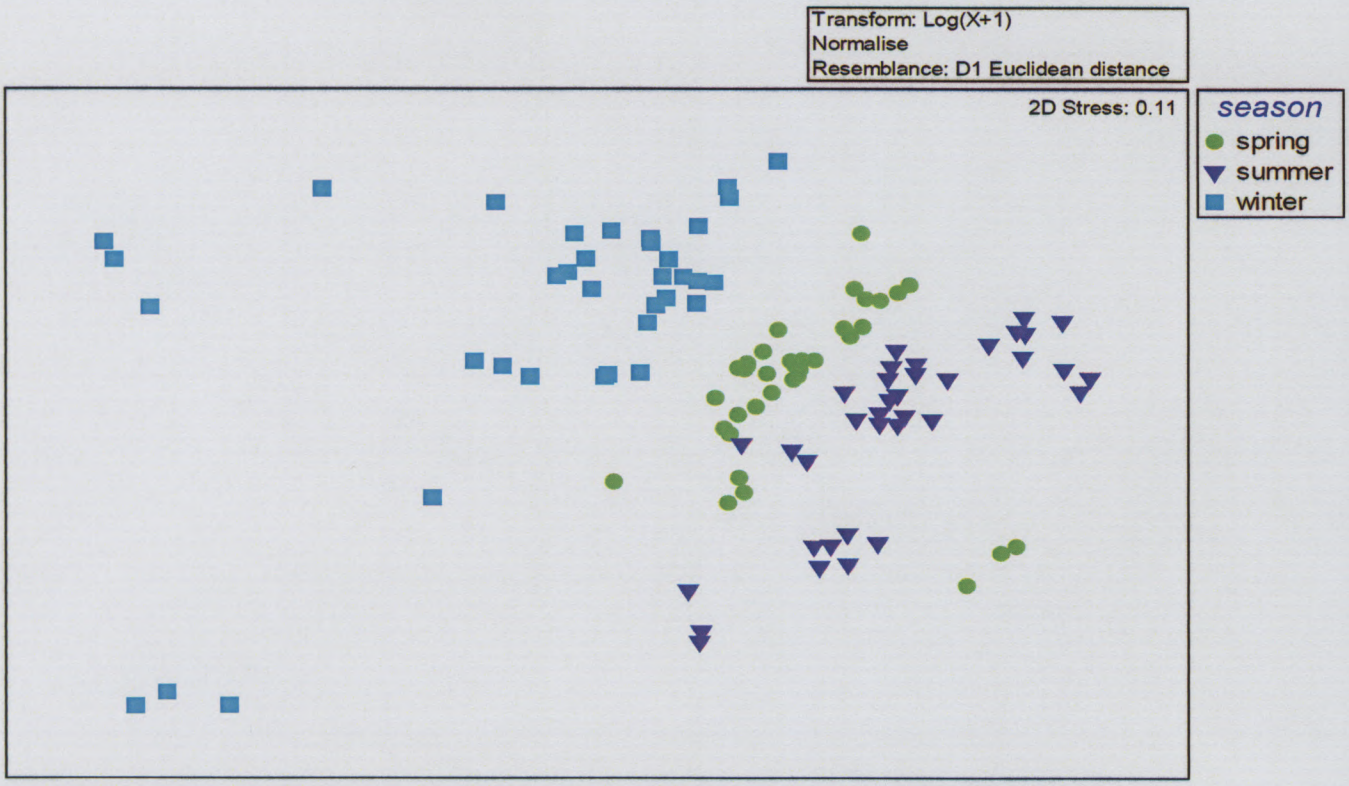


Figure 4.24. PCA showing annual seasonal distribution of heavy metals in Cape Town harbour water



F igure 4.24b. MSD plots showing the disribution of the heavy metals accross seasons.

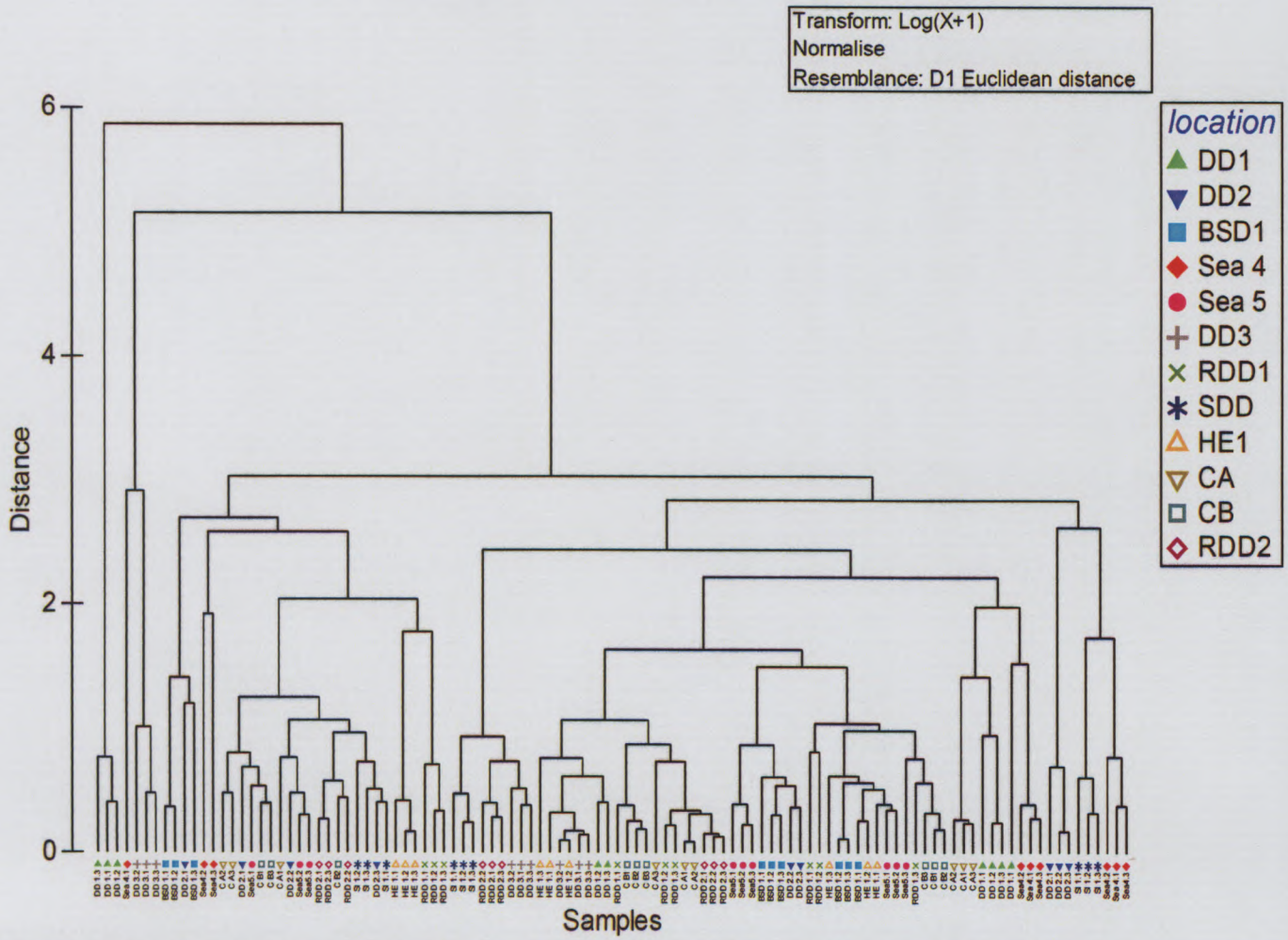


Figure 4.24c: Cluster analysis graph showing heavy metals distribution across locations in Seawater from Cape Town harbour.

4.9: Bioaccumulation of metals in mussel (*Mytilus galloprovincialis*)

Concentrations of elements in mg/kg in tissues and shells of different sizes are shown in Table 4.27 while the concentrations recorded in group D (whole mussels) are shown in Table 4.28. EDXRF analysis showed the presence of K, Ca, Fe, Cu, Zn, Si, Sr, Al and Au in the tissue of the mussel while K, Ca, Fe, Cu, Zn, Si and Sr were found in all sizes of mussel shell. Al was additionally detected in the largest size class. Cu and Zn exhibited the highest concentration in tissue of small size mussel while lowest concentrations values were measured for Cu and Zn in the shells as shown in Figure 4.26. Indexing the concentration of Cu and Zn detected in mussel against the FAO (FAO, 1983) recommended guideline concentrations for mussels, there is an indication that Cu and Zn most probably share the same accumulation process in the mussel tissue (Stanciu *et al.* 2004).

Standard two-dimension PCA for five essential elements plus silicon and strontium (K, Ca, Sr, Si, Fe, Cu and Zn) measured in black mussels (*Mytilus galloprovincialis*) collected from Cape Town Harbour, South Africa were plotted (Figure, 4.26, 4.27). The

distribution of K, Zn, Fe, and Cu components were highly concentrated in the soft tissue of the black mussels while Ca, was found at normal concentrations in the shell PCA was also used to show the distribution of all the essential elements plus silicon and strontium, and trace heavy metals analyzed in different sizes (Figure 4.25). Size has sometimes been shown to be an important variable. In this study, effects of size were investigated on metal contents in the black mussel (*Mytilus galloprovincialis*) (Cevic *et al.,* 2008). Significant variation was obtained at 0.05 levels, the results confirmed that the correlated metals exhibit a common accumulation process in mussel tissues (Saavedra *et al.,* 2004; Cevic *et al.,* 2008). The order of metal accumulation amongst the various sizes of mussels follows the order: C (large size) > B (medium size) > A (small size) as shown in Table 4.27. The results obtained with the PCA are in agreement with Pearson correlation analysis. The percentage distributions of essential elements plus silicon and strontium, and trace heavy metals are presented in Figure 4.28 and 4.29. The percentage accumulation of Ca, Si, and Al in the shell is 49%, 16%, and 5%, respectively. The data confirms that Ca is mainly found in the shell as $CaCO_3$ than in the tissue (Cevic *et al.*, 2008). Ca was found most abundantly in the shell while in the tissue K is the most abundant with a percentage of 23% while Fe, Cu and Zn recorded 4%, 2% and 1% respectively.

The high concentration of Fe recorded in *M. galloprovincialis* indicates a high impact of terrigenic particles, which are generally rich in iron. Similar results were found by Ugur *et al.* (2002) for *M. galloprovincialis* collected from Aegean coast of Turkey. High concentration levels can be attributed to the fact that iron occurs naturally in the environment and may come from different backgrounds. These results agreed with results obtained with the PCA. The PCA information further allows the classification of the metals into two main groups and these are the Sn-Cd-As- Hg-Pb, and Mn-V-Cr-Co-Ni-Pb groups. While Sn, Cd, As, Hg and Pb appeared to bioaccumulate more in the tissues, V, Cr, Mn, Co, Ni, and Pb are predominantly found in the shell (Figure 4.27). This is in agreement with the results of Saavedra *et al., (*2004). From the obtained data using ICPMS, the average metal concentrations found in the mussels are as follows: Pb (7.30 ± 0.67), Cd (1.98 ± 0.13), Hg (4.92 ± 0.60), As (6.94 ± 0.04), Sn (2.63 ± 0.13), Ni (1.88 ± 0.05), Cr (3.54 ± 0.05), V (4.17± 0.23), Co (0.74±0.01) and Mn (35.20 ± 1.46) ppm.

The order of the metal concentrations is Mn > Pb> As> Hg> V> Cr> Sn > Cd> Ni > Co. Heavy metal concentration levels in mussels collected from the Cape Town harbour are much higher than those collected the Black Sea. Mn and Cd values were the same between the two areas. Besides, significant correlation was observed between Zn and Cu, this finding is in agreement with Cevic *et al.* (2008). Metal accumulation in the gills and visceral mass of *M. galloprovincialis* is of ecotoxicological interest. The distribution of metals among the

various organs seems to depend on the species and on the metals. Serra *et al.* (1999) observed that cadmium preferentially accumulates in the gills of *M. galloprovincialis*. In this study Cu and Cd concentration levels are higher in mussel tissues than in their shells and these results are in agreement with those obtained by Stanciu *et al.* (2004). The elevated concentration levels of Cu, Cd and Zn may be attributed to the traffic of tanker and large freight vessels docking in the Cape Town harbour. These ship vessels may bring metallic pollutants to the harbour environment which may become bioavailable to the mussels. These results are in agreement with those obtained by Romeo *et al* (2005).

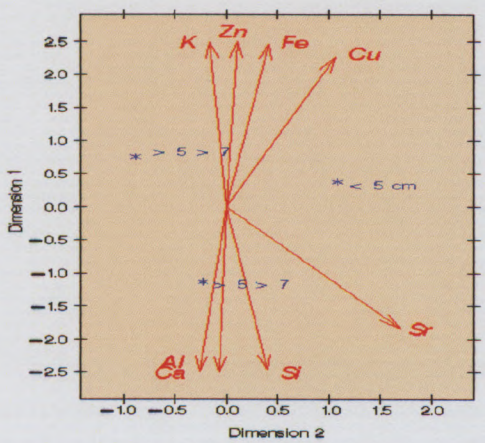


Figure 4.25: Bioaccumulation of non- toxic elements in different sizes of *M galloprovincialis* collected from Cape Town harbour

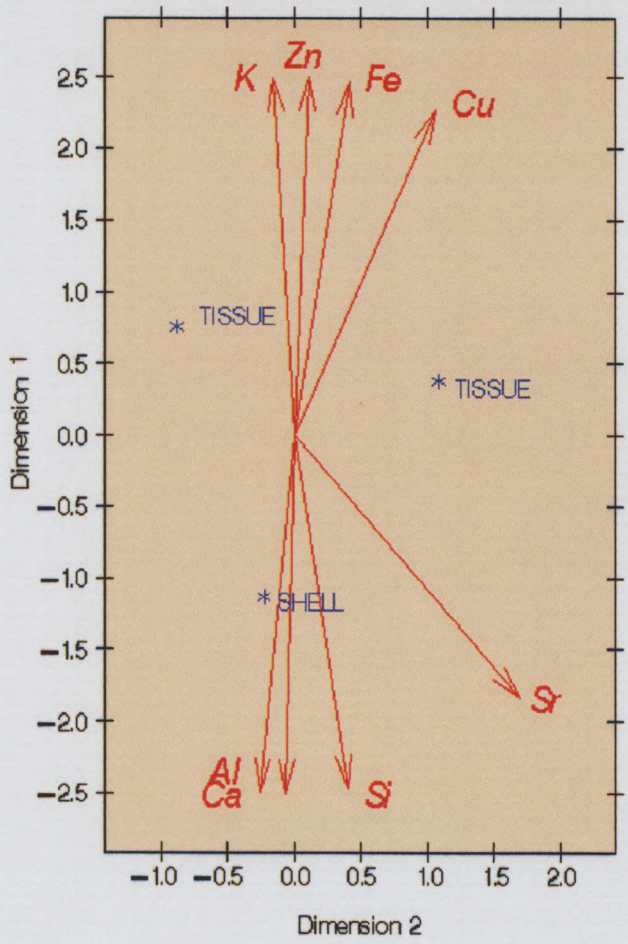


Figure 4.26: Bioaccumulation of non- toxic elements in shell and tissues of *M galloprovincialis* collected from Cape Town harbour

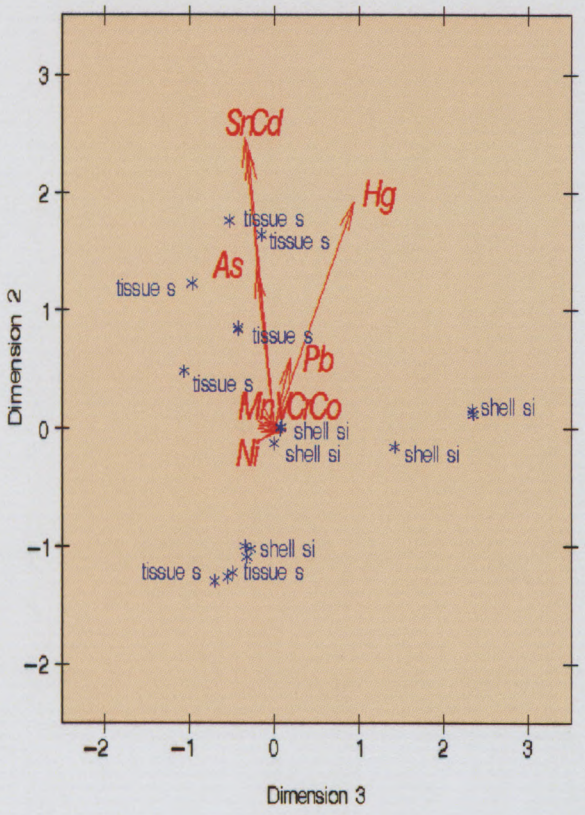


Figure 4.27: Bioaccumulation of toxic elements in tissue and shell of *M galloprovincialis* collected from Cape Town harbour

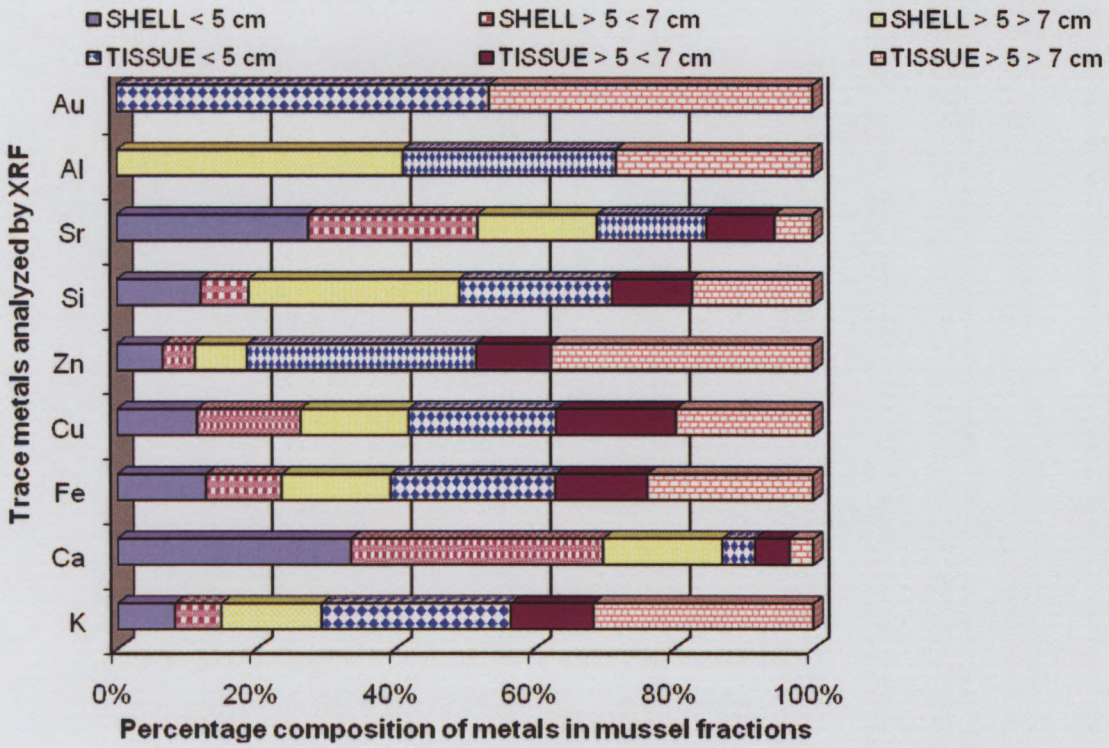


Figure 4.28: Percentage of non- toxic elements in shell and tissue of *M galloprovincialis* by EDXRF

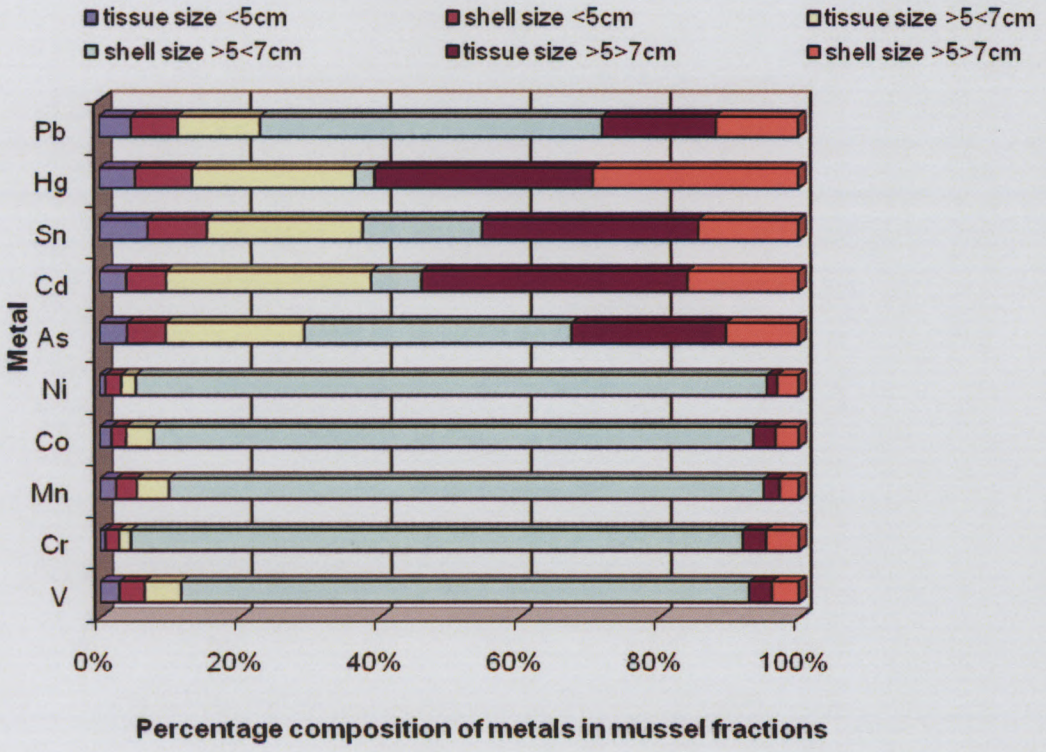


Figure 4.29: Percentage of toxic elements in shell and tissue of mussel by ICP-MS.

Table 4.27: Concentration of toxic and non-toxic elements found in mussel samples (*M galloprovincialis)* collected from Cape Town harbour in mg/kg

	Samples	Group A		Group B		Group C	
EDXRF	Elements	Tissue	Shell	Tissue	Shell	Tissue	Shell
	K	9898.52±216.05	2441.03±131.46	11530.65±247.83	2997.9±133.35	4327.46±178.87	5263.34±165.93
	Ca	34704.51±442.98	267036.59±2160.3	24840.04±361.69	247661.98±2056.23	36971.67±485.6	126410.04±1223.78
	Fe	2047.84±133.27	943.06±108.46	2067.39±137.21	1106.8±116.05	1149.78±150.61	1358.64±134.68
	Cu	913.77±36.25	643.78±31.5	851.58±36.12	496.84±28.73	743.58±34.42	668.8±32.28
	Zn	620.28±23.39	87.52±11.13	710.3±25.66	124.39±12.31	204.02±15.23	141.71±13.17
	Si	10531.84±501.21	3309.28±378.51	8343.73±471.28	5804.67±396.36	5552.45±373.43	14539.49±490.88
	Sr	334.64±6.57	516.67±8.98	116.36±3.6	585.83±9.96	208.9±5.06	364.49±7.11
	Al	2769.86±995.95	<DL	2562.63±969.62	<DL	<DL	3728.72±964.01
	Au	26.47±8.89	<DL	23.01±8.96	<D	<DL	<DL
ICP-MS	Pb	2.4423±0.2367	3.5714±0.0466	6.2797±0.6963	26.0436±3.0231	8.6612±0.9445	6.2664±0.6479
	Cd	0.2737±0.0165	0.4055±0.0169	2.0838±0.1290	0.5117±0.0248	2.7017±0.2489	1.1275±0.0816
	Hg	0.4866±0.0769	0.7748±0.0453	2.2117±0.3465	0.2639±0.0321	2.9563±0.3971	2.7856±0.3859
	As	1.6337±0.0306	2.3136±0.0493	8.2736±0.0710	15.8943±0.2643	9.1893±0.1319	4.3310±0.0550
	Sn	0.7806±0.0325	0.9663±0.0816	2.5191± 0.1951	1.9254±0.1022	3.5010±0.2513	1.6183±0.1131
	Ni	0.9744±0.0141	2.6779±0.07575	2.4602±0.0829	105.9829±2.3046	1.7756± 0.0167	3.5615±0.0395
	Cr	1.3511±0.0750	3.5567±0.0217	3.0176±0.0826	157.4157±5.5977	5.9805±0.2073	8.3655±0.07048
	V	2.8076±0.19847	3.6946±0.05080	5.3466±0.3430	82.9255±6.24058	3.32278±0.29208	3.92638±0.2023
	Co	0.3699±0.0060	0.4618±0.0114	0.8655±0.0148	18.8634±0.3534	0.7010±0.0208	0.7215±0.0091
	Mn	23.4963±1.3876	30.2436±0.3734	46.9127±2.2171	867.581±85.2730	23.2456±1.5058	27.7060±1.3050

The results are reported as average of three subsamples (in a sample of 12 mussels) with the relative standard error, DL, detection limit

Group A: < 5 cm (small size): Group B: > 5 < 7 cm (medium size); Group C: > 5 > 7 cm (big size).

Table 4.28: Concentration of Metals found in whole mussel samples (group D) in ppm

EDXRF	Non Toxic Elements	Concentration (ppm)	ICP-MS	Toxic elements	Concentration (ppm)
	K	2417.97±133.6		Pb	7.30476± 0.67543
	Ca	217314.781±1837.48		Cd	1.98878±0.13866
	Fe	1377.49±139.14		Hg	4.92801±0.60223
	Cu	616.76± 31		As	6.94597±0.041492
	Zn	153.32±13.22		Sn	2.63842±0.13684
	Si	10211.85± 439.67		Ni	1.88415±0.059810
	Sr	397.6±7.51		Cr	3.54399±0.058432
	Al	2625.87±996.41		V	4.17057±0.23966
	Au	ND		Co	0.74681±0.014233
	Cl	30629.61±274.77		Mn	35.2071±1.46026
	Mo	27.31±2.31			

Table 4.29: Pearson correlation Coefficients between metals levels in mussels by EDXRF

	K	Ca	Fe	Cu	Zn	Si	Sr	Al
K	1.00000	-0.78490 0.0644	0.98822 0.0002	0.85429 0.0303	0.97813 0.0007	0.45031 0.3702	-0.72618 0.1022	0.99627 0.0550
Ca		1.00000	-0.74836 0.0870	-0.85214 0.0312	-0.74367 0.0901	-0.43464 0.3891	0.91397 0.0108	0.99687 0.0504
Fe			1.00000	00.0385 0.0016	0.96754 0.3116	0.50077 0.1762	-0.63424 0.0911.	0.98978 0.83510
Cu				1.00000	0.85330 0.0307	0.32434 0.5306	-0.76896 0.0739	0.91551 0.2636
Zn					1.00000	0.27444 0.5987	-0.68019 0.1371	0.99981 0.0124
Si						1.00000	-0.27162 0.6026	0.98232 0.1199
Sr							1.00000	0.71828 0.4899
Al								1.00000

KEY: Upper value=correlation coefficient r

Lower value=Significance level P≤ 0.05; <.0001

Table 4.30: Pearson correlation Coefficients between metals levels in mussels by ICP-MS

	V	Cr	Mn	Co	Ni	As	Cd	Sn	Hg	Pb
V	1.00000	0.99823	0.99975	0.99810	0.99689	0.82534	-0.31742	0.02437	-0.51460	0.94862
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1993	0.9235	0.0289	<.0001
Cr		1.00000	0.99700	0.99910	0.99877	0.82557	-0.31353	0.03285	-0.49630	0.95931
			<.0001	<.0001	<.0001	<.0001	0.2052	0.8971	0.0362	<.0001
Mn			1.00000	0.99656	0.99504	0.82208	-0.32072	0.01978	-0.51917	0.94312
				<.0001	<.0001	<.0001	0.1944	0.9379	0.0273	0.94312
Co				1.00000	0.99961	0.83071	-0.30903	0.03666	-0.50546	0.96137
					<.0001	<.0001	0.2121	0.8852	0.0324	<.0001
Ni					1.00000	0.81971	-0.32613	0.01970	-0.51418	0.96008
						<.0001	0.1866	0.9381	0.0290	<.0001
As						1.00000	0.26667	0.57099	-0.04395	0.91443
							0.2848	0.0133	0.8625	<.0001
Cd							1.00000	0.93194	0.83928	-0.08553
								<.0001	<.0001	0.7358
Sn								1.00000	0.68644	0.26428
									0.0017	0.2893
Hg									1.00000	-0.29024
										0.2427
Pb										1.00000

KEY: Upper value=correlation coefficient r

Lower value=Significance level P≤ 0.05; <.0001

Pearson correlation coefficients for metals in black mussels (*Mytilus galloprovincialis*) collected from Cape Town harbour are presented in Tables 4.29 & 4.30. Strong correlation (r > 0.8) was calculated between (Cu and K) and (between Cu and Zn). High correlations (r > 0.9) were also calculated between (Zn and K), (between Fe and K), (between Ca and Sr) (between Ca and Al) and (between Zn and Al) which indicate strong association between these metals. Al, Sr, K, Cu, Ca, Fe and Zn would most likely be of natural origin. High correlations (r > 0.9) were also calculated between (V and Cr, Mn, Ni, As, Pb) between (Cr and Mn, Co, Ni, Pb), between (Mn and Co, Ni, Pb), (between Co and Ni, Pb) and between (Pb and Ni, As) and also strong correlation coefficients (r > 0.8) were calculated between (As and V, Cr, Mn, Co, Ni) depicting strong association and hence would most probably be of anthropogenic origin. These findings are in agreement with the results obtained from the PCA. These results suggest that the correlated metals share a common accumulation process in tissue and shell of mussels. Tables 4.32 and 4.33 show the metal concentrations in water and sediment samples from the same locations where mussels were collected. It is apparent from the table that the Sn concentration in sediment samples is quite high.

Table 4.31: Comparison of metal concentrations (mg/kg) in mussels with other Guidelines

Heavy Metal	Cd	Cr	Cu	Fe	Ni	Zn	Pb	References
Current study	1.98±0.13	3.54±0.05	616.76±0.31	1377.49±139.14	1.88±0.05		7.30±0.67	
UNEP	0.3	-	-	-	-		0.3	(UNEP, (1985))
IAEA-407	0.18	0.73	3.28	146	0.60		0.12	(Azemard, (2003))
TFC	0.05	-	20	-	-		0.3	(TFC,23,September 2002,)
Directives 2005 /78/EC	0.05	-	-	-	-		0.2	(Commission)
FAO	0.01	0.005	0.05-0.15			0.2-0.5	0.005-0.03	(FAO, 1983)

Table 4.32: Metal concentrations (ppm) in sea water collected from the sampling site

Heavy metals	Concn. In ppm
Cd	0.31263±0.02341
As	2.13804±0.04466
Pb	9.8034±0.18950
Hg	5.7410±0.37807
Sn	9.23±0.134
Cu	18.144±0.19193
Zn	311.253±7.566
Al	303.645±6.0634
Si	2000.53±19.631
Fe	625.135±4.5264

Table 4.33: Metal concentrations (mg/kg) in sediment collected from the sampling site

Heavy metals	Concn. In mg/kg
Cd	0.696±0.047
Pb	158.519±12.715
Hg	0.650±0.096
Sn	34.336±2.707
Cu	1.511±0.112
Zn	0.854±0.04
Al	17.625±0.970
Si	17.625±0.970
Fe	28.753±1.850

The average metal concentration levels in mussels were compared with the national and international standards for metals in molluscs compiled by UNEP, IAEA, TFC, EC Directives and FAO as shown in Table 4.24. The mean metal concentration values obtained were higher than those recorded in the literature. Cu, Fe and Zn concentration values found in mussel samples are far higher than the permissible limits allowed in national and international guidelines. The abnormally high values for the three metals could be due to contamination from industrial and domestic sources. In Western Cape, the Diep River is one of the major rivers. This river passes various pollution sources like agricultural lands, industrial areas and landfill site. Moreover, a wastewater treatment plant also discharges in the river in the lower reaches. This river eventually joins Table Bayin which the harbour is situated. Nevertheless, it is necessary to step-up the regular monitoring and adequate control measures in order to ensure compliance with national and international regulations on the protection of the marine water system. Moreover harbour activities such as stripping and ships painting could also contribute to metal pollution of the harbour.

4.10: Assessment of physico–chemical parameters of seawater and their annual distribution patterns

Sea water quality depends on the anthropogenic discharges as well as the natural physico–chemical features of the environment (Efe *et al.*, 2005). Cape Town harbour was chosen for this study because it is the busiest harbour use for shipping activities in Africa and also has high commercial activity. The results of seasonal variation of some physico – chemical parameters of water samples from Cape Town harbour have been summarised in table below 4.34, 4.35 and 4.36, respectively.

Table 4.34: Seasonal and annual mean (±SD) concentration of pH in seawater of Cape Town harbour

Item		Seasons			
	Locations	Spring	Summer	Winter	Mean location
pH	Duncan Dock 1	8.40±0.01	7.8±0.14	7.71±0.01	7.96±0.34
	Duncan Dock 2	8.10±0.01	7.75±0.35	7.88±0.19	7.91±0.24
	Benshoeman Dock	8.30±0.01	7.80±0.01	7.64±0.10	7.91±0.30
	Inside Sea 1	8.40±0.01	7.00±0.01	7.94±0.01	7.78±0.63
	Inside Sea 2	8.00±0.01	7.10±0.10	8.02±0.01	7.70±0.47
	Duncan Dock 3	8.20±0.01	7.50±0.10	6.84±0.28	7.51±0.61
	Robinson Dry Dock 1	8.20±0.01	7.9±0.42	7.87±0.02	7.90±0.25
	Synchrolift	8.20±0.01	8.1±0.01	7.82±0.03	8.03±0.21
	Entrance to Harbour	8.30±0.00	8.55±0.35	7.98±0.02	8.27±0.29
	Control A	8.40±0.01	8.15±0.35	8.05±0.01	8.20±0.22
	Control B	8.15±0.02	0.31± 0.10	8.96±0.17	7.81±0.24
	Robinson Dry Dock 2	8.20±0.01	8.00±0.01	7.72±0.01	7.97±0.21
	Mean seasons	8.19±0.22	7.79±0.46	7.79±0.33	
	CV5	2.007%			
	P≤0.05			****	

4.10.2: Effects of pH

The annual mean pH data of seawater ranges between 7.51 to 8.27. The results indicate that the seawater is alkaline. No significant variation was observed for winter and summer. High values were recorded during spring (Table 4.27, Figure 4.30). The target water Quality Range (TWQR) for pH in seawater varied from 6–9. This study reveals that maximum and minimum pH values lie in the range 6-9, which indicates that water from Cape Town harbour is of high quality pH of a water body is an important parameter since it can affect the toxicity of metals as well as their solubility (Fatoki *et al.*, 2001).

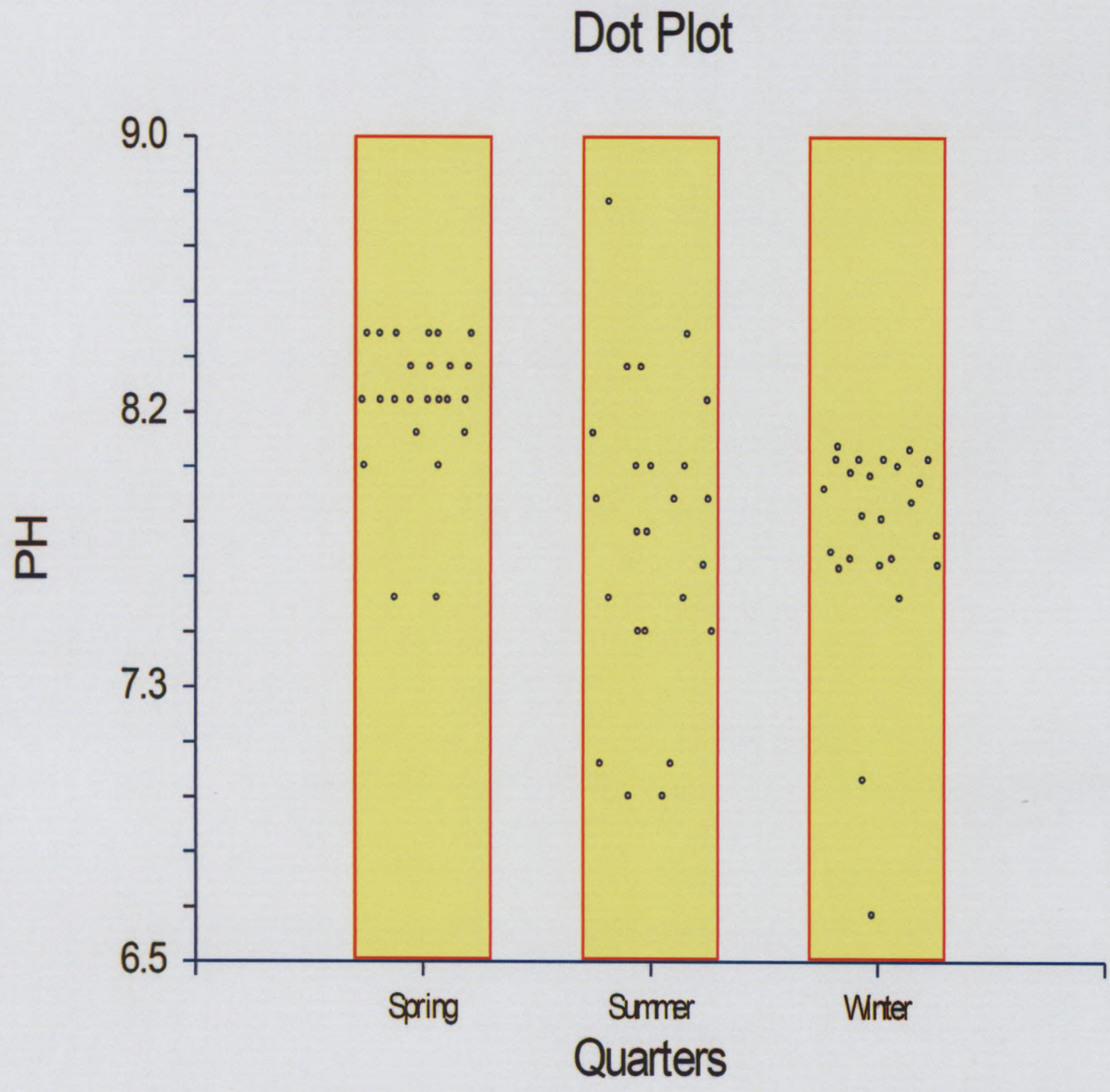


Figure 4.30: Seasonal variation of pH in seawater from Cape Town harbour

4.10.3: Effects of TDS

The Total Dissolved Solids (TDS) concentration in a body of water is affected by many different factors. High concentration of dissolved ions is not an indication that seawater is contaminated or unhealthy. Certainly, it is normal for flowing seawater to dissolve and accumulate fairly high concentrations of ions from the minerals in the rocks and soils over which they flow (Altman and Parizek, 1995). The TDS distribution varies with location. The concentration ranges between 12.91 to 25.34 ppt. High concentration values were recorded at location 4. Anthropogenic sources of ions may be cause of elevated TDS at this location. .

Seasonal variations of TDS were also investigated. Significant different of p (≤0.05) was observed between seasons. The annual mean values recorded for spring, summer and winter are; 21.3, 1.51, and 22.27 ppt respectively as shown in Table 4.28 and Figure 4.31. Highest mean concentration value was observed during winter periods. The order of increase in TDS concentrations is winter > spring > summer. Organic matter from wastewater treatment plants may also contribute higher levels of nitrate or phosphate ions (Adekunle, 2009; Shuping *et al.*, 2011). Amongst other factors is irrigation water that enters the sea from the river mouth, which often has higher concentration of sodium or chloride ion. Dissolved gases like CO_2, NO_2 in acidic rainwater could also yield elevated H^+ ions concentration. High levels of TDS affects aquatic life and other dissolved ions affect the pH of a body of water which in turn may influence the health of aquatic species (Rao and Mamatha, 2004; Adekunle, 2009).

Table 4.35: Seasonal and annual mean (±SD) concentration of TDS in seawater of Cape Town harbour

		Seasons			
		Spring	Summer	Winter	Mean locations
TDS (ppt)	Duncan Dock 1	24.03±0.09	2.35±0.01	19.30±0.05	15.23±10.20
	Duncan Dock 2	24.60±0.04	2.34±0.01	19.55± 0.01	15.5±10.44
	Benshoeman Dock	24.51±0.53	2.32±0.01	24.02±0.19	16.95±11.33
	Inside Sea 1	24.40±0.36	3.41±1.51	48.22±0.82	25.34±20.07
	Inside Sea 2	24.75±0.83	2.31±0.04	19.43±0.01	15.50±10.49
	Duncan Dock 3	19.03±0.09	1.24±1.52	19.26±0.02	13.18±9.27
	Robinson Dry-dock 1	19.07±0.08	2.29±0.02	19.31±0.01	13.56±8.73
	Synchrolift	19.01±0.05	0.19±0.06	19.38±0.12	12.86±9.81
	Entrance to Harbour	19.14±0.07	1.25±1.53	19.52±0.11	13.31±9.36
	Control A	19.12±0.02	0.05±0.01	19.43±0.01	12.9±9.88
	Control B	19.09±0.01	0.06±0.01	19.48±0.06	12.911±9.88
	Robinson Dry Dock 2	19.02±0.01	0.15±0.01	20.37±1.45	13.24±10.17
	Mean seasons	21.33±2.72	1.51±1.24	22.27±8.10	
	CV	3.67%			
	P≤0.05	***			***
	Interaction P≤0.05	***			

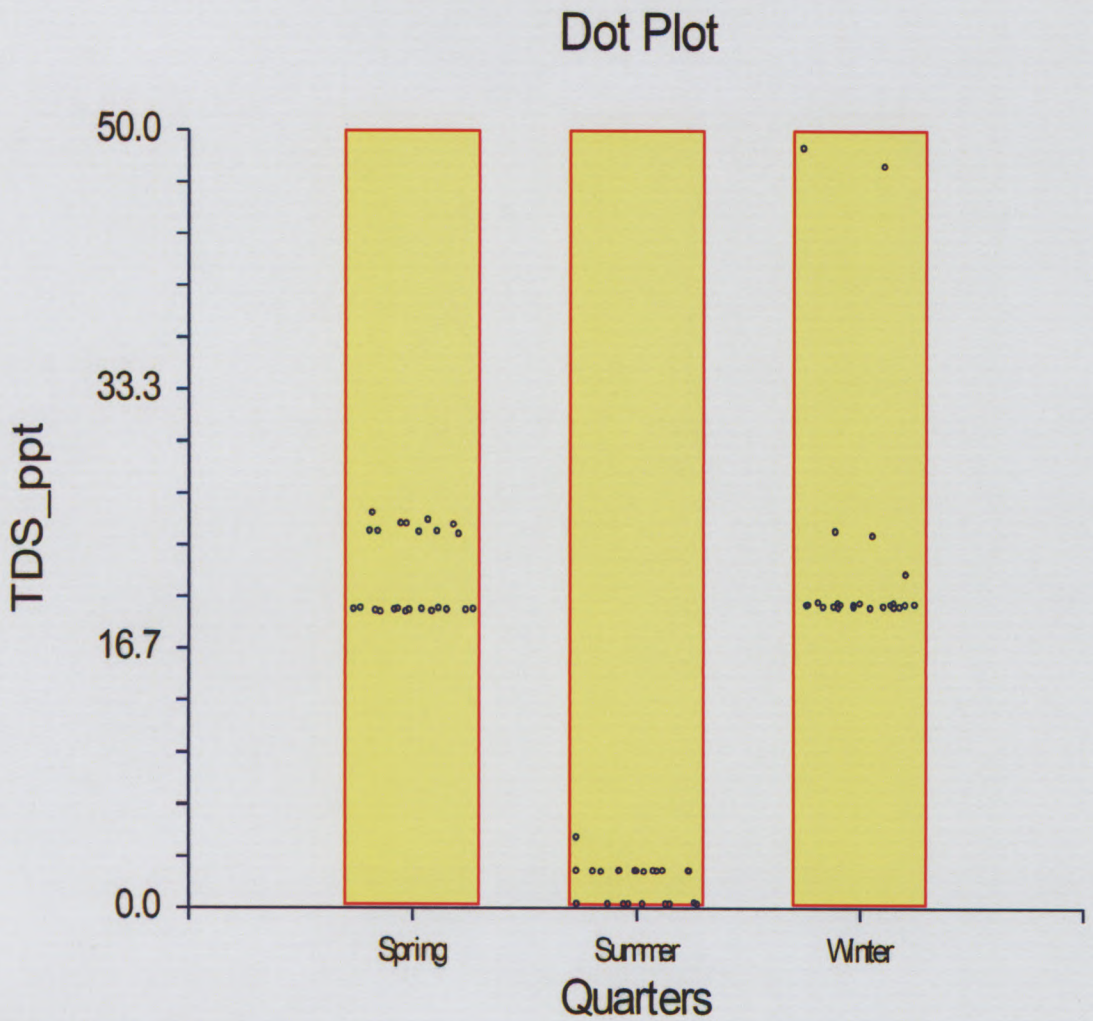


Figure 4.31: Seasonal variation of TDS in seawater from Cape Town harbour

4.10.4: Conductivity

Electrical conductance of water in a measure of its ability to carry electric current as a result of dissolved salts in the water. The annual mean electrical conductivity recorded in this study ranges between 7.97–33.54 µg/l as shown in Table 4.35 and Figure 4.32. A higher conductivity value of 33.54 µg/l was recorded at location 3. This could be the result from industrial activities like ship repair, blasting, pollutants and seawater influx at high tides etc. taking place at this location (Grindley and Dudley, 1988; Ren *et al.*, 2006). Low conductivity values recorded at location 12 (Robinson dry-dock 2) may presumably be due to dilution effect of the rainwater on the sea during the rainy season. High significant variation $p(\leq 0.05)$ was observed between seasons. The order of decreasing conductivities is spring > winter > summer.

Table 4.36: Seasonal and annual mean concentration of conductivity and temperature in seawater of Cape Town harbour

		Seasons			
	Locations	**Spring**	**Summer**	**Winter**	**Mean location**
Conductivity (μcm^{-1})	Duncan Dock 1	47.99±0.21	4.80±0.01	38.55±0.14	30.44±20.30
	Duncan Dock 2	49.00±0.26	4.69±0.01	39.13±0.01	31.02±20.89
	Benshoeman Dock	49.06±1.11	4.64±0.04	46.91±0.33	33.54±22.41
	Inside Sea 1	48.71±0.60	3.84±1.73	47.77±0.01	33.32±23.12
	Inside Sea 2	49.67±1.35	4.60±0.09	32.34±8.54	28.87±20.69
	Duncan Dock 3	38.01±0.08	2.46±3.01	38.15±0.70	26.21±18.44
	Robinson Dry Dock	38.16±0.12	4.58±0.03	38.64±0.02	27.13±17.46
	Synchrolift	38.04±0.09	0.31±0.01	39.01±0.01	25.79±19.73
	Entrance to Harbour	33.27±7.02	2.49±3.08	38.90±0.01	24.88±17.86
	Control A	38.23±0.02	0.30±0.01	38.99±0.17	25.84±19.78
	Control B	38.15±0.02	0.31±0.01	38.96±0.16	13.16±1.96
	Robinson Dry Dock 2	38.41±0.01	0.29±0.01	48.12±0.91	7.97±0.21
	Mean seasons	42.25±6.13	2.75±2.16	40.46±4.93	
	CV5	7.142%			
	P≤0.05	***			
	Interaction P≤0.05	***			***
Temp(°C)	Duncan Dock 1	13±0.01	18±0.01	12.50±0.71	14.5±2.70
	Duncan Dock 2	12±0.01	16.50±0.71	8.50±0.71	12.33±3.61
	Benshoeman Dock	12±0.01	14±0.01	9.5±0.71	11.83±2.04
	Inside Sea 1	14±0.01	11.5±0.71	10.50±0.71	12±1.17
	Inside Sea 2	13±0.01	12±0.01	10.50±0.71	11.83±1.17
	Duncan Dock 3	13±0.01	14±0.01	10.5±0.71	12.5±1.64
	Robinson Dry Dock 1	16.5±0.71	9.50±0.71	9.5±0.71	13±3.16
	Synchrolift	13±0.01	16±0.01	9.5±0.71	12.83±2.93
	Entrance to Harbour	12±0.01	16.5±0.71	10.50±0.71	13±2.83
	Control A	13±0.01	15±0.01	11.50±0.71	13.16±1.60
	Control B	14±0.01	15±0.01	11±1.41	13.33±1.97
	Robinson Dry Dock 2	13±0.01	14±0.01	10.5±0.71	12.5±1.64
	Mean seasons	12.91±0.65	14.92±1.91	10.38±1.17	
	CV5	4.033%			
	P≤0.05	***			***
	Interaction P≤0.05	***			

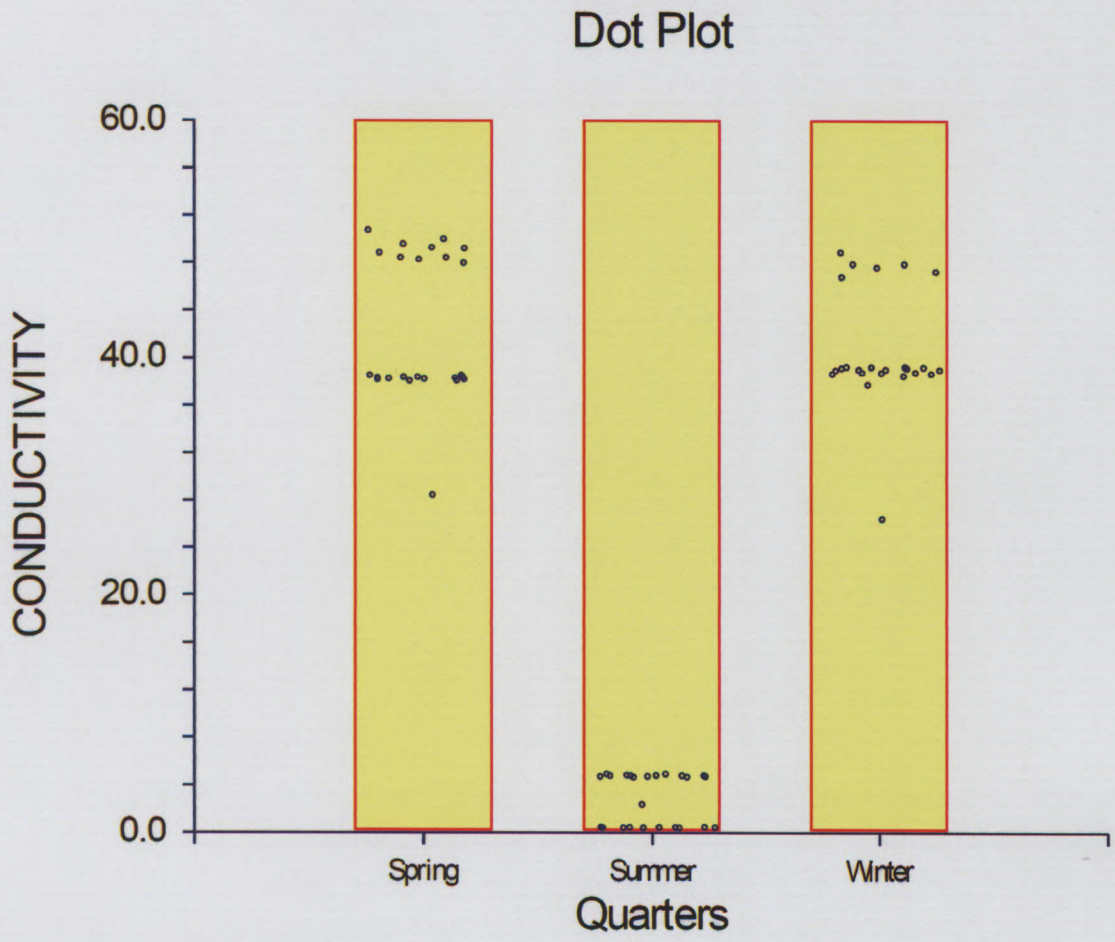


Figure 4.32: Seasonal variation of conductivity in seawater from Cape Town harbour

4.10.5 Effects of Temperature

Annual temperature distribution is represented on dot plot shown in the Figure 4.35. Water temperature during the study period ranged between 11.83 to 14.50°C. High significant variation of ($p \leq 0.05$) was observed for the seasons. Highest mean value of (14.92°C) was recorded during summer and the least value (10.38°C) in winter (Table 4.33). High values recorded in summer can be associated to climatic conditions of the Western Cape which are characterized generally by the higher temperature during the summer seasons. According to South African water quality guidelines for aquatic ecosystems, large, rapid shifts in temperature are lethal to aquatic organisms (DWAF, 1996). Other suspected factors for the high temperatures recorded in summer are heat transfer between the seawater and the land surface due to the release of industrial effluents into the sea (Jackson *et al.*, 2009).

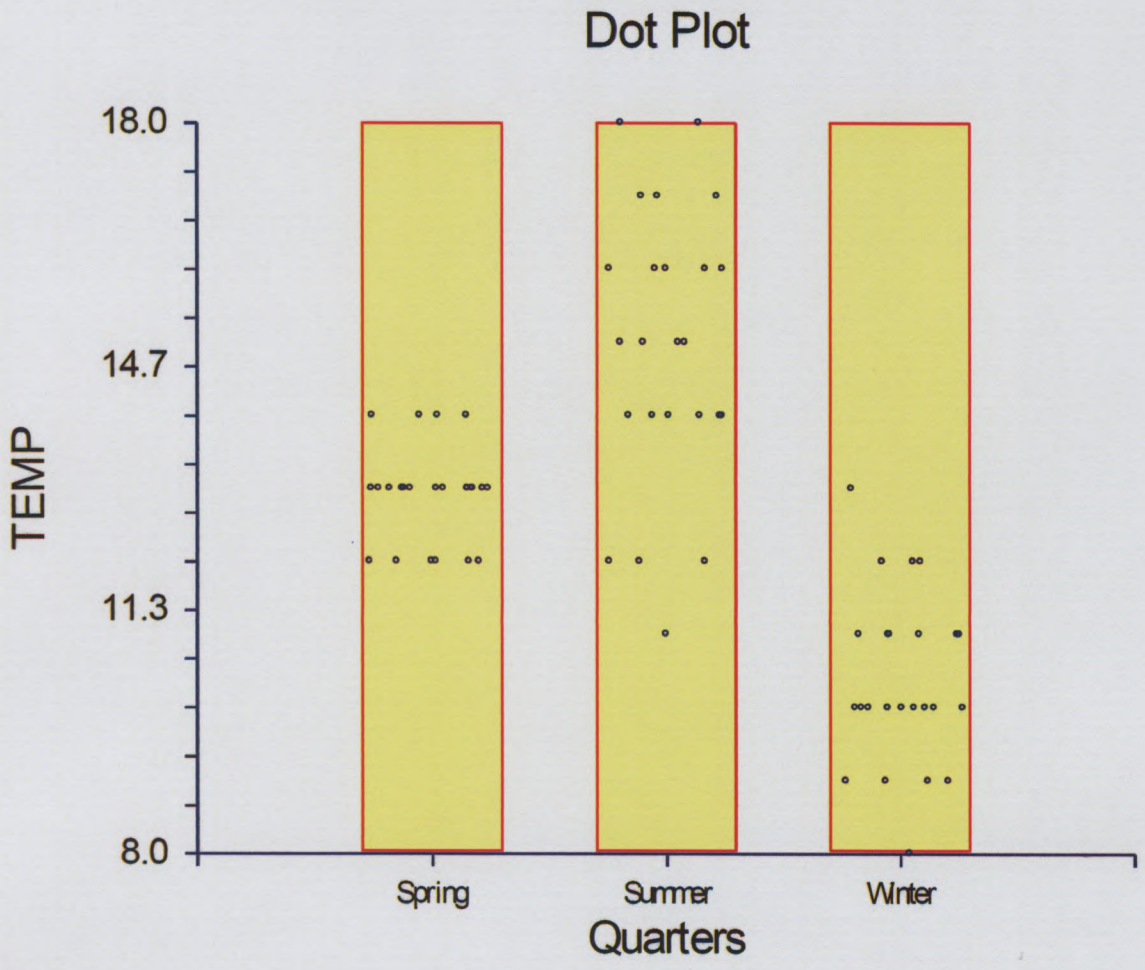


Figure 4.33: Seasonal variation of temperature in seawater from Cape Town harbour

4.10.6: Total Organic Content

The annual mean concentration recorded during the study period ranged between 1.398% to 15.135% as depicted in Table 4.37. The highest value was recorded in location 2 (Duncan dock). This high result suggests that intensive ship repair, painting and other boating activities taking place at this location might be the source of elevated values of TOC at this location (Fatoki *et al.,* 2012). A significant difference of $p \leq 0.05$ was observed between the seasons. High TOC values were recorded during summer. This might be due to stable or steady flow of water during summer and in which most of the organic matter is leached into the sediments.

Table 4.37: Seasonal and annual mean concentration of total organic content in sediment

		Seasons			
	Locations	Spring	Summer	Winter	Mean location
TOC	Duncan Dock 1	3.57±0.98	19.3±24.06	1.0335±0.002	11.435±8.305
	Duncan Dock 2	3.45±1.01	26.82±30.25	1.031±0.0014	15.135±11.038
	Benshoeman Dock	1.47±0.22	26.47±32.98	1.032±0.001	13.97±11.83
	Inside Sea 1	1.04±0.22	25.32±17.96	1.031±0.002	13.177±8.718
	Inside Sea 2	0.17±0.125	4.57±4.43	1.0305±0.001	2.845±1.624
	Duncan Dock 3	7.62±5.47	18.165±0.10.71	1.0305±0.001	12.893±4.618
	Robinson Dry Dock	5.93±1.52	11.115±5.35	1.032±0.001	8.525±2.194
	Synchrolift	3.95±0.04	1.105±0.014	1.031±0.00	2.527±0.822
	Entrance to Harbour	0.49±0.014	2.305±1.265	1.034±0.002	1.398±0.638
	Control A	0.365±0.07	11.56±0.00	1.031±0.00	5.96±3.2318
	Control B	0.725±0.77	8.91±0.00	1.031±0.00	4.81±2.373
	Mean seasons	2.702±0.573	14.149±3.25		
	CV5		3.67		
	P≤0.05		***		***
	Interaction P≤0.05		***		

CHAPTER FIVE

Conclusion and Recommendation

5.1 Conclusion

A simple, fast, precise and accurate method has been developed for the simultaneous determination of organotin compounds in water, sediment and mussel samples. Three different derivatization procedures have been described. The GC-MS–TOF confirmed the presence of the organotins in both real and standard samples and their characteristic ions were established. The mechanical shaking method for extraction of target organotin compounds in sediments was optimized which gave a better recovery than the sonication method. The cleanup of the extracts was carried out on a glass column packed with activated silica and anhydrous sodium sulphate. From all the solvent tested, toluene and N-hexane were found to give a better recovery. The idea of blowing the final extract to dryness and reconstitute to 1 ml gives a proper account of the actual volume of the final extract used for GC analysis. The developed method was applicable to real samples.

The annual distribution of OTCs in the seawater of Cape Town harbour has been investigated. The seasonal variation of TBT was also investigated; significant variation of $P \leq 0.05$ was found after the statistical analysis. Seasonal variation in TBT and TPT concentrations with a higher level in summer than in winter and spring has been observed. Apparently, the observed high or low value recorded for TBT compound in Cape Town harbour could be as a result of increase or decrease in the traffic of ships and boats. Moreover, dilution due to increase in water volume results in decrease in concentration of TBT in winter and spring. High values observed for TBT in summer could be associated to steady flow of water, while in spring, the ocean tide effect may suggest reason why we have more TBT in spring or low but this also depends on the direction of the tide.

TBT were detected in all the sediment samples analysed except for location 9 (entrance to harbour), the two control sites (which are located far away from the inner harbour where boating activities is taking place), and location 12 (Robinson dry dock 2) where the samples were not found at all. For the control sites, antifouling compound TBT and TPT were not detected throughout except for TBT that was found in control A during summer. The seasonal variation of OTCs abundance to sediment was also investigated, the results indicated that TBT is presents throughout the seasons but predominantly present in this order summer > winter > spring. High variation recorded during summer is associated to steady flow of water during summer which enhances siltation of TBT in water column into sediment. The least value recorded for TBT and TPT in winter could be as a result of erosion due to increase in water flow remove, the OTCs from sediment while the ocean tidal effect might be the reason while the least value was recorded during spring throughout the studies.

The hemocytes in marine mollusc are stress indicators and they may predict the health condition of the host animal. The results of the present study support the use of lysosomal NRR time assay for assessing the toxic effects of TBT. A strong negative correlation was observed for the entire experimental period between TBT body loads and lysosomal responses of *Mytillus galloprovincialis* over time. This shows that hemocytic lysosomes are target organelles for TBT toxicity in *Mytilus galloprovincialis*. This study also identified *Mytilus galloprovincialis* as a marine invertebrate that can be used as a biomonitor species and in which NRRT assay can be employed as a toxicity monitoring technique. The lysosomal response in hemocytes of *Mytilus galloprovincialis* and measuring the NRR time assay could be considered as a useful cellular biomarker of stress due to tributyltin exposure.

Metal concentration levels in sediments from Cape Town harbour varied across locations. The results revealed the heavy metal pollution level of Cape Town harbour. Heavy metal contamination could be from industrial and domestic sources and other non-point sources such as storm water discharged from the storm water/stream that comes from residential and industrial areas in and around the harbour. The results also showed that while metals such as Pb and Cd were below the recommended sediment quality of guidelines of South Africa but above EPA, Cd, Cu, Zn and Hg exceeded the EPA guideline limit. Based on the findings of this study, there is the need to step-up the regular monitoring of these metals and other potential harmful pollutants within the marine ecosystem. More so, there is need for adequate control measures to be put in place to ensure compliance with national and international regulations on the protection of marine water systems.

The present study showed qualitative mineral identification of Cape Town harbour sediment using FTIR and XRD techniques respectively. The study reveals the levels of contamination of Cape Town harbour. The results are indication of the contributions of heavy metals which carry runoff from the waste water treatment plant that discharges into the Diep River, as well as domestic, ocean current, and storm water inflow into the harbour. Ship reparation is a major suspected factor responsible for the higher metal contamination in dockyard areas of the harbour. The enrichment factor recorded for Sn, Pb, Zn, Fe, Cd, Al and Hg in locations 7 and 8 were higher, metal contamination in these locations is due to activities previously mentioned in this section. Copper concentration was below 1, meaning that its presence was due to natural changes mentioned above. Since EF for Sn was high, government should monitor the docking of ships coated with antifouling agent known as organotins into the harbour. The ban on the use of these antifouling agents has been put under control in European and other developed countries but in Africa the ban has not been effected. The FTIR and XRD analyses indicate the presence of quartz, pyrite, calcite and

carrolite in the harbour sediments. The combination of the two techniques showed that they are useful techniques for mineral analysis.

One year monitoring study of pollution of priority heavy metals As, Cd, Hg, Pb and Sn in seawater from Cape Town harbour was determined successfully with the aid of ICP-MS technique. The concentrations of heavy metals were found to be high. These could be as a result of industrial activities, storm water inflows, and discharge from waste water treatment plant from urban/industrial area around the harbour. In additions, harbour activities such as striping and ship painting could also contribute to metal pollution of the harbour

This study has further supported the importance of mussel as a useful organism for measuring the pollution levels of aquatic environment. The soft tissues accumulate metals more efficiently than the shells. However, shells may also give information about pollution levels in environment. Sn, Cd, As, Hg and Pb bioaccumulated more in tissue while V, Cr, Mn, Co, Ni and Pb bioaccumulated majorly in the shell depicting strong association and hence would most probably be of anthropogenic origin. These findings are in agreement with the results obtained from the PCA. This study further revealed that the average metal concentration levels in the mussel (*Mytilus* galloprovincialis) were higher than the national and international standards for metals in molluscs complied by UNEP, IAEA, TFC, EC Directives and FAO. It is therefore important that Cape Town harbour need regular monitoring in order to meet national and international guidelines on the protection of marine environment.

5.2 Recommendation

The ban on the use of antifouling agents has been enforced in European and other developed countries but this is not the case in Africa. There is a need to for the government legislate banning of importation and use of ship made of antifouling agent in our marine ecosystem. Thus, if this law is put in place it will minimise the level of organotin pollution. With regards to further research other biomarkers studies using also other key species are suggested to further use to check their suitability in monitoring the toxic effects of organotin compounds. As for organotin research, the measurement of concentration of organotin pollutants in different matrices i.e. water and sediment and their toxicity were carried out in this work. Other related studies that need to be undertaken include:

(1).The absorption kinetics in humans, mechanism of action, and human exposure levels, along with the burdens of the organotins.

(2)Studies to define levels of organotin compounds that exist in foodstuffs

(3). Studies to better define the toxic responses of marine species to TBT residues

(4) Investigation on the extent of human exposure to OTCs in the atmospheric environment.

Moreover, the level of heavy metal in Cape Town harbour is very high, which confirms that the harbour is highly polluted contamination. Based on the findings from these studies, there is need to step-up the regular monitoring of these metals and other potential harmful pollutants within the marine ecosystems. More so, there is also a need to put in place adequate control measures to ensure compliance with national and international regulations on the protection of marine water systems. The concentration of heavy metals in water and sediment and their bioaccumulation in Cape Town harbour has been investigated in this study. It is therefore suggested that biomarkers or risk assessment software should be used to test both the risk and toxic effects of these contaminants to aquatic life. There is also a need for adequate marine water and sediment quality guidelines for South Africa.

REFERENCES

Abalos, M., Bayona, J.M., Compano, R., Granados, M., Leal, C. and Prat, M.D. (1997). Analytical Procedures for the determination of Organotin in sediment and biota: A Critical Review. *Journal of Chrom.* 788: 1-49.

Abbott, A., Abel, PD., Arnold., D.W. and Milne, A. (2000). Cost benefits analysis of the use of TBT: The case for a treatment approach. *Science of the Total Environment*, 258: 5-19.

Adams F. and Slaets, S. (2000). Improving the reliability of speciation Analysis of Organometallic Compounds. *Trends anal. Chem.,* 19: 80-5.

Adams, SM. (1990) Status and use of biological indicators for evaluating the effects of stress on fish. *Amer Fish Soc Symp* 81: 1-8.

Adedayo A, Darning L, Don S. F., Valerie C., Gerard MC., Johanna B., Daniel GC., Jacquette MT., Trasler, Bernard R. and Barbara, F Hales. (2003). Effects of in Utero Tributyltins chloride exposure in the rat on pregnancy outcome. *Toxicol. Sci.* 74: 407- 415.

Adekunle, A.S. (2009). Effects of Industrial Effluent on Quality of Well Water within Asa Dam Industrial Estate, Ilorin, Nigeria. Nature and Science. 7(1).

Adepoju-Bello, .A.A., O.M. Alabi, O.M. (2005). Heavy metals: A review. *The Nig. J Pharm.* 37:41 – 45.

Akan, J. C., Abdurrahman, F.I., Sodipo, O.A., Ochanya, A.E. and Askira, Y.K. (2010). Heavy metals in sediments from River Ngada, Maiduguri Metropolis, Borno State, Nigeria. *J of Environ Conta and Toxicol* 2 (9): 131-140.

Albalat, A, Potrykins J., PempoKwiak, J. and Porte, C. (2002). Assessments of organotin pollution along the polish coast (Baltic Sea) by using mussels and fish as sentinel organisms. *Chemosphere* 47: 165 – 71.

Alessandro Delle Site, 2001. Factors Affecting Sorption of Organic Compounds in Natural SorbentÕ Water Systems and Sorption Coefficients for Selected Pollutants: A Review. J. Phys. Chem. Ref. Data, Vol. 30, No. 1,: 187-430

Altman, SJ., Parizek, RR. (1995). Dilution of nonpoint source nitrate in ground water. Journal of Environmental Quality. 24: 707-717.

Alzieu C Sanjan J., Deltreil JP. and Bonel, M (1986) Tin contaminations in Archachon Bay-Effects on oyster shell anomalies. *Mar Pollut Bull* 17: 494- 8.

Alzieu C., Michel P., Tolosa I., Bacci E., Mec LD. and Readman JW (1991) Organotin compounds in the Mediterranean: a continuing cause of concern. *Mar Environ Res* 32: 261-270.

Alzieu C., Sanjuan J., Michel P., Borel M. and Dieno, JP. (1989). Monitoring and assessment of Butyltins in Atlantic coastal waters. *Mar Pollut. Bull.* 208: 22-6.

Alzieu, C (2000). Impact of tributyltin on marine invertebrates. *Ecotoxicology* 9: 71-76.

Anderson, MJ., Gorley, RN., Clarke, KR. (2008) PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK.

Anglin-Brown, B., Armour-Brown, A., Lalor, G.C (1995). Heavy metal pollution in Jamaica 1: Survey of cadmium, lead and zinc concentrations in the Kintyre and hope flat districts. *Environ. Geochem. Health,* 17: 51 -56.

ANZECC (2000) Australian and New Zealand Environment and Concervation Councils. Organotin Analysis in Marine samples.

Archibald, C., and Parsons, G. (1998). Sediment assessment programme for the port of Richards Bay. Heavy Metal Survey. Final Report. CSIR, Durban.

ATSDR, (1994). US Department of Health and Human Service, Toxicological profile for zinc. US Department of Health and Human Service, Public Health Service. (205): 88 – 608.

ATSDR, Agency for Toxic Substances and Disease Registry, Atlanta, Toxicological Profile for *Chromium* GA: U.S. Department of Health and Human Service, Public Health Service. 1600 Clifton Road N.E, E-29 Atlanta, Georgia. 30333(6-9) (2000) 95 - 134.

ATSDR (2005) Agency for Toxic Substances and Diseases Registry. Toxicological profile for tin and tin Compounds. US Department of Health and Human Services. http://www.atsdr.cdr.gov/ tox profiles/ tp.55.pdf.

Awan, A.M., Baigl, M.A., Igbal, J., Aslam, M.R. and Ijaz, N. (2003). Recovery of chromate for tannery waste water. Electron. *J. Environ. Agric. Food Chem.* 2(5): 543 –548.

Azuela M and Vasconcelos, MT. (2002). Butyltins compounds in Portuguese wines. *J Ag Food Chem* 50 : 2713-6.

Azumi K., Nakamura S., Kitamura S., SJ., Keisuke K., Hisato I, Shinsuke, T and Satoru, S. (2007). Accumulation of organotin compounds and Marine birnavirus detection in Korean ascidians. *Fisheries* 731: 263 – 269.

Baeyens, W., Monteny, F., Leermaakers, M. and Boullion, S (2003). Evaluation of Sequential extraction on dry and wet sediments. *Anal. Bioanal. Chem* 376:890 - 901.

Bakare-Odunola, M.T. (2005). Determination of some metallic impurities present in soft Drinks Marketed in Nigeria. *The Nig. J. Pharm.* 4(1): 51 - 54.

Bartley, G.E (1996). The distribution and fate of tributyltin in the marine environment, In: De Mora, S .J Ed, Tributyltin: a case study of an environmental contaminant Cambridge Environmental Chemistry Series, Cambridge, Univer press, Cambridge.139-165.

Barreiro, R., Gonzalez, R., Quintela M and Ruiz, M. (2001).Imposex organotin bioaccumulation and sterility of female *Nastarius reticulates* in polluted areas of NW Spain. *Mar Ecol Pro. Series* 218: 203- 212.

Beaumont, AR. and Budd, MD. (1984). High mortality of the larvae of the common mussel at low concentrations of tributyltin. *Mar Pollut Bull* 15: 402- 5.

Becker G, Janak K, Colmsjo, A and Ostman, C. (1997). Speciation of organotin Compounds released from poly (vinylchloride) at increased temperatures by gas chromatography with atomic emission detection. *J Chromato A.* 775: 295 -306.

Bekri, K., Pelletier, E. (2004). Trophic transfer and in vivo immunotoxicological effects of tributyltin (TBT) in polar seastar Leptasterias Polaris. Aquatic toxicology. 66 (1): 39- 53.

Bella J, Beiras R, Marino-Balsa JC, Fernandez N (2005a) Toxicity of organic compounds to marine invertebrate embryos and Larvae: A Comparison between the Sea Urchin Embryogenesis Bioassay and Alternative Test Species. *Ecotoxicology* 14: 337-353.

Bennett, RF. (1983). Industrial development of organotin compounds *Indu. Chem. Bull.,* 2: 171,176.

Bennett. RF. (1996) Industrial manufacture and applications of tributyltin compounds. In : De Mora, S.J (Ed), tributyltin: a case study of an Environmental contaminant, Cambridge Univ. press, Cambridge. 21-61.

Bervoets, L., Panis, L and Verheyen, R. (1994). Trace metal levels in water, sedimentand Chironomus grthumni, from different water courses in Flanders (Belgium). Chemosphere. 29(8) 1591 - 1601.

Blabber, SJM. (1970). the occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus*. The Malocological Society of London. London.

Biselli, S., Bester, Kai., Huhnerfussi, H., Fent, K., (2000). Concentrations of the Antifouling Compound Irgarol 1051 and of Organotins in Water and Sediments of German North and Baltic Sea Marinas. Mar. Poll. Bull. 40(3): 233-243.

Blanca, AL. (2008). Environmental levels toxicity and human exposure to TBT- contaminated environment. A review. *Environ Int* 34: 292 – 308.

Blunden, SJ, Evans, CJ. (1990). Organotin compounds. In: Hitzinger O (ed) The Hand-book of Environmental Chemistry Vol 3 Part E. Anthropogenic Compounds. Springer, Berlin pp 1-44.

Blunden, SJ, Hobbs LA, Smith PJ Craid (eds) (1986). Organometallic compound in environment, Longman, Harlow, pp 111.

Bodin, N, Burgeot T, Stanisiere JY, Bolquene G., Menard D., Minier Boutet I., Amat A., Chard, Y and Budzinski, H (2004). Seasonal variations of a battery of biomarkers and Physiological indices for the mussel *Mytilus galloprovinciallis* transplanted into the Northwest Mediterranean Sea. Comp Biochem PhysiolToxicol: *Pharmacol* 138 (4): 411- 427.

Bordas, **F** and Bourg, A.C.M (1998) A critical evaluation of sample for storage of Contaminated Sediments to Be investigated for the potential mobility of their heavy metal load. *Water. Air. Soil. Poll.* 103 (1 - 4):137-149.

Borghi, V and C. Porte, Environ. *Sci. and Techno.*3 6(20): 4224, 4228 (2002).

Brack, K. (2002). Organotin Compounds in Sediments from the Gota Alv estuary. *Water, Air Soil Pollut* 135: 131-140.

Bravo M., Lespes G., Gregory DI., Pinochet H. and Potin-Gautier M. (2005) Determination of Organotin compounds by headspace solid Phase micro extraction-gas Chromatography-pulsed Flame -photometric detection (HS-SPM-GC-PF) *Anal. Bioanaly. Chem.* 38; 1082-9.

Bruder-Hubscher, V., Lagarde, F. Leroy, M.J.F., Conghanowr, C and Engelhard, F (2002) Application of a Sequential extraction procedure to study the release of element from municipal solid waste Incineration bottom ash. *Anal Chim Acta* 451: 285 - 295.

Brunori C., Ipolyi I., Massanisso P., and Morabito R. (2005). New trends in sample preparation methods for the determination of organotin Compounds in marine matrices. In Handbook of environmental chemistry, Vol 5. *Water pollution 50 edition* Springer *Verlag Berlin Heidelberg*. 51-70 .

Brunori, C., Ipolyi, I., Massanisso, P and Morabito, R. (2006). New Trends in Sample Methods for the of Organotin Compounds in Marine Matrices. *Hdb Environmental Chemistry Springer-Verlag, Berlin.* 1-70.

Bryan, GW, Gibbs, PE and Burt GR (1988). A Comparison of the effectiveness of tri-n-butyltin chloride and five other organotin compounds in promoting the development of imposex in The dogwhelk, I. J Mar Bio Assoc. UK 68: 733- 44.

Bubb, J.M., J.N., Lester, J.N. (1991). The impact of heavy metals in low land riversand the implications for Man in the environment. *Sci. Total. Environ.* 100: 207 – 233.

Bulut, VN et al., (2007)"A multi-element solid-phase extraction method for trace metals determination in environmental samples on Amberlite XAD-2000," *J. Hazard. Mater*, vol. 146, pp. 155-163.

CAI Y., Rapsomanikis, S. and Andrea M.O (1993). Determination of butyltin compounds in Sediment using gas chromatography – atomic Absorption spectrometry. Comparison of sodium and sodiumTetraethylborate derivatization methods .*Analytical Chim. Acta.* 274; 243-25.

Canadian Environmental Quality Guidelines. (2002). Canadian Sediment quality guidelines for The Protection of Aquatic life. Updated 2002.

Camilla, J. Quadros, I.B. Castro and M.A. Fernandez, Imposex in Chais rustica Mollusca; Neogastropoda (Lamarck, 1822) as an indicator of organotin compound Pollution in Macelo coast (Northeastern Brazil, Brazilian *.J. of Oceano.*, 52 (2) ; 101-105 (2004).

Capalat, C., Texier, H., Barillier and D., Lelievre (2005). Heavy mobility in Harbour Contaminated Sediments: The case of Port-en-Bessin. *Mar. Poll. Bull.* 50: 504 – 511.

Carlier–Parnassian C., Lespes G., and Astrul M (2002). Determination of butyl-and Phenyltin Compounds in sediments by GC-FPD after $NaBEt_4$ ethylation. *Talanta.* 44: 1171.

Castro IB., Camilla E., Quadros J., and M.A. Fernandez, MA. (2004). Imposex in Chais rustica Mollusca; Neogastropoda (Lamarck, 1822) as an indicator of organotin compound Pollution in Macelo coast (North-eastern Brazil, Brazilian *.J. of Oceano.* 52 (2); 101-105 (2004).

Centineo, G., Rodriguez –Gonzalez, P., Blanco, E.G., Garcia Alonso, J. I. and Sanz M., Font, C.N., Aranda Mare, J.L. and Ballester Nebot, N. 2006. Isotope Dilution GC –MS Routine Method For the Determination of Butyltin Compounds in Water. *Analytical Bioanalytical Chemistry.* 384: 908 – 914.

Ceulemans, M and Adams, FC. (1995). Evaluation of sample preparation methods for organotin speciation analysis in sediments- focus on MBT extraction. *Anal Chim Acta* 317: 161-170.

Cevik, U., Damla, N., Kobya, A.I., Bulut, VN., Duran, C., Dalgic, G., Bozaci, R. (2008). Assessment of metal element concentrations in mussel (M. Galloprovocialis) in Eastern Sea, Turkey. Journal of Harzadous Materials. 160: 396-401.

Chapman, P.M and Wang, F. (2001). Assessing Sediment Contamination in Estuaries. *Environ Toxicol and Chem.* 20(1): 3 - 22.

Champ, MA (2000) A review of organotin regulatory strategies pending actions, related costs and benefits. *Sci Tot Env* 258: 21- 71.

Champ MA (2003). Economic and environmental impacts on ports and harbours from the Convention to ban harmful marine antifouling systems. Mar Pollut Bul. 46: 935 - 40.

Champ M.A and P.F Seligman (1996). An introduction to organotin compounds and their use in antifouling coatings .In: Champ, M.A Seligman, P.F (Ed), Organotin, Environmental fate and effects. Chapman and Hall London. 1-25. 62 G.

Chang D., Jianzhong He (2010). Effects of antibiotics in the environment of microbial populations. Applied Microbiology and Biotechnology. 87(3): 925-941.

Cheam, V., Lechner, J., Sekerka, I., Desrosiers, R., Nriagu, J and Lawson, G (1992). Development of laser- excited atomic fluorescence spectrometer and a method for theDirect determination of lead in Great Lake waters. *Anal. Chim. Acta.* 269: 129 - 136.

Chen Chem., Chih- Ming K., Cheng-Di D and Chieu- Wen C. (2010). Butyltin contamination in Sediments and seawater from Kaohsiung Harbours, Taiwan. *Environ Monit and Assess.* 169: 75 -87.

Chieu LC., Hung TC., Choang KY., Yeh CY., Meng PJ., Sheih MJ and Hem, BC. (2002). Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Sci Tot Env.* 285: 177- 85.

Chima, F., Marin, M.G., Mattozzo, V., Ros, D.L and Ballarin, L. (1999). Biomarkers for TBT Immunotoxicity Studies on the Cultivated Clam *Tapes philippinarum* (Adams and Reeve, 1850). *Mar Pollut Bull* 39 (1-12): 112 - 115.

Chiron S, Roy S., Cottier R and Jeannot, R. (2000). Speciation of butyl and phenyl-tin compoundsIn sediments using pressurized liquid extraction and liquid chromatographyICP-MS. *J Chromato A*879:137- 145.

Clark EA, Sterrit RM, Lester JN (1988). Environmental Science and Technology. (22) 600 In: Dubey SK and Roy U. (2003) Biodegradation of tributyltins by marine bacteria. *Appl OrganChem* 17: 3-8.

Cooke, MG (2002). Effect of organotins on human aromatase activity in vitro. *Toxicol Let, Elsevier.* 126:121 – 130.

Coates JP (1977) The IR analysis of Quartz and Asbestos, Nelioth Offset Ltd.,

Chesham, England.
Coetzee, PP. (1993). Determination and speciation of heavy metals in sediments of the HartebeespoortDam By sequential extraction. Water SA. 19 (4), 291- 300.

Comber, S.D.W., Gunn, A.M., C. Whalley, C (1995). Comparison of the Partitioning of tracemetals in the Humber and Mersey estuaries. *Mar. Poll. Bull.* 30(42): 851 - 860.

Cuong, T.D and Obbard, J.P (2006) Metal speciation in coastal marine sediments from Singapore from Singapore using a modified BCR- sequential extraction procedure. *Appl. Geochemistry* 21: 1335 - 1346.

Dapaah, R.K., Takano, N and Ayame, A (1999). Solvent extraction of Pb (II) from acid medium with zinc Hexamethylenedithiocarbamate followed by back-extraction and subsequent determination by FAAS. *Anal. Chim. Acta* 386:281 - 286.

Dassenakis, M., Degaita, A., M and Scoullos, M (1995). Trace metals in sediments of aMediterranean estuary affected by human activities (Acheloos river estuary, Greece). *The Sci. Total. Environ.* 168: 19 - 31.

David, J.M. and Anisha, R. 2003. Imposex in the indigenous *Nassarius Kranssianus* (Mollusc: Neagastropoda) from South African harbours. *Marine Pollution Bulletin.* 46: 1150-1155.

Delucchi F, Tombesi N. B, Freije RH, Marcovecchio JE (2007) Butyltin compounds in sediments of the Bahia Blanca Estuary, Argentina. *Environ Moni Asses.* 132: 445 –451.

Department of Water Affairs and Forestry (1996) South African Water Guidelines for Aquatic Ecosystems. Volume 7. Second Edition

Depledge MH, Fossi MC (1994) the role of biomarker in environmental assessment (2). *Invertebrat Ecotoxicol* 3:161 -72.

De Mora SJ, Stewart C, Philips D (1995). Sources and rate of degradation of tri (n-butyl) Tin in marine sediments near Auckland, New Zealands. *Mar Pollut Bull* 30:50- 7.

D. Mora, S.J., fowler, S., Cassi, R. and I. Tolosa I (2003). *Mar. poll. Bull.,* 146; 401-409.

Dietz C., Sanz J., Sanz E., Munoz –Olivas R., and Camara C. (2007). Current perspectives in analyte extraction strategies for tin and arsenic speciation. *J. of Chro.,* A., 1153: 114-29.

Donard, OFX and R., Ritsema, R., (1993) Hyphenated Tech applied to the speciation of organometallic compounds in the Env. In Barcelo (ed) . Environmental analysis (Tech, application and quality assurance 2nd edition. *Elsevier Amsterdam.*, 549.

Donard, OXF., Lalere B., Martin F. and Lobinski R. (1995) Microwave assisted leaching of organotin compounds from sediments for speciation analysis. *Anal. Chem.,* 67: 4250-4.

Donard, OF., Lespes X., Amorous, G., Morabito, R., (2001) In Trace metal speciation for environment, food and health, edited by Ebdon L, Pitts, Cornels, Crews, H.O.F. Donard and P Quevanviller. *Royal society of chemistry*. 142, 175.

Dowson, PH, Bubb JM. and Lester, JN. (1993). Temporal distribution of organotins in the aquatic Environment: Five years after the 1987 UK retail ban on TBT based antifouling paints. *Mar Pollut Bull* 26: 482 -94.

Dubey, SK and Roy, U. (2003). Biodegration of tributyltins (organotins) by marine bacteria. *Appl Organo Chem* 17:3-8.

Dumbleton MJ and West G, (1966). Some factors affecting the relationship between clay mineral in soil and their plasticity. Clay mineral, 6: 179-193

Dun JG, Gong W, Shi D (1992) A FTIR study of oxidation of pyrites. J Thermochimica Acta 208: 293 -303.

DWARF (2010). SouthAfrican Marine Living Resources Act, 1988 (ACT No 18 of 1988). European communities EC(2001). Commission Regulation, 2001/22EC NO: 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs.

Eaton, A.D (2005). Standard Methods for the Examination of Water and Waste Water.21st

Edn. American Public Health Association, Washington, ISBN. 0875530478: 343 - 453.

Efe, SI., Ogban, FE., Horsfall, MJ., Akporhonar, EE (2005). Seasonal variations of physicochemical characteristics in Water resources, Ouality in Western Niger Delta Region, Nigeria. J. Appl. Sci. Environ. Mgt. 9(1): 191-195.

EFSA, (2004). Scientific panel on contaminants in the food chain, opinion on the health risks Assessment to consumers associated with the exposure to organotins in foodstuffs. The EFSA Journal 102 : 1-119. http : // www.efsa. Europe. Eu /eu/ science/ contaminant / contaminant opinions / 658. Html.

Encinar JR., Ahuso, JIG, Sanz –Medel (2000). A synthesis and application of isotropically Labelled dibutyltin for isotope dilution analysis using gas Chromatography ICP-MS. *of Anal. Atom Spect.,* 15: 1233-9.

Esslemont, G (2000). Heavy metals in seawater, marine sediments and corals from the

Townsville section, Great Barrier Reef Marine Park, Queensland. *Marine Chemistry.*71: 215 231.

EU- SCOOP (2006). Revised assessment of the risks to health and the environment associated with the use of organotin of the four organotin compounds TBT, DBT DOT and TPT). Directorate General Health and consumer protection. http :// ec. Europa. Eu /health /ph –risk- committees/ 04 –scher / docs /scher-0-047.pdf Accessed.

Evans DA., Huggett, RJ., (1991). Statistical modelling of intensive TBT monitoring data in two tidal creeks of the Chesapeake Bay. Mar Environ Res 32: 169- 186.

EVISA (European virtual Institute for speciation analysis). (2010). Human exposure to Organotin compounds via consumption of fish. Http: // www.speciation .net/ Public/ info/ contact form. Html. 2010 / 03/ 08.

Ewa, C., Luke, C., Hermogene., N and Valerie., K. (2004). Application of supported liquidmembrane probe for extraction and preparation of organotin compounds from environmental water samples. *Analytical Chimica, Acta.* 523: 141- 147.

FAO (Food and Agriculture Organization), (1983) "Compilation of legal limits for hazardous substances in fish and fishery products'," no. FAO fishery circular, No 464, pp. 5–100, 983.

Fanfandel M., Muller, W.E.G, Batel., R (2003). Molecular response to TBT stress in marine Sponge (Suberites domuncila: proteolytical cleavage and phosphylation of KRS.SD Protein kinase. *J Exp Mar Bio Ecology* 297 (2): 239 -252.

Fang GC, Wu YS, Chang, SY (2006) Size distribution of ambient and enrichment factor Analysis. Atmospheric Research 81(4): 320 – 333.

Fatoki, O.S and Mathabata, S. (2001). An assessment of heavy metals pollution in the East London and Port Elizabeth harbours. *Water SA.* 27(2): 233 - 240.

Fatoki, O.S, Okoro, H.K, Adekola, F.A, Ximba, B.J, Synman, R.G (2012). Bioaccumulation of Metals in black mussels (*Mytillus* galloprovincialis) in Cape Town Harbour, South Africa ". *The Environmentalist.* 32: 48-57 Published by Springer Science + Business Media LLC 2011: (DOI 10.1007/s10669-011-9370-5.

Federico, D., Noma, T.B., Ruben, H., Freije, H and Jorge, E.M. (2007). Butyltin compounds in Sediments of the Bahia Blance Estuary, Argentina. *Environment Monitoring Assessment.* 132: 445 -451.

Fent, K. (1996). Ecotoxicological of organotin compounds. *Crit Rev Toxicol* 26: 1-117.

Fent, K. and Muller, MD. (1991). Occurrence of organotins in municipal wastewater and Sewage Sludge and behaviour in a treatment plant. *Environ Sci Technol.* 25: 489 – 93.

Fent, K. and Hunn, J (1995). Organotin in freshwater harbours and rivers: temporal distribution Annual trends and fate. *Environ Toxicol Chem* 14:1123-32.

Fernely, PW., Moore, MN., Lowe, DM., Donkin, P., Evans, S. (2000). Impact of the Sea Empress oil spil on lysosomal stability in mussel blood cells. Marine Environ. Res. 50, 451-55.

Fernandez, M.A., Wagener, ALR, Limaverde, AM., Scofield, AL., Pinheiro, F., and Rodriguez. Imposex and surface sediment speciation: A combined approach to Evaluate organotin contamination in Guanabara bay, Rio de Janeiro *Brazil. J. of Mar. Environ. Res.* 59: 435, 452 (2005).

Fischer, E and Van Den Berg, C.M.G (1999) Anodic Stripping Voltammetry of Pb and Cd using a Hg film electrode and thiocyanate. *Anal. Chim. Acta.* 385: 273 - 280.

Forsyth, D.S D. Weber, and C. Cleronx., (1992) Determination of butyltin Cyclohexyltin and phenyltin compounds in beers and wires. *Food Add contain* 9: 161-169.

Forsyth, D.S. and B.Jay. (1997). Organotin leachates in drinking water from chlorinated Polyvinylchloride (CPVC) Pipe. *Applied Organo. Chem.,* 11: 551, 558.

Forstner, U and Wittmann, G.T.W .1981, Metal Pollution in the Aquatic Environment Springer- Verlag, Berlin. Springer- Verlag, Heidelberg P. 486.

FSA. Food Standard agency (2005) Survey of organotins in Shellfish, http: // www. Food. Gov. UK./ Multimedia/ pdfs/fsis8105. Pdf Accessed.

Gibbs, P.E., Pascoe, P.L. and Bryan, G.W. (1991a). Tributyltin-induced imposex in stenoglansang gastropods: pathological effects on the female reproductive system. *Comp. Biochem Physiol.* 100C., 231, 235 (1991a).

Gibb's P.F. and G.W Bryan, G.W (1996). Reproductive failure in the gastropods Nuclella Capillus associated with imposex caused by tributyltin pollution: A review, In: Champ, M.A Seligman, P.F (Eds), Organotin -Environmental fate and effects, Chapman and hall, London. 259- 281.

Gil R, Avital G, and Stewart I., Yehuda, B (2000). Unregulated use of TBT-based Antifouling paints in Israel: high contamination and imposex levels in two species of marine gastropods. *Mar Ecol Progress Series.* 192: 229 -239.

Grindley, LA., Dudley, S. (1988). Estuaries of the Cape: Part II: Synopses of available information on individual systems In: A.E.F. Heydom, P.D. Morant (Eds), Report No. 28 Rietvlei/Diep CW24/25.CSIR Research Report 427. Stellenbosch, South Africa.

Gopalakrisshnan, S., Huang, W., Wang, Q., Wu, M., Liu, J., Wang, K. (2011). Effects of tributyltin and benzo [a] pyrene on the immune-associated activities of hemocytes and recovery responses in the gastropod abalone, *Haliotis diversicolor*. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 154(2), 120-128.

Gomez S.R (2008) Study of the cyto –toxic activity of di and triphenyl (IV) Carboxylate Complexes. *J. of inorganic Biochemi.,* 102: 2087.

Gomez-Ariza, J.L., Giraldez, I., Sanchez-Rhodes, D and Morales, E (1999). Metal readsorption and re-distribution during analytical fractionation of trace elements in oxic estuarine sediments. *Anal. Chim. Acta* 399 295 - 307.

Gomez – Ariza, J.L., Giraldez, I., Sanchez- Rodas, D and Moralesm, E. (2000). Metal Sequential Extraction Procedure optimized for heavy metal polluted and iron- oxide rich sediments. *Anal. Anal. Chim.* Acta 414: 151 - 164.

Godoi, FL., Montone, RC., and S.M. Santiago, SM. (2003). Determination of butyltin compounds In surface sediments from the Sao panlo state coast (Brazil) Ygas Chromatography pulse flame photometric detection. *J. of Chrom. A.* 185: 205, 210.

Guerra-Garcia JM, Garcia-Gomez JC (2005) Assessing pollution levels in sediments of a harbour with two opposing entrances. Environmental implications. *J of Environ Management.* 77, 1 – 11.

Guevara-Riba, A., Sahuquillo, A., Rubio, R., Rauret, G. (2004). Assessment of Metal mobility in dredged Harbour sediments from Barcelona, Spain. Sci. Total. Environ. 321: 241 – 255.

Guevara- Riba, A., Sahuquillo, A., Rubio, R., Rauret, G (2005) Effect of chloride on heavy Metal Mobility of harbour sediments. *Anal. Bioanal. Chem.* 382: 353 - 359.

Gummy, C. (2008). Dibutyltin disrupts glococorticoid receptor function and impaired glucocorticoid. Induced suppression of cytokine production plos one 3.dot: 10, 1371/Journal, Pone 000354.

Haggera, JA., Depledge, MH., Galloway TS (2005). Toxicity of tributyltin in the marine mollusk *Mytilus edulis*. *Mar Pollut Bull* 51: 811 -6.

Hammer, M. JM. J. Hammer, Water Quality. In: Water and Wastewater Technology. 5th Edn. New Jersey: Prentice-Hall, (2004) 139 - 159.

Harino, H., Fukushima, MH., Kawai, S (2000). Accumulation of butyltin and phenyltin compounds in various fish species. *Arch Environ Contam Toxicol* 39: 13-19.

Harino, H., O'Hara, S.C.M., Burt, G.R., Chessman, B.S., Pope, N.D. and Langston, W.J. (2003). Organotin compounds in Mersey and Thanes Estuaries a decade after UK TBT Legislation. *Journal of Marine Biology Association UK.* 83: 11-22.

Harino, H., Iwasaku, N., Arai, T., Ohji, M., Miyazaki, N. (2005). Accumulation of organotin compounds in the Deep- Sea Environment of Nankai Trough, Japan. *Arch Environ Contam Toxicol.* Vol 49: 497 - 503.

Harino, H, Ohji M., Wattayakorn G, Arai T., Rungsupa S., Muyazaki, N. (2006). Occurrence of antifouling biocides in water and sediments from the Port of Osaka, Japan. *Arch Environ Contam Toxicol* 48 3: 303 -310.

Harino H, Madoka O, Wattayakorn G, Adulyyanukosol K, Arai T, Miyazaki N, (2008). Accumulation of organotin compounds in Tissues and organs of Dolphin from the Coasts of Thailand. *Arch Environ Contam Toxicol.* 54: 146 -153.

Hawkins SJ, Gibbs PE, Pope ND, Burt GR, Chessman BS, Bray S, Proud SV, Spence SK, Southward AJ, Southward GR, Langston WJ (2000). Recovery of polluted ecosystems: the case for long-term studies. *Mar Environ Res* 54 3-5:215- 222.

Hermogenes, N., Ewa., M.C., Andrew. C., Emanuel, T and David, A. 2009. In situ ethylation of organolead, organotin and organomercury species by bromomagnessium Tetraethylborate prior to GC –ICP-MS analysis. *Journal of Separation Science.* 32: 24226 - 2433.

Hermosin, MC., Martin, P. and Cornejo J (1993). Adsorption mechanisms of monobutyltins in clay minerals. *Environ Sci Technol* 27: 2606 – 2611.

Hiavay J, Jonas K, Elek S, inczedy J (1978) Characterization of the particle size and the crystallinity of certain minerals by infrared spectrophotometry and other Instrumental Methods-II. Investigation on Quartz and feldspar. Clay Minerals 26: 139 -143.

Hintze, J (2012). NCSS 8. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com .

Hirade, M., Chen, Z., Sugimoto, K. and Kawaguchi, H. (1980). Co precipitation with tin (IV) hydroxide followed by removal of tin carrier for the Determination of trace heavy metals by

Graphite-furnace atomic absorption Spectrometry. *Anal. Chim. Acta* 302:103 - 107.

Hoch M (2001). Organotin compounds in the environment- an overview. *Appl Geochem* 16: 719 – 743.

Hongwen, S., Guolan, H., Shugui., D. (1996). Adsorption behaviour and OSPR studies of organotin compounds on estuarine sediments. *Chemosphere* 33 (5):831 – 838.

Horiguchi T, Imai T., Cho HS, Shiraishi H, Slubata Y and Morita M (1998). Acute toxicity of organotin compounds to the larvae of the rock shell Thais Clarivigera, the disk abalone, Halitosis madaka. *Mar Environ Res* 46: 469 -73.

Horiguchi T., Li, Z., Uno S., Shimizu M., Shirashi V., Morita M., Thompson JAJ, Lerings, CD. (2004) Contamination of organotin compounds and imposex in Mollusc from Vancouver, Canada. *Mar Environ Res* 52 1-2: 75 -88.

Horio, BT., Iwanoto, Y., and Shiga J.I The nitrate corrosion of canned orange juice Drinks In Japan communication presented at the 5^{t} int.*Congtres on canned foods Vienna,* 12: 3-6 (1967).

Hu, J., Zhen H., Wan Y., Gao J, An W., An L., Jin P., Jin X (2006). Tropic Magnification of Triphenyitin in a Marine food Web of Bohai Bay, North China: Comparison to TBT. *Environ Sci Techno* 40 10: 3142 -3147.

Huggett, RJ., Unger MA., Seligman PF., Valkirs, AO. (1992). the marine biocide tributyltin: assessing and managing the environmental risks. *Environ Sci Technol* 26: 232 -237.

IAEA, International Atomic Energy Agency. The IAEA database on natural matrix Reference Materials; 2003; http// naweb.iaea.org/nahu/nmrm/nmrm2003/Index.htm Accessed; 2007.

Idris, A.M., Eltayeb, M.A.H., Potgieter- Vermaak, S.S., Grieken, R., and Potgieter, J. H (2007) Assessment of heavy metal pollution in Sudanese harbours along the Red Sea coast. *Microchemical. J.* 87: 104 - 112.

Ikeda, M., Yamada, T., Koyama H., Kankyo J., Kayeku. (2002). In Chamdana 5 Organotin compounds in the Marine Environment., 12 : 1: 105, 114.

Ikonomu MG., Fernandez MP., He T. and Cullon D (2002). Gas chromatography–high-resolution mass spectrometry based method for the simultaneous determination of nine organotin compounds in water, sediment and tissue. *J of Chrom. A.* 975:319

IMO, (2001). International Maritime Organisation. International convention on the control of the harmful antifouling systems on ships : http : // www. Imo.orglconventions / Mainframe .asp1topic_id -59. Accessed 2007.

Iwata, H., Tanabe S., Mizuno T. and Tatsukawa, R. (1995). High accumulation of toxic butyltins in marine mammals from Japanese coastal waters. *Environ Sci Technol* 29: 2959 - 2962.

Iwegbue, C.M.A., Egobueze, F.E K. and Opuene, K. (2006). Preliminary assessment ofHeavy Metals Levels of soils of an oil field in the Niger Delta. *Int. J. of Sci. and Technol.* 3(2):167 – 172.

Isani, G., Andreau G, Kindt M, Carperie, E. (2000). Metallothioneins (MTs) in marine molluscs. *Cell Mol Biol* 46 2: 304 -313.

Jackson, VA., Paulse, AN., Odendaal, JP., Khan, W (2009). Investigation into the metal contamination of the Plankemburg and Diep River. Western Cape, South Africa, Water SA 35(3): 289-299.

Jiang, G.B., Liujy. and Yang K.W (2000). Anal chem. Acta. 421:67.

Jianying, HU. Huajun YI., Zhen J., Wan W., GAO A.W., Lihui AN, JIN F., and J. XIAOHUI., Trophic magnification of Triphenyitin in a marine food web of Bohai bay, North china: comparison to tributyltin *J. of Environ. Sci. and Techno.* 40 (10) : 3142-3147. (2006).

Kannan K, Lee RF (1996) Triphenyitin and its degradation products in foliage and soils from sprayed pecan orchards and in fish from adjacent ponds. Environ Toxicol Chem 15: 1492 - 1499.

Kersten, M and Frostner, U. (1986).Chemical fractionation of heavy metals in anoxic estuarine and coastal sediments. *Water. Sci. Technol.* 18: 121 - 130.

Konstantinou, IK., Albanis TA (2004). Worldwide occurrence and effects of antifouling Paint Booster biocides in the aquatic environment: a review. *Environ Int* 30 235 – 48.

Koukouzika, K., Dimitriadis, VK. (2005). Multiple Biomarker Comparison in *Mytilus galloprovincialis* from the Greece Coast "Lysosomal Membrane Stability, Neutral Red Retention, Micronucleus Frequency and Stress on Stress. Ecotoxicology. 14: 449-463

Kress, N., Herut, B., Galil, B.S (2004). Sewage sludge impact on sediment quality and benthic assemblages off the Mediterranean coast of Israel—a long-term study. *Mar. Environ. Res.* 57: 213 - 233.

Kuballa J, Jantzen, RD, Wilken. (1996). In: Calmano W, Forstner U (eds) Sediments and Toxic Substances, Vol 10 Springer, Berlin, 1996, p245.

Lalere, B., Szpunar J., Badzinski H., Garrigues P., Donnard, OFX. (1995). Speciation analysis for organotin compounds in sediments by capillary gas Chromatography with flame photometric detection after microwave-assisted acid Leaching. *Analyst* 1995; 120: 2665-73 (1995).

Langston, WJ, Popoe, ND. (1995) .Determination of TBT absorption and desorption in Estuarines Sediments. *Mar Pollut Bull* 31 1-3:32 – 43.

Lagadic, L., Caquet, T. And Ramade, F. 1994. The role of biomarkers in environmental\assessment. *Invertebrates Populations and Communities Ecotoxicolgy*. 3: 193 – 208.

Laughlin RB (1996) Bioaccumulation of TBT by aquatic organisms. In: Champ MA., Seligman PF (eds) Organotin Environmental Fate and effects. Chapman and Hall, London 331-357.

Lawson, G Organometallic compounds in polymers, their interaction with the Environ. In : Craig, PJ. (Ed), Organometallic compounds in the environment. Principles and reactions. Longman London. 1986. 308-344 (1986).

Leinio, S, Lehtonen KK. (2005). Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Marcona baltical* from the northern Baltic Sea. Comp Biochem Physiol C *Toxical Pharmacol* 140 3-4:408 – 421.

Liang, Y., Wong, M.H, (2003). Spatial and temporal organic and heavy metal Pollution at Mai Po Marshes Nature Reserve, Hong Kong '*Chemosphere.* 52: 1647-1658.

Lisicio, C., Carro, DM. and Magi, E. (2009). Comparison of two analytical methods for the determination of organotin compounds in marine organisms. Comptes Rendus. Chimie. 12: 831-840.

Lionetto, MG, Giord nano ME., Caricato, R., Pascariello, MF., Marinosci, L., Shettino T (2001)Biomonitoring of heavy metals contamination along the salento coast (Italy) by metallothionen evaluation in *Mytilus galloprovincialis* and *Mullus barbatus.* Aquatic Consv Mar Fresh Eco 11: 305 – 310.*Chimie.* 12: 831 - 840.

Lo S, Allera A., Alberts P., Heimbrechi J, Janzen E, Klingmuller D, Stockclbroeck, S (2003). Diethoerythritol (DTE) prevents inhibitory effects of TPT on the key enzymes of the human setsteroid hormone metabolism. J Steroid Biochem 41:1560 -5.

Lowe, DM., Fossato VU., Depledge, NH. (1995b) Contaminant-induced lysosomal membrane damage in blood cells of mussels *Mytilus galloprovincialis* from the Vennice Lagoon: an in vitro study. Marine Ecology Prog Ser. 129: 189-196.

Lowe, DM., Pipe, RK. (1994). Contaminant induced lysosomal membrane damage in marine mussel digestive cells: an invitro study. Aquatic Toxicology. 30: 357-365.

Lowe, D.M., Soverchia, C., Moore, M.N. 1995a. Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluaranthene *Aquatoxicology.* 33: 105 – 112.

Lyle. W.H (1958). Lesiours of the skin in process workers caused by contact with butyltin Compounds. *Brazilian. J. of Int. Med.,* 15:193,196.

Manoharan SS, Kumari NRS, Patil KC (1990) Preparation of fine particles Chromite: A Combination approach. J of Material Res bull 25 (6): 731- 738.

Magi E., Liscio, C., Pistarino E., Santamaria, B., Carro, DM., Tiso, M., Scaloni, A., Renzone, G., Cosulich, EM. (2008). Interdisciplinary study for the evaluation of biochemical alterations on mussel *Mytilus galloprovincialis* exposed to a tributyltin-polluted area, Anal Bioanal Chem. 391: 671-678.

Maguire R J (1996) the occurrence, fate and toxicity of tributyltin and its degradation products in fresh water environments. In: de Mora SJ (ed., Tributyltin: Case Study of an Environmental Contaminant. Cambridge University Press, Cambridge, UK. 94- 98.

Magnusson, K, Ekelund, R, Dave, G. Granmo, A. Forlin, L. Wennberg, L. Samuelsson, M-O Berggen, M. and Brorstrom-Lunden, E. (1996). Contamination and correlation with toxicity of sediment samples From the Skagerrak and Kattegat. Journal of Sea Research. 35, (1-3) 223- 234.

Magni, P., De Falco, G., Falugi C., Frauzoui, M., Monteverdi M, Perrone E, Squo M, Bolognesi (2006). Genetoxicity biomarkers and acetyl cholinesterase activity innatural populations *Mytilus galloprovincialis* along a pollution gradient in the Gulf of Oristano. *Environ Pollut.* 142 (1): 65- 72.

Marshall, D.J., Rajkumar, A. (2003). Imposex in the indigenous *Nassarius kranssianus* (Mollusca: *Neagastropoda*) from South African harbours. *Mar Pollut Bull* 46:115 –1155.

Matozzo, V., Ballarin L, Marin MG (2002). In vitro effects of tributyltin on functional Responses of haemocytes in the clam *philippinarum. Appl Organic Chem*16: 169 -174.

Matozzo, V., Tomei A., Marin, MG. (2005). Acetyl cholinesterase as biomarkers of exposure to neurotoxic compounds in the clam *Tapes philippinarum* from the Lagoon of vernice. *Mar Pollut Bull* (50) 12: 1686 -1693.

Maureen, EC., Willingham GL. (1996). Biodegration of TBT by marine bacteria. *Biofueling.*10: 239.

Meng- Pei, H, Shin- Mei, L. (2003). Accumulation of organotin compounds in Pacific Oysters, Crassostrea gigas, collected from aquaculture sites in Taiwan. *Sci Total Env.* 313: 41 -48.

Meng, P., Lin, J., Liu, L., (2009). Aquatic organotin pollution in Taiwan. Journal of Environmental Management. 90: S8-S15.

Meador, JP., Rice, CA. (2001). Impaired growth in the polychaete *Armandia brevis* exposed to TBT in sediments. *Mar Environ Res* 51: 43-29.

Mee, D (1992). The black sea in crisis: A need for concerted international action. *Ambio,* 21(3): 278.

Moetes L., Smaele D., Dams T., Van Den R., Broeck P and Sandra P 1997 Sensitive, simultaneous Determination of organomercury, lead and tin compounds with Headspace solid phase micro extraction capillary gas chromatography combined with Inductively coupled plasma mass spectrometry. *Anal. Chem.* 69: 1604-11.

Montes, B., Camblor M.G., Gaarcia M., Alouso J.L and Janz-Medel., An alternative GC-ICP-MS interface design for trace element speciation. *Journal of* Analytical *Spect.,* 14: 1317-22 (1999).

Morabito, R, Quevauviller, P (2002). Performances of spectroscopic methods for TBT determination the 10 years of the EU-SM and T organotin programme. *Spectroscopy Europe* 14: 18 -28.

Morabito, R In: Mester Z, Sturgeon R (eds) sample preparation for trace element analysis comprehensive Anal. Chem. Vol XLI. *Elsevier, Amsterdam.* 991 (2003)

Morabito, R., P. Massanisso, P and Quevauviller P. (2000). Derivatization methods for the determination of organotin compounds in environmental samples. *Trends in Anal. Chem.*, 19: 113-9.

Morales, M.M., Mart, P Llopis, A. Compos, Land Sagrado, S. (1999). An environmental study by factor Analysis of surface sea waters in the Gulf of Valencia (Western Mediterranean). *Anal. Chim. Acta* 394 (1):109 - 117.

Morillo, J., Usero, J., Gracia, I (2004). Heavy metal distribution in marine sediments fromthe Southwest Coast of Spain. *Chemosphere.* 55:431 - 442.

Mourgand, Y., Martinez, E., Geffard, A., Andradal, B., Stanisiere JY, Amiard JC,(2002) Metallothionein concentration in the mussel *Mytins galloprovincialis* as a biomarker of response to metal contamination: validation in the field. *Biomarkers.* 7 6: 479- 490.

Micic M., Bihari N., Labura Z., Muller WEG, Batel, R (2001). Induction of apoptosis in the mussel *Mytillus galloprovincialis* by tri-n-butyltinchloride. *Aquatic Toxicol* 55: 61- 73.

Muller G (1969) Index of geo-accumulation in sediments of Rhine River. *Geol Journal* **2**:108--118

Nakanish, T (2007). Potential toxicity of organotin compound via nuclear receptor signallingin mammals. *J Health Sci* (5) 1-9.

Nemanic T.M., Milacic R., and Scancar, J. (2007). Critical evaluation of various extraction procedures for the speciation of butyltin compounds in sediments. *Int. J. Environ Anal Chem.*, 87: 615, 25.

Nicholson S, Lam PKS (2005). Pollution monitoring in Southeast Asia using biomarkers inIn the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia). Environ Int (31): 121- 132Ohji M., T. Arai, N. Miyazaki., (2007) Comparison of organotin accumulation in the Masusalmon on corhynchns masou accompanying migratory histories. *Estuainer Coast shelf Sci.,* 72: 721,731.

Okoro HK, Fatoki OS, Adekola FA, Ximba BJ, Snyman RG (2011a). Sources, Environmental Levels and toxicity of organotins in marine environment- a review. *Asian J. of Chem.* 23(2): 473 –482.

Okoro HK, Fatoki OS, Adekola FA, Ximba BJ, Snyman RG, Opeolu B. (2011b). Human Exposure, Biomarkers and Fate of Organotins in the Environment. *Reviews of Environ; Contam and Toxicol Springer.* 213: 27 -54.

Olsson, PE., Kling, P., Hongstrand, C. (1998). Mechanisms of heavy metal contamination and toxicity in fish. In: Langston W J, Bebianno MJ (eds) Metal metabolism in aquatic environments. Chapman and Hall, London 321-350.

Pann R, Anu T, Pia K., Verkasalo K, Satu M., Terttu, V (2008). Blood levels of organotin compounds and their relation to fish consumption and Finland, Sci Total Env http: // dx. doi. org/ 10.101b / J Scitotenv.

Pardo, R., Vega, M., Deban, L., Cazurro, C and Carreretero, C. (2008). Modelling of Chemical Fractional Patterns of metals in soils by two-way and three-way principalcomponent analysis. *Anal Chim Acta* 606:26 -36.

Paton, GI., Cheewasedtham, IL., Marr, Dawson (2006). Degradation and toxicity of phenyltin compounds in soil. *Environ Pollut* 144: 746 – 751.

Perez-lopez M, Alonso J, Novoa-Valinas MC, Melgar MJ (2003) Assessment of Heavy Metal Contamination of Seawater and Marine Limpet, Patella vulgate L., from Northwest Spain. Journal of Environmental Science and Health. Vol A38 (12): 2845-2856.

Peachery, RBJ. (2003). Tributyltin and polycyclic aromatic hydrocarbon levels in Mobile Bay Alabama: a review. *Mar Pollut Bull* 41:1305 – 1371.

Pellegrino C., Massanisso P and Morabito R. (2000). Comparison of twelve selected extraction methods for the determination of butyltin and phenyltin compounds in mussel samples. *Trend Anal. Chem.*, 19: 97-106.

Pe´rez, M., Usero, J.,I and Gracia, I. (1991). Trace metals in sediments from the "Rı´a de Huelva." *Toxicol. Environ. Chem.* 31(32): 275 - 283.

Perez-Lopez M., Alonso J., Novoa-Valinas MC and Melgar J.M. (2003). Assessment of heavy metal contamination of seawater and marine Limpet, Patella vulgate L., from Northwest Spain. Journal of Environ. *Sci and Health.* 38 (12): 2845-2856.

Pereiro IR and A.C Diaz AC (2002) *Ana. Bioanal. Chemi.* 372:74 (2002).

Pempkowlak, J., Sikora, A., Biernacka, E. (1999). Speciation of heavy metals in marine

Sediments VS their bioaccumulation by mussels. *Chemosphere.* 39(2): 313 - 321.

Pessoa M.F, Fernando A, Oliveira (2001). Use of imposex (Pseudohermaphroditism) asindicator of the Occurrence of Organotin Compounds in Portuguese Coastal Waters-Sado and Mira Estuaries. *John Wiley and Sons, Inc.* 234 -241.

Petrovic S., Ozretic B., Krajnovic- Ozretic M., Bobinac, D (2001). Lysosomal membranestability and metallothionein in digestive gland of mussels (*Mytilus galloprovincialis*) as biomarkers in a field study. *Mar Pollut Bull* 42 12: 1373 -1378.

Pfeiffer$_S$, Doris S. and Dippner, JW (2005). Effects of temperature and salinity on Acetylcholinesterase activity, a common Pollution biomarker, in *Mytilus* sp. from the south-western, Baltic Sea. *J Exp Mar Bio Ecol* 320 1: 15 93 -103.

Pizzaro, I., Gomez, M., Camara, C. and Palacios, M.A (2003). Arsenic speciation in Environmental and biological samples –Extraction and stability studies. *Anal. Chim. Acta.* 495: 85-98.

Puri, BK, Murioz-Olivers, R, Camara, C. (2004). A new polymeric adsorbent for screening and Pre-concentration of organotin compounds in sediments and seawater samples. Spectr. *Chimica. Acta. Atomic. Spect.* 59 (2): 209 -214.

Quevanviller P., Bruchet, A., O.F.X. and Donard O.F.X. (1991). Leaching of organotin compounds from poly (Vinchloride) (PVC) material. *Appl. Organo. Chem.,* 5: 125,129 (1991).

Quevauviller, P and Olazabal, J (2002). Links between the water framework directive, the thematic strategy Soil protection and Research trends with focus on pollution issues. *J. of soil and Sed.* 3(4): 243 - 244.

Rao, SM., Mamatha, P. (2004). Water quality in sustainable water management, Current science. 87(7).

Raspor B, Dragun Z, Erk M, Ivankovie D, Pavii J (2004) Is the digestive gland of *Mytilus galloprovincialis* a tissue of choice for estimating cadmium exposure by means of Metallothioneins? *Sci Total Env* 333 1- 3: 99 -108.

Ravisankar R, Senthikumar G, Kiruba S, Chandrase karan A J, Prince P (2010) Mineral Analysis of coastal sediment samples of Tuna, Gujarat, India. India J of Sci and Technol 3(7): 774- 780.

Rebeiro C.A.O, Schwartzman M., Silva de Assis H.C, Silva P.H, Pelletier E. and Alkaishi M. (2002). Evaluation of TBT subchronic effects in tropical freshwater fish (*Astyanax bimaculatus*, Linnaeus, 1758). *Ecotoxicol Environ* Saf 51 (3): 161 -167.

Rees, CM, Brachy BA, Fabus, GJ. (2001). Incidence of imposex in Thais orbital from Port Phillip Bay (Victori, Australia), following 10years of regulation on use of TBT. *Mar Pollut Bull* 42 10 : 873 – 878.

Ren, NH., Wang, JD., Zhang, XL. (2006). Assessment of soil lead exposure in children in Shenyang, China. Environmental Pollution. 144(1): 327-335.

Reinecke, SA., Reinecke, AJ., (1999). Lysosomal response of earthworm coelomocytes induced by long-term experimental exposure to heavy metals. Pedobiologia. 43: 585- 593.

Riba, I., García-Luque, E., Blasco, J. and Del Valls, T.A. (2003). Bioavailability of heavy metals bound to estuarine sediments as a function of pH and salinity values.15 (4), 101 – 114.

Riba I Delvalls TA Forja JM Gomez-Parra A (2004) The influence of pH and salinity on the Toxicity of heavy metals in sediments to the estuarine *Calm Ruditape Philippinarium Environmental Toxicology and Chemistry.* 23 (5), 1100-1107.

Riba, I., García-Luque, E., Maz-Courrau, A., de Canales, G. M. L.M. and DelValls, T.A.(2010). Influence of Salinity in the Bioavailability of Zn in Sediments of the Gulf of Cadmium (Spain).*Water Air and Soil Pollution.* 212: 329 - 336.

Riedel, GF (1993). The annual cycle of Arsenic in a Template ESstuary. Estuaries 16(3A): 533-540.

Ridout, P. S and Jones, H. R. (1988). Determination of trace elements in marine reference materials of Lobster Hepatopanceas (TORT - 1) using inductively coupled plasma mass spectrometry. *Analyst.* 113: 1383 - 1386.

Ringwood AH., Conners, DE., Hoguet, J (1998). Effects of natural and anthropogenic stressors on lysosomal destabilization in oysters *Crassostrea virginica* . Marine Ecology Ser. 166: 163-171.

Rodriguez -Ortega MJ, Alhama T, Fues V, Romero-Ruiz A, Rodriguez -Ariza A, Lopez-Barea J (2002) Biochemical's biomarkers of pollution in the clam chamaclea Gallina From South- Spanish littoral. *Environ Toxicol Chem* 21:542 -549.

Roeva, N.N, Sidrov AV, Ymovitskii YG. (1999). Metallothioneins, proteins binding Heavy Metals in fish. *Bio Bull* 26 6: 617 -622.

Romeo Met al., (2005) "Biomonitoring of trace metals in the Black Sea (Romania) using mussels *Mytilus galloprovincialis*," Water Res., vol. 39, pp. 596-604.

Ross, A. Industrial applications of organotin compounds. Ann. N.Y *Acad. Sci.*, 125; 107 (1965).

Ross KS, Cooper N, Bidwell JR, Elder J (2002) Genetic diversity and metal tolerance of two Marine species: Comparisons between populations from Contaminated and reference sites. *Mar Pollut Bull* 44: 671 – 679.

Ruiz, JM., Bachelet, G., Canmett, P., Donard, OFX. Enviro. *Poll.* 93: 195-203.

Ruiz, J.M., Bryan, G.W. and Gibbs, P.E. 1995. Effects of TBT exposure on the velyer larvaedevelopment of the bivalve Scrobiculana plana (da costa). *Journal of Experimental Marine Biology and ecology*. 186: 53 -63.

Russell JD (1987) Infrared methods, a hand book of determinative methods in clay mineralogy, Wilson MJ(Ed)., Blackie & Son Ltd, NY. Pp: 11-67.

Sadiki AI. and Williams, DT (1999). A study on organotin levels in Canadian drinking water distributed through PVC Pipe. Chemosphere 38: 1541 -1548.

Sahuquillo, A., Rigol, A., Rauret, G. (2003). Overview of the use of leaching, extraction tests for risk assessment of trace metals in contaminated soils and sediments. *Trend Analytical Chemistry* 22(3) 152 - 159.

Sakai, H., Kojima, Y., and Saito, K (1986) Distribution of heavy metals in water and sieved sediment in the Toyoher River, *Water Res.* 20: 559 - 567.

Sakao, S.Y., Ogawa, Y. and Uchida, H. (1999). Determination of trace elements in seaweed samples by inductively coupled plasma mass spectrometry. *Anal. Chim. Acta.* 355: 121 - 127.

Sander, H.I., Thoomen, V. Berth- Jan, Van Koton, G. (2005). Synthetic aspects of Tetraorganotins and organotin (V) halides. *Journal of organometallic Chemistry.* (689): 2145-2157.http: / dspace –test. Library.Uu.nl/kew/ Chem/ 2005-0426-083436// 3093.Pdf.

Sarkar A, Ray D, Amulya, NS Subhodeep S (2006). Molecular Biomarkers; Their significant and application in marine pollution monitoring: *Ecotoxicology* 15: 333 –340.

Sarradin PM, Lapaquellerie Y, Astruc A, Lactouche C, Astruc, M. (1995). *Sci Total Env* 170: 59.

SAS, 9. (2002). SAS Institute Inc., Cary, NC, USA.

Saavedra, A, Gonzalez, P, Fernandez, J, Blanco Y, "The effect of size on trace metal levels in raft cultivated mussels (*Mytilus galloprovincialis*)," The Sci of the Total Environ, vol. 318, pp. 115-1124, 2004.

Schroth W., Ender A. and Schierwater B. (2005). Molecular Biomarkers and adaptation to Environmental stress in Moon Jelly (*Aurelia spp.*). *Mar Biotech* (7): 449- 461.

Schubert P., Rosenberg E., and Grasserbauer M Comparison of sodium tetraethylborate and sodium tetra (n-propyl) borate and derivatization reagent for the speciation of organotin and organolead compounds in water samples. Fresenius. *J. of Anal. Chem.* 366. 350, 360.

Sentosa AP, Bachri M. and Sadijah, A. (2009). Speciation of organotin compounds with Ion-Pair-Reversed Phase Chromatography Technique. *Eurasian J Anal Chem* (EJAC) 4 2: 215 - 225.

Serra, R., Isani, G., Tratamontano, G., Carpene, E. (1999)"Seasonal dependence of cadmium accumulation and Cd-binding protein in *Mytilus galloprovincialis* exposed to cadmium. ," *Comp. Biochem. Physiol*, vol. 123C, pp. 165-174,

Sergi D, Silva L, Panta V, Damia Barcelo, Josep MB. (2005). Survey of organotin compounds in rivers and coastal environment in Portugal 1999- 2000. Environ Pollut (136) 525 – 586.

Schintu, M. and Degetto, S. (1999). Sedimentary records of heavy metals in the industrial harbour of Portovesme, Sardinia (Italy). Science of the Total Environment, 241 129-141.

Shuping SL., Snyman, RG. Odendaal JP., Ndakidemi PA (2011). Accumulation and Distribution of Metals in *Bolboschoenus maritimus* (Cyperaceae), from a South African River. Water Air. Soil. Pollut. 216: 319-328.

Smaele, D., Moens, T., Dams, L., Sandra R., Vandereycken P. and Vandy-ck J., Jis., (1998) Sodium Tetra (n-propyl) borate: a novel aqueous in situ derivatization Reagent for the Simultaneous determination of organomercury, lead and tin compounds with Capillary gas chromatography-inductively coupled plasma mass spectrometry. *J. of Chrom. A.* 793:99-106.

Smith, BS (1981). Tributyltin compounds induce male characteristics on female mud Snails *Nassarius obsoletus (Say) J App Toxicol* 1: 141 – 4.

Snaz-Medel (1998). A trace element analytical speciation in biological systems importance, Challenges and trends. *Spectr. Chim. Acta. B.* 53: 197-211.

SNMMC, 2002. Soil structure and soil structure. A report of Soil Management for Mau County. Archived from tp://www.aehsmag.com/issues/2002/june/soilclays.htm on 02 November 2012.

Snyman, RG., Reinecke, SA., Reinecke, AJ. (2000). Hemocytic Lysosome Response in the Snail *Helix aspersa* After Exposure to the Fungicide Copper Oxychloride. Arch. Environ Contam. Toxicol. 39: 480- 485.

Stanciu, G., Mititelu, M., Gutaga, S., (2004)"Analysis of Heavy metals and organic pollutants from black sea mussels," *Turkey proceedings*, no. 221, pp. 1-3.

South African National Water Act (1998). No 36 Vol 398.

St- Jean, SD., Pelletier, E., Courtenay, SC. (2002). Hemocyte functions and bacteria clearance affected in vivo by TBT and DBT in the blue mussel *Mytilus edulis. Marine Ecology Progress Series. 236; 163-178.*

Stalinon, In: http; // www.in chem.org/document/ech/ech/ech0.15.hm 7/7/2009..

Strand J., Larsen MM., Lockyer, C (2005). Accumulation of organotin compounds and mercuryin harbour porpoises (*phocoena phocoena*) from the Danish waters and West Greenland. SciTotal Env. 350:59-71.

Stoner In: http // www.inchem /ech /ech 0.15.hotmail /7/7 2009

Takahashi S, Mukai H, Tanabe S, Sakayana K, Miyazaki T, Masuno H (1999) Butyltins residues in livers of humans and wild terrestrial mammals and in plastic products. Environ Pollut 106: 213 – 218.

Surent E., et al (2007). Concentrations of cadmium and lead heavy metals in Dardanelles seawater. *Environ Monit and Assess*. 125: 91.

Suzuki, T., Yamada, H., Yamamoto, I., Nishimura, K., Kondo, K., Murayama, M., and Uchiyama, M (1996). Chemical Species of Organotin Compounds in Seawater and Their Seasonal Variations. J. Agric. Food Chem. 44: 3989-3995.

Svendsen, C., Weeks, JM. (1995). The use of a lysosome assay for the rapid assessment of cellular stress from copper to the freshwater snail *Viviparus contectus* (Millet). Marine Pollution Bulletin. 31(1-3): 139-142.

Svendsen, C., Weeks, JM (1997). Relevance and applicability of a simple earthworm biomarker of copper exposure. I . Links to ecological effects in a laboratory study with *Eisenia andrie*. Ecotoxicol Environ Saf. 36: 72 -79.

Takahashi S, Tanabe, S, Kubodera, T (1997). Butyltin residues in deep-sea organisms collected from Suruga Bay, Japan. *Environ Sci Technol* 31:3103 – 3109.

Takahashi S, Mukai H, Tanabe S, Sakayana K, Miyazaki T, Masuno H (1999) Butyltins residues in livers of humans and wild terrestrial mammals and in plastic products. Environ Pollut 106: 213 – 218.

Tao H., Rajendraw R.B., Quetel C.R., Nakazato T., and Tominaga, M (1999) Tin speciation in the fentogram range in open ocean seawater by gas chromatography inductively coupled plasma mass spec using a shield torch at normal plasmas Conditions. *Anal. Chem.,* 71: 4208-15.

Templeton, D. M., Ariese, F., Cornels, R. (2001) IUPAC guidelines for terms related tochemical Speciation and Fractionation of elements. *Pure. Appl. Chem.* 72: 1453 –1470.

Tessier, A., Campbell, P.G.C. and Bisson, M., (1979). Sequential extraction procedures for the speciation of Particulate trace metals. *Anal Chem.* 51: 844 - 851.

Thompson, SA. Luoma, SN. Johansson, CE. and Cain, DJ. (1984). Comparison of sediments and organisms and organisms in identifying sources of biologically available trace metals concentrations. Water Research, 18 (6), 755- 765.

Tsuda T, Inone T, Kojima M, Aoki S (1995) Daily intakes of tributyltins and Triphenyitin Compounds from metals. J ADAC Int. 78: 941 – 943.

Tsunoi, S, Matoba, T; Shioj, H; Huong- Giang, L.T; Harino, H; Tanaka, M. 2002. *J. of chro., A.* 962: 197.

Turkish Environmental Guideines (TSE-266) (1988) Publication of Turkish Foundation of Environmentw

Turkish Food Codes, TFC, Official Gazette, no. 24885., 23 September 2002.

Turkoglu M., Parlak H., Buyukisik B (1992). A comparative study on Crtot concentrations of water, sediment and some benthic organisms of Izmir Bay. In : 2002 Rapp Comm Int Mer Medit (International symposium). 33: 86 Trieste, Italy: CIESM.

Tutschku S., Mothers S., and Wennrich R. (1996). Preconcentration and determination of Sn and Pb –organic species in environmental samples by SPME and GC /AED Fresenius. *.J. of Anal. Chem.,* 354: 587- 591.

Ugur, A. Yener, G., Basasari, A.(2002) "Trace metals and Po , Pb concentrations in mussels (*Mytilus galloprovincialis*) consumed at western Anatolia," *Appl. Radiat.Isotopes*, vol. 257, pp. 565-571.

UNEP, (1985). "Reference Methods for Marine Pollution Studies, Determination of total Hg in marine sediments and suspended solids by cold vapour AAS," vol. 26.

US. United State Code (1988) Organotin paint controls. http:// uscodehouse. Gov/download / Pls/ 33C37.txAccessed. 2007.

USEPA (1996) Method 3050B, Acid digestion of sediments, sludge, and soils. Revision 2, December 1996. Newyork.

US Environmental Protection Agency. Office of the Air quality planning and standards. Bioaccumulation summary –Tributyltin. Available at http: // www.epa.gov/ ttu/ uatw/ hlthef/ eth-phth.html. Updated April 2001.

Usero, J., M. Gamero, M., Morrillo, J., Gucia, I (1998) Comparative study of the sequential. Extraction Procedures for metals in marine sediments. *Environ. Int.* 24: 487 - 496.

Valkirs, AO., Seligman, PF., Haslbeck, E, Caso JS. (2003). Measurements of copper release rates from antifouling paint under laboratory and insitu conditions: Implications for loading estimation to marine water bodies. *Mar Pollut Bull* 46: 763 -779.

Van den Hurk, P. Eertman, RHM. and Strokhorst, J. (1997). Toxicity of harbour canal sediments before dredging and after off-shore disposal. Marine Pollution Bulletin, 34 (4), 244- 249.

Van- Gestel, CAM., Van Brummelen, TC (1996). Incorporation of the biomarkers concept in Ecotoxicology calls for a redefinition of terms. *Ecotoxicology* 5: 217- 225. -225.

Vella AJ., Mintoff B and K.V Axia, *Sci. Tot. Environ.* 258: 81(2000). Vidal MJL, Vega B, Arrebola FJ, Gonzalez Rodriguez, MJ, Morales Sánchez MC, Garrido

Vidal MJL., Vega B., Arrebola FJ., Gonzalez Rodriguez MJ., Morales Sánchez MC., Garrido Frenich, MC. (2003) Trace determination of organotin compounds in water, sediment and mussel samples by low-pressure gas chromatography coupled to tandem mass Spectrometry. Rapid Comm. Mass Spectrometry. 17 (18), 2099-2106.

Visoottiviseth P, Kruawan K, Bhumiratana A, Wileirat, P (1995). Isolation of bacterial culture capable of degrading triphenyltin pesticides. *Appl Organic Chem* 9: 1-9.

Wadlock, MJ. and Thain, JE. (1983). Shell thickening in *Crassotreagigas*. Organotin antifouling or sediment induced. *Mar. Pollut. Bull.* 14 411-415.

Wagner, G. (1993). Plants and soils as specimen types from terrestrial ecosystems in the environmental specimen banking program of the Federal Republic of Germany. *Sci Total Env* 139-140, 213-224.

Weeks, JM., Svendsen, C. (1996). Neutral red retention by lysosomes from earthworm (Lumbricus rubellus) coelomocytes: a simple biomarker of exposure to soil copper. Environ Toxicol Chem . 15: 1801-1805.

Wepener, V. and Vermeulen, L.A (2005). A note on the concentrations and bioavailability of selected metals in sediments of Richards Bay Harbour, South Africa. *Water SA* 31(4): 589 - 595.

Wenshi, LGB (1983) Quantitative infrared spectroscopic analysis of calcite, feldspar and Quartz in Loess. J of Acta Mineralogical Sinica. 1: 01-009.

Williamson, R., Van Dam, L.F., Bell, Grenn, M.O. and Kim, J.P. (1996). Heavy metal and suspended sediments fluxes from a contaminated intertidal inlet (Manukau Harbour, New Zealand). *Mar Pollut Bull.* 32(11): 812 - 822.

World Health Organization., (1990) Tributyltin compounds. UNEP WHO, Environmental Health Criteria 116, WHO, Geneva, Switzerland.

World Health Organization (WHO) (1993). 41st Report of the join Expert committee on food additives (JEFCA).

WHO-IPCS (1999). World Health Organization. International Programme on Chemical Safety. Tributyl Compounds. Environmental health Criteria 116. Http: // www. In chem.org/Documents ehc/ehc /ehc/ 116. Htm Assessed 2007.

Worldwide fund for Nature (1995). Marine Pollution by Triorganotins. Marine update WWF UK Godalining. P 4.

Yilmaz, S. and Sadikolglu, M. (2011). Study of heavy metal pollution in seawater of Kepez harbour of Canakkale (Turkey). *Environ Monit Assess.* 173: 899-904.

Yuan, C., Shi, J., He, B., Liu, J., Liang, L. and Jiang, L (2004). Speciation of heavy metals in marine sediment From the East China Sea by ICP-MS with sequential extraction. *Environ Int.* 30: 769 - 783.

Zhang, G, Yan J, Fu JM., Parker A, Li X.D., Wang, ZS. (2003). Butyltins in sediments and Biota from the Pearl River Delta, South China. *Chem Spec Bioavail* 14 35 -42.

Zhang, M., Wang, M., Xuebao, T, (2003), Potential leachability of heavy metal in urban soils from Hangzhou City. *Acta Pedological Sinica*. 40(6):915 - 920.

APPENDIX A: XRD DIFFRACTOGRAM FOR LOCATIONS 1-9

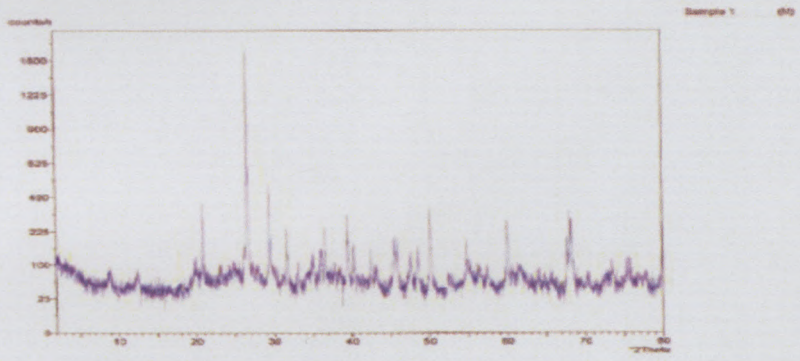


Fig. VIII Powder diffraction pattern of location 1

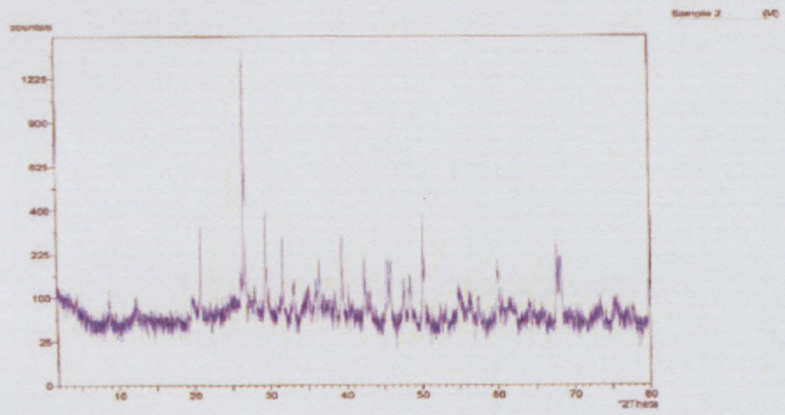


Fig. IX Powder diffraction pattern of location 2

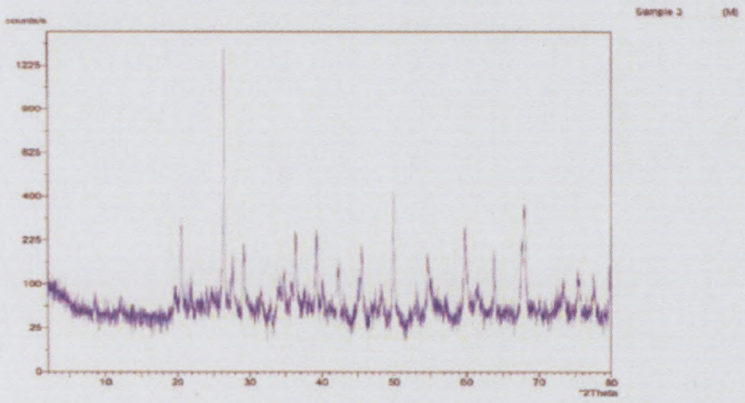


Fig. X Powder diffraction pattern of location 3

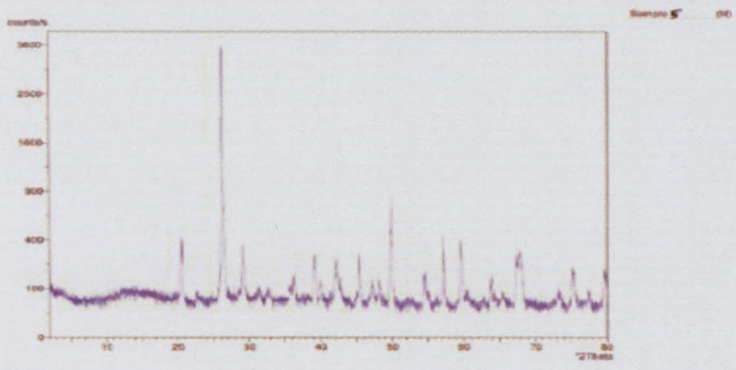


Fig. XI Powder diffraction pattern of location 4

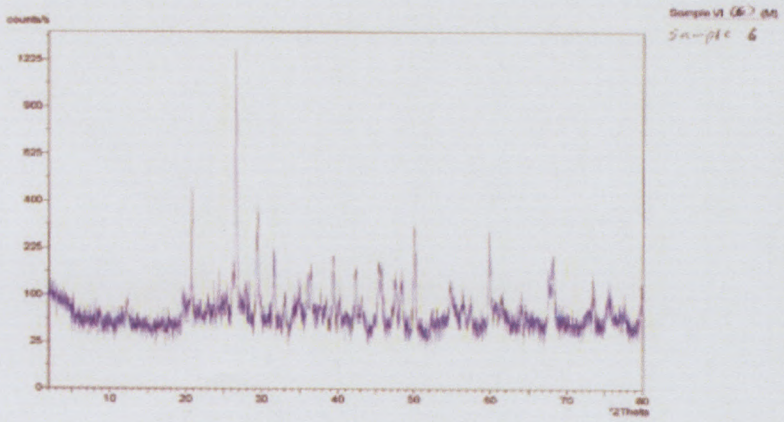


Fig. XII Powder diffraction pattern of location 5.

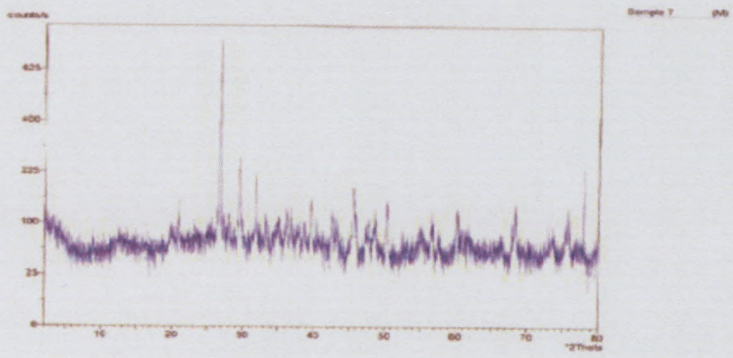


Fig. XIII Powder diffraction pattern of location 6

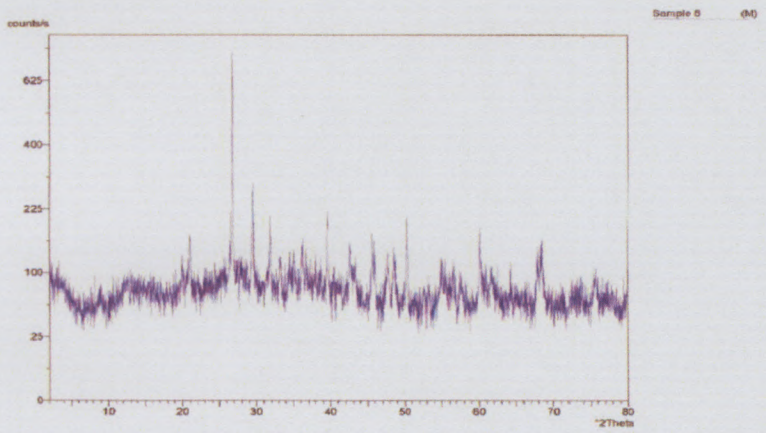


Fig. XIV Powder diffraction pattern of location 7

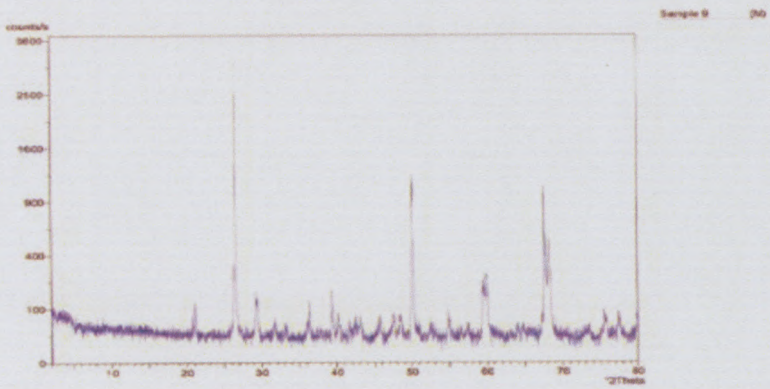


Fig. XV Powder diffraction pattern of location 8

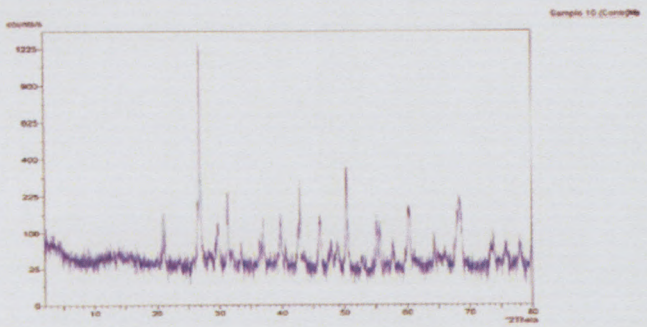


Fig. XVI Powder diffraction pattern of location 9

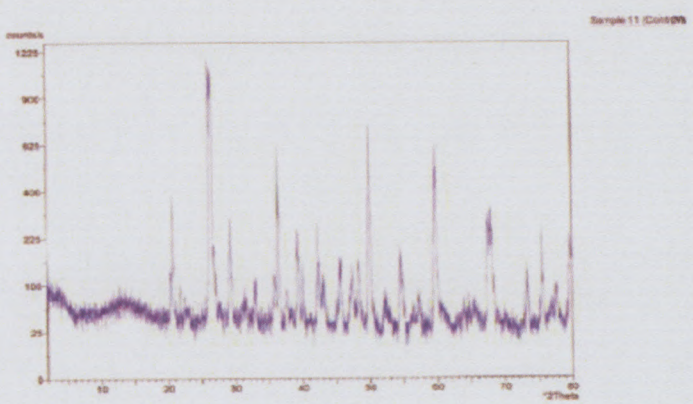


Fig. XVII Powder diffraction pattern of location 10

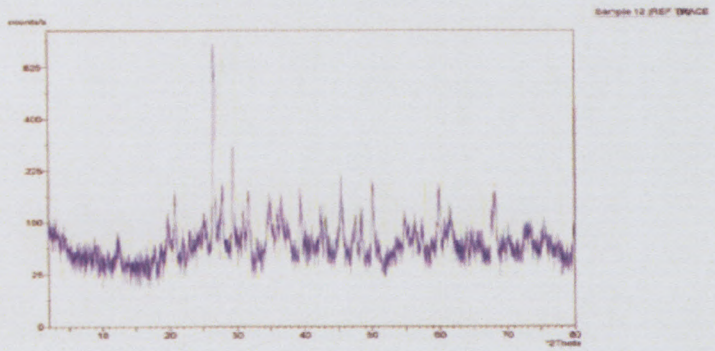


Fig. XVIII Powder diffraction pattern of location 11

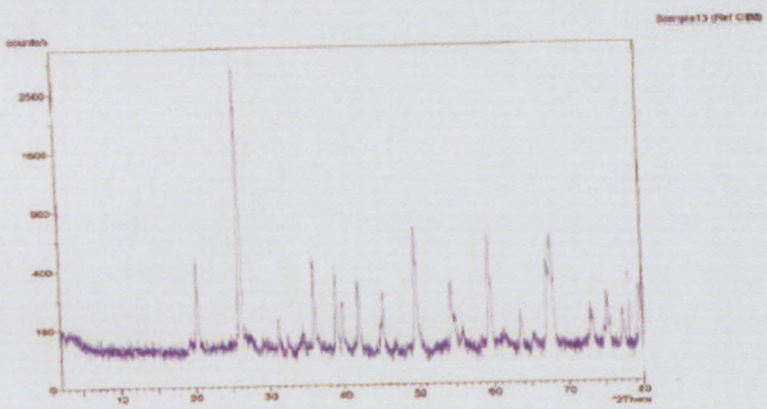


Fig XIX. Powder diffraction pattern of certified trace element.

TYPICAL XRD DATAS FROM PXRD INSTRUMENT FOR LOCATION 4

HR-XRDScan
FileName, w11.x00
FileDateTime, 24-Oct-2012,11:55
Sample, w11
Reflection, 0 0 0
Wavelength, 1.540560
GenkVmA, 10, 10
Omega, 18.75250
TwoTheta, 37.5050
X, 0.00
Y, 0.00
Phi, 0.0
Psi, 0.000
ScanType, CONTINUOUS
ScanAxis, 2Theta/Omega
FirstAngle, 5.01500
ScanRange, 64.98000
StepWidth, 0.03000
TimePerStep, 0.40
NrOfData, 2167
ScanData

Cps	2theta
77.5	5.015
57.5	5.045
57.5	5.075
45	5.105
52.5	5.135
50	5.165
55	5.195
75	5.225
50	5.255
50	5.285
70	5.315
65	5.345
35	5.375
52.5	5.405
82.5	5.435
50	5.465
60	5.495
35	5.525
50	5.555
72.5	5.585
65	5.615
50	5.645
50	5.675
67.5	5.705
45	5.735
47.5	5.765
72.5	5.795
47.5	5.825
55	5.855
57.5	5.885
47.5	5.915
55	5.945
65	5.975
52.5	6.005
52.5	6.035
75	6.065
55	6.095
65	6.125
65	6.155
47.5	6.185
65	6.215
50	6.245
50	6.275
62.5	6.305
57.5	6.335
47.5	6.365
45	6.395

55	6.425
42.5	6.455
47.5	6.485
40	6.515
42.5	6.545
60	6.575
65	6.605
35	6.635
57.5	6.665
65	6.695
50	6.725
62.5	6.755
60	6.785
62.5	6.815
37.5	6.845
52.5	6.875
55	6.905
57.5	6.935
45	6.965
67.5	6.995
55	7.025
50	7.055
30	7.085
32.5	7.115
42.5	7.145
42.5	7.175
45	7.205
45	7.235
32.5	7.265
47.5	7.295
42.5	7.325
62.5	7.355
62.5	7.385
27.5	7.415
80	7.445
47.5	7.475
45	7.505
35	7.535
62.5	7.565
67.5	7.595
37.5	7.625
77.5	7.655
65	7.685
62.5	7.715
45	7.745
70	7.775
45	7.805
45	7.835
70	7.865

APPENDIX B

CONTROL		P≤0.05 YES/NO	LOW		P≤0.05 YES/NO	HIGH		P≤0.05 YES/NO
WEEK 0	WEEK 1	NO	WEEK 0	WEEK 1	YES	WEEK 0	WEEK 1	YES
WEEK 1	WEEK 2	NO	WEEK 1	WEEK 2	YES	WEEK 1	WEEK 2	YES
WEEK 2	WEEK 3	NO	WEEK 2	WEEK 3	YES	WEEK 2	WEEK 3	YES
WEEK 3	WEEK4	NO	WEEK 3	WEEK 4	YES	WEEK 3	WEEK 4	YES
WEEK4	WEEK0	NO	WEEK4	WEEK0	YES	WEEK4	WEEK0	YES

Dose- Response Comparison Table over Weeks for Each Exposure Group

	WEEK 1	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	YES

	WEEK 0	P≤0.05 YES/NO
CONTROL	LOW	NO
CONTROL	HIGH	NO
HIGH	LOW	NO

	WEEK 2	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	YES

	WEEK 3	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	YES

	WEEK 4	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	YES

Comparison of Dose- Response value with NRRT Over 4Weeks

Control Group Comparison Table over 4Weeks

Mean week	Concentration	NRR
Mean Week 0	ND	30.000
Mean Week 1	ND	26.333
Mean Week 2	ND	23.333
Mean Week 3	ND	22.000
Mean Week 4	ND	18.000

Low Group Comparison Table over Weeks

Week	Concentration	NRR
Mean Week 0	0	30.000
Mean Week 1	0.020	21.333
Mean Week 2	0.050	22.000
Mean Week 3	0.06	18.000
Mean Week 4	0.080	14.000

High Group Comparison Table over Weeks

Week	Concentration	NRR
Mean Week 0	0	30.000
Mean Week 1	0.30	21.000
Mean Week 2	0.35	22.000
Mean Week 3	0.50	14.000
Mean Week 4	0.70	10.000

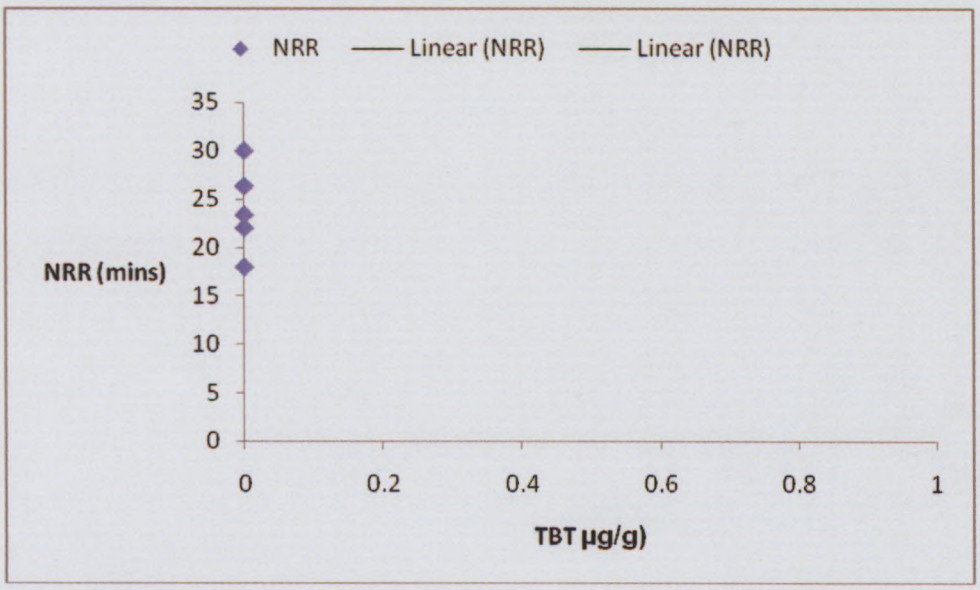


Figure 1: Regression for Control over 4weeks

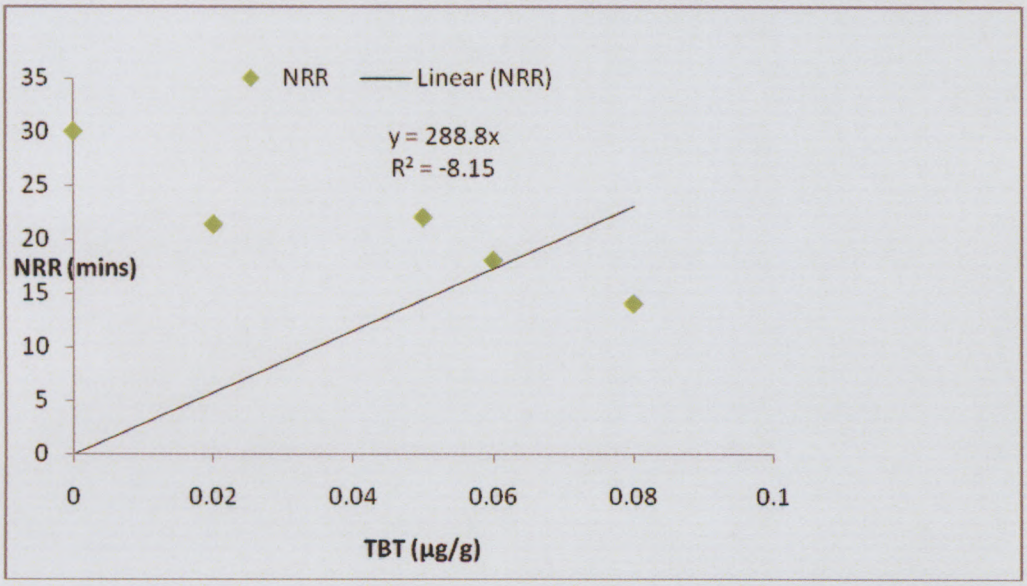


Figure 2: Regression for Low over 4weeks

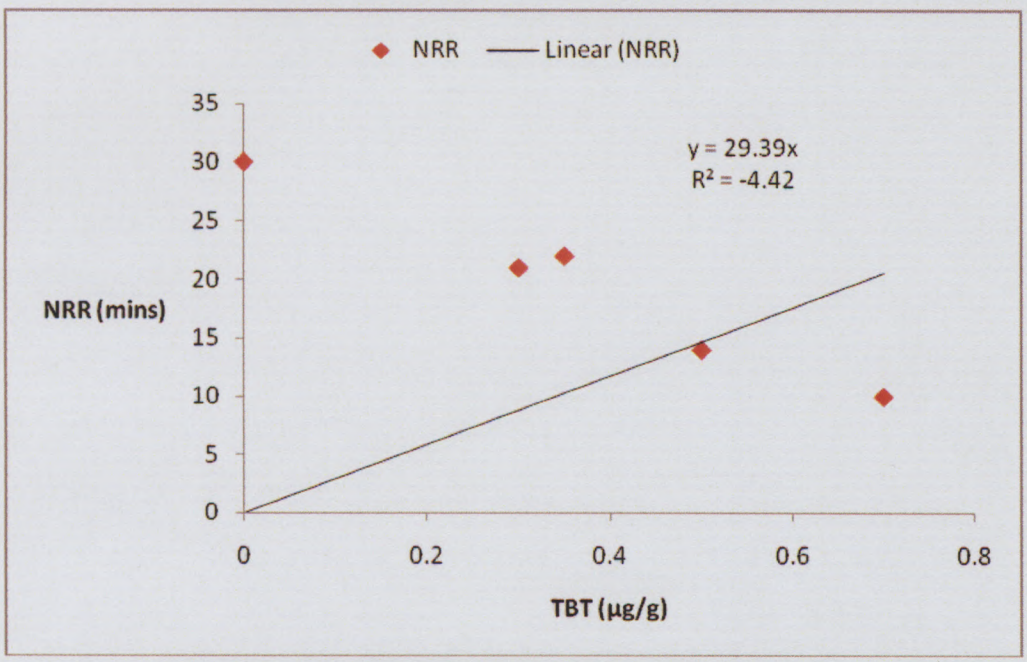


Figure 3: Regression for High over 4weeks

NRRT Results Comparison over Weeks for Neutral Red Assays

CONTROL		P≤0.05 YES/NO	LOW		P≤0.05 YES/NO	HIGH		P≤0.05 YES/NO
WEEK 0	WEEK 1	YES	WEEK 0	WEEK 1	YES	WEEK 0	WEEK 1	YES
WEEK 1	WEEK 2	NO	WEEK 1	WEEK 2	NO	WEEK 1	WEEK 2	NO
WEEK 2	WEEK 3	NO	WEEK 2	WEEK 3	YES	WEEK 2	WEEK 3	YES
WEEK 3	WEEK 4	YES	WEEK 3	WEEK 4	YES	WEEK 3	WEEK 4	YES
WEEK4	WEEK0	NO	WEEK4	WEEK0	YES	WEEK4	WEEK0	YES

NRRT Results Comparison over Weeks for Neutral Red Assays

	WEEK 0	P≤0.05 YES/NO
CONTROL	LOW	NO
CONTROL	HIGH	NO
HIGH	LOW	NO

NRRT Results Comparison for Week

NRRT Results Comparison for Week 1

	WEEK 1	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	NO

NRRT Results Comparison for Week 2

	WEEK 2	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	NO

NRRT Results Comparison for Week 3

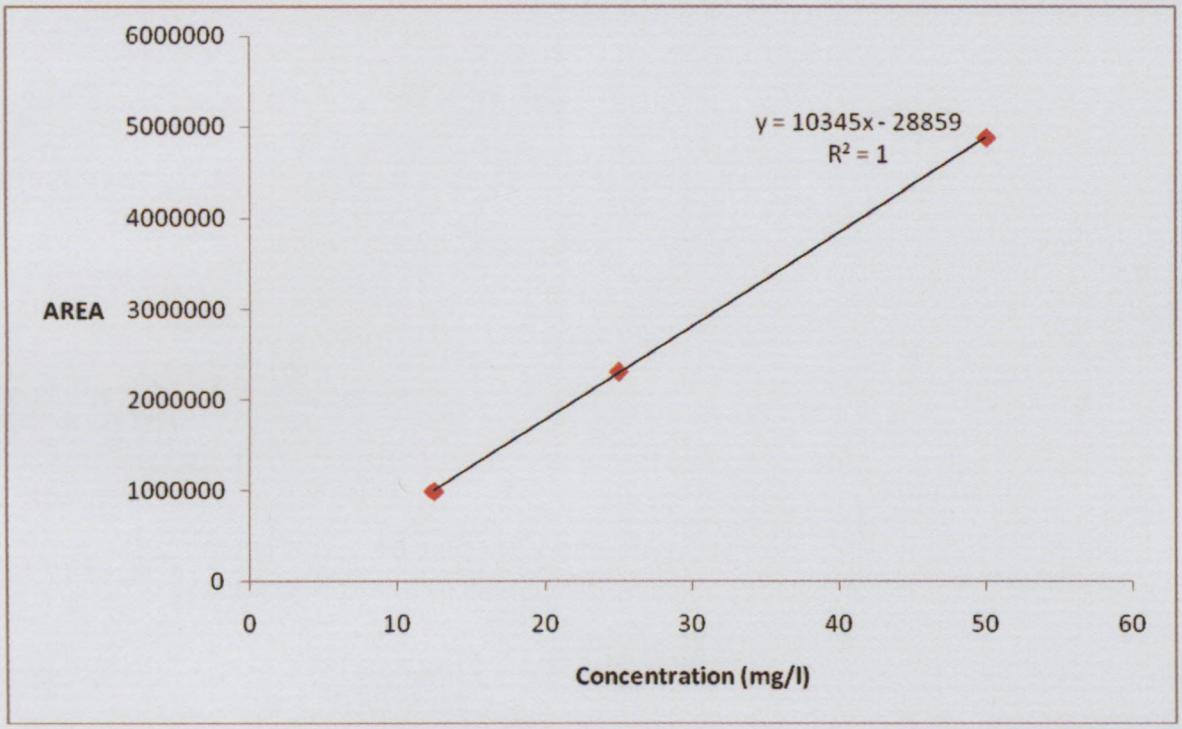
	WEEK 3	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	YES

NRRT Results Comparison for Week 4

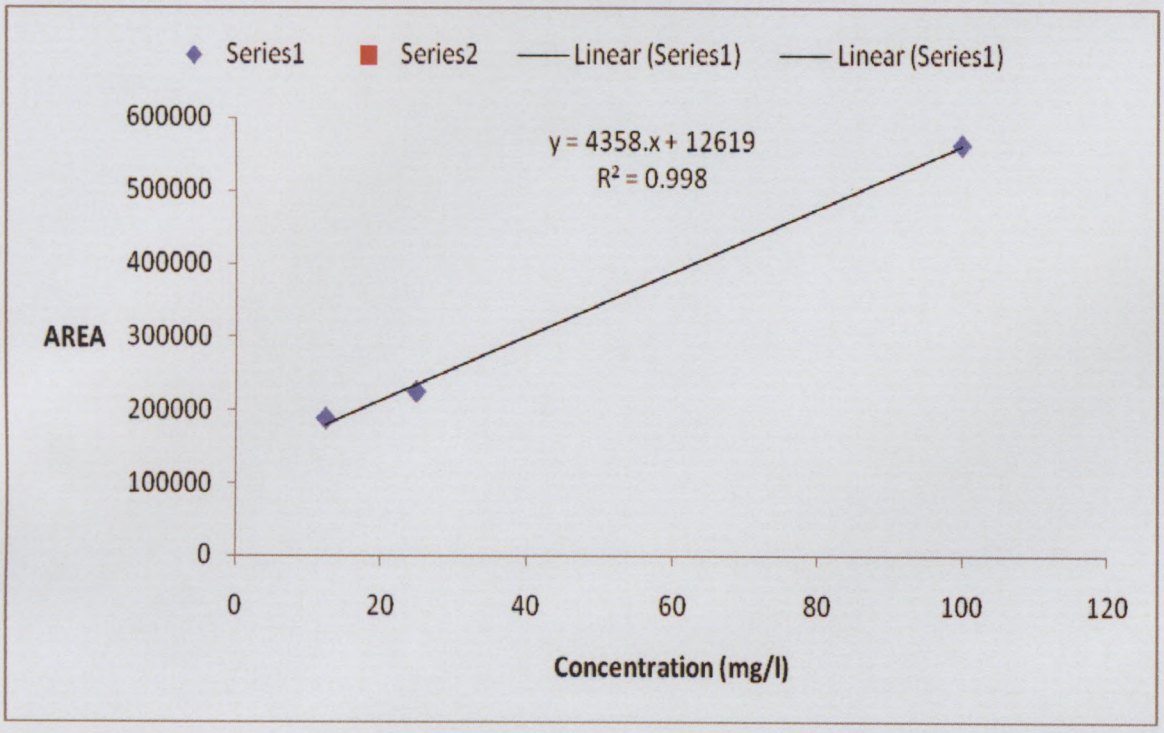
	WEEK 4	P≤0.05 YES/NO
CONTROL	LOW	YES
CONTROL	HIGH	YES
HIGH	LOW	YES

APPENDIX C: CALIBRATION PLOT FOR TPT AND TBT

CALIBRATION FOR TPT



CALIBRATION FOR TBT



Appendix D

Table 1. 1 a: raw data of organotin in sea water from Cape Town harbour

Sampling date	Sampling locations	TBT (µg/l) x 10^{-6}	TPT (µg/l)	Sampling date	Sampling locations	TBT (µg/l)	TPT (µg/l)	Sampling date	Sampling locations	TBT (µg/l)	TPT (µg/l)
SEPTEMBER 23, 2011	1-11	0.750	209.5	March 10,2012	1-11	0.700	366.700	June, 20, 2012	1-11	0.300	0.010
SEPTEMBER 23, 2011	1-12	0.752	209.8	March 10,2012	1-12	0.740	366.900	June, 20, 2012	1-12	0.290	0.010
SEPTEMBER 23, 2011	1-13	0.750	209.000	March 10,2012	1-13	0.730	415.000	June, 20, 2012	1-13	0.280	0.010
SEPTEMBER 23, 2011	2-11	0.010	0.010	March 10,2012	2-11	0.010	166.460	June, 20, 2012	2-11	0.250	0.010
SEPTEMBER 23, 2011	2-12	0.010	0.010	March 10,2012	2-12	0.010	166.480	June, 20, 2012	2-12	0.350	0.010
SEPTEMBER 23, 2011	2-13	0.010	0.010	March 10,2012	2-13	0.010	213.000	June, 20, 2012	2-13	0.310	0.010
SEPTEMBER 23, 2011	3-11	0.605	127.900	March 10,2012	3-11	0.400	68895.00	June, 20, 2012	3-11	0.010	0.010
SEPTEMBER 23, 2011	3-12	0.258	127.500	March 10,2012	3-12	1.120	68896.00	June, 20, 2012	3-12	0.010	0.010
SEPTEMBER 23, 2011	3-13	0.500	127.600	March 10,2012	3-13	0.550	68896.00	June, 20, 2012	3-13	0.010	0.010
SEPTEMBER 23, 2011	4-11	0.343	476.9	March 10,2012	4-11	0.800	0.010	June, 20, 2012	4-11	0.250	0.010
SEPTEMBER 23, 2011	4-12	0.326	0.010	March 10,2012	4-12	0.700	0.010	June, 20, 2012	4-12	0.240	0.010
SEPTEMBER 23, 2011	4-13	*0.350*	0.010	March 10,2012	4-13	1.780	0.010	June, 20, 2012	4-13	0.270	0.010
SEPTEMBER 23, 2011	5-11	1.570	0.010	March 10,2012	5-11	970.000	0.010	June, 20, 2012	5-11	0.280	133.000
SEPTEMBER 23, 2011	5-12	14.530	0.010	March 10,2012	5-12	0.010	0.010	June, 20, 2012	5-12	0.300	0.010
SEPTEMBER 23, 2011	5-13	14.500	0.010	March 10,2012	5-13	0.010	129.800	June, 20, 2012	5-13	0.41	143.300
SEPTEMBER 23, 2011	6-11	0.514	0.010	March 10,2012	6-11	0.010	0.010	June, 20, 2012	6-11	0.010	0.010
SEPTEMBER 23, 2011	6-12	0.280	0.010	March 10,2012	6-12	0.010	0.010	June, 20, 2012	6-12	0.010	0.010
SEPTEMBER 23, 2011	6-13	0.507	0.010	March 10,2012	6-13	0.010	140.020	June, 20, 2012	6-13	0.010	0.010
SEPTEMBER 23, 2011	7-11	0.500	0.010	March 10,2012	7-11	0.010	0.010	June, 20, 2012	7-11	0.190	0.010
SEPTEMBER 23, 2011	7-12	0.454	0.010		7-12	0.010	0.010		7-12	0.250	0.010

Table 1.1 b: Raw data of organotin in sea water from Cape Town harbour

Sampling date	Sampling locations	TBT (µg/l) x 10^{-6}	TPT (µg/l)	Sampling date	Sampling locations	TBT (µg/l)	TPT (µg/l)	Sampling date	Sampling locations	TBT (µg/l)	TPT (µg/l)
SEPTEMBER 23, 2011	7-13	1.162	0.010	March 10,2012	7-13	0.000	152.590	June, 20, 2012	7-13	.0.29	0.010
SEPTEMBER 23, 2011	8-11	0.223	0.010	March 10,2012	8-11	0.800	0.010	June, 20, 2012	8-11	0.010	0.010
SEPTEMBER 23, 2011	8-12	0.010	0.010	March 10,2012	8-12	0.780	0.010	June, 20, 2012	8-12	0.010	
SEPTEMBER 23, 2011	8-13	0.010	0.010	March 10,2012	8-13	0.190	137.000	June, 20, 2012	8-13	0.010	0.010
SEPTEMBER 23, 2011	9-11	0.010	0.010	March 10,2012	9-11	0.000	0.010	June, 20, 2012	9-11	0.17	0.010
SEPTEMBER 23, 2011	9-12	0.010	0.010	March 10,2012	9-12	0.000	15680.00	June, 20, 2012	9-12	0.19	0.010
SEPTEMBER 23, 2011	9-13	0.010	0.010	March 10,2012	9-13	0.000	270.600	June, 20, 2012	9-13	0.18	0.010
SEPTEMBER 23, 2011	10-11	0.169	0.010	March 10,2012	10-11	0.000	0.000	June, 20, 2012	10-11	0.010	0.010
SEPTEMBER 23, 2011	10-12	0.160	0.010	March 10,2012	10-12	6.390	36900.000	June, 20, 2012	10-12	0.010	0.010
SEPTEMBER 23, 2011	10-13	0.120	0.010	March 10,2012	10-13	2.750	136.260	June, 20, 2012	10-13	0.010	0.010
SEPTEMBER 23, 2011	11-11	0.010	0.010	March 10,2012	11-11	1.130	0.010	June, 20, 2012	11-11	0.21	0.010
SEPTEMBER 23, 2011	11-12	0.010	0.010	March 10,2012	11-12	1.090	2791.700	June, 20, 2012	11-12	0.19	0.010
SEPTEMBER 23, 2011	11-13	0.010	0.010	March 10,2012	11-13	1.840	0.010	June, 20, 2012	11-13	0.18	0.010
SEPTEMBER 23, 2011	12-11	2.664	0.010	March 10,2012	12-11	0.960	0.010	June, 20, 2012	12-11	0.010	0.010
SEPTEMBER 23, 2011	12-12	1.390	0.010	March 10,2012	12-12	0.000	0.010	June, 20, 2012	12-12	0.010	0.010
SEPTEMBER 23, 2011	12-13	1.500	0.010	March 10,2012	12-13	6.310	0.010	June, 20, 2012	12-13	0.010	0.010

Table 2.1 a: Raw data of organotin in sediment samples from Cape Town harbour

Sampling date	Sampling locations	TBT (µg/g) x 10-6	*TPT (µg/g)*	Sampling date	Sampling locations	TBT (µg/g)	TPT (µg/g)	Sampling date	Sampling locations	TBT (µg/g)	TPT (µg/g)
SEPTEMBER 23, 2011	1-11	0.035	*0.010*	March 10,2012	1-11	0.010	0.010	June, 20, 2012	1-11	0.300	0.010
SEPTEMBER 23, 2011	1-12	0.036	*0.010*	March 10,2012	1-12	0.010	0.010	June, 20, 2012	1-12	0.290	0.010
SEPTEMBER 23, 2011	1-13	0.035	*0.010*	March 10,2012	1-13	0.010	0.010	June, 20, 2012	1_13	0.280	0.010
SEPTEMBER 23, 2011	2-11	0.010	*0.010*	March 10,2012	2-11	ND	0.010	June, 20, 2012	2-11	0.250	0.010
SEPTEMBER 23, 2011	2-12	0.010	*0.010*	March 10,2012	2-12	ND	0.010	June, 20, 2012	2-12	0.350	0.010
SEPTEMBER 23, 2011	2-13	0.010	*0.010*	March 10,2012	2-13	ND	0.010	June, 20, 2012	2-13	0.310	0.010
SEPTEMBER 23, 2011	3-11	0.014	*0.010*	March 10,2012	3-11	0.010	0.010	June, 20, 2012	3-11	ND	0.010
SEPTEMBER 23, 2011	3-12	0.012	*0.010*	March 10,2012	3-12	0.010	0.010	June, 20, 2012	3-12	ND	0.010
SEPTEMBER 23, 2011	3-13	0.016	*0.010*	March 10,2012	3-13	0.010	0.010	June, 20, 2012	3-13	ND	0.010
SEPTEMBER 23, 2011	4-11	NF	*0.010*	March 10,2012	4-11	ND	0.010	June, 20, 2012	4-11	0.04	0.010
SEPTEMBER 23, 2011	4-12	NF	*0.010*	March 10,2012	4-12	ND	0.010	June, 20, 2012	4-12	0.02	0.010
SEPTEMBER 23, 2011	4-13	*NF*	*0.010*	March 10,2012	4-13	ND	0.010	June, 20, 2012	4-13	0.04	0.010
SEPTEMBER 23, 2011	5-11	NF	*0.010*	March 10,2012	5-11	ND	0.010	June, 20, 2012	5-11	0.280	0.010
SEPTEMBER 23, 2011	5-12	NF	*0.010*	March 10,2012	5-12	ND	0.010	June, 20, 2012	5-12	0.300	0.010
SEPTEMBER 23, 2011	5-13	NF	*0.010*	March 10,2012	5-13	ND	0.010	June, 20, 2012	5-13	0.41	0.010
SEPTEMBER 23, 2011	6-11	0.0.10	*0.010*	March 10,2012	6-11	0.010	0.010	June, 20, 2012	6-11	2.60	0.010
SEPTEMBER 23, 2011	6-12	0.010.	*0.010*	March 10,2012	6-12	0.010	0.010	June, 20, 2012	6-12	2.30	0.010
SEPTEMBER 23, 2011	6-13	0..010	*0.010*	March 10,2012	6-13	0.010	0.010	June, 20, 2012	6-13	2.50	0.010
SEPTEMBER 23, 2011	7-11	0.024	*0.010*	March 10,2012	7-11	0.149	2.249	June, 20, 2012	7-11	0.190	0.010
SEPTEMBER 23, 2011	7-12	0.020	*0.010*		7-12	0.161	1.953		7-12	0.250	0.010

Table 2.1 b: Raw data of organotin in sediment samples from Cape Town harbour

Sampling date	Sampling locations	TBT (µg/g) x 10^{-6}	*TPT (µg/g)*	Sampling date	Sampling locations	TBT (µg/g)	TPT (µg/g)	Sampling date	Sampling locations	TBT (µg/g)	TPT (µg/g)
SEPTEMBER 23, 2011	7-13	**0.023**	*0.010*	March 10,2012	7-13	**0.161**	**1.93**	June, 20, 2012	7-13	**.0.29**	0.010
SEPTEMBER 23, 2011	8-11	**0.040**	*0.010*	March 10,2012	8-11	**0.029**	0.010	June, 20, 2012	8-11	0.010	0.010
SEPTEMBER 23, 2011	8-12	**0.045**	*0.010*	March 10,2012	8-12	**2.69E-08**	0.010	June, 20, 2012	8-12	0.010	0.010
SEPTEMBER 23, 2011	8-13	**0.046**	*0.010*	March 10,2012	8-13	**2.69E-08**	**0.010**	June, 20, 2012	8-13	0.010	0.010
SEPTEMBER 23, 2011	9-11	ND	*0.010*	March 10,2012	9-11	ND	ND	June, 20, 2012	9-11	**ND**	ND
SEPTEMBER 23, 2011	9-12	ND	*0.010*	March 10,2012	9-12	ND	**ND**	June, 20, 2012	9-12	**ND**	ND
SEPTEMBER 23, 2011	9-13	ND	*0.010*	March 10,2012	9-13	ND	**ND**	June, 20, 2012	9-13	**ND**	ND
SEPTEMBER 23, 2011	10-11	**ND**	*0.010*	March 10,2012	10-11	**9.04E-08**	ND	June, 20, 2012	10-11	ND	ND
SEPTEMBER 23, 2011	10-12	**ND**	*0.010*	March 10,2012	10-12	**8.49E-08**	**ND**	June, 20, 2012	10-12	ND	ND
SEPTEMBER 23, 2011	10-13	**ND**	*0.010*	March 10,2012	10-13	**8.49E-08**	**ND**	June, 20, 2012	10-13	ND	ND
SEPTEMBER 23, 2011	11-11	ND	*0.010*	March 10,2012	11-11	**ND**	ND	June, 20, 2012	11-11	**ND**	ND
SEPTEMBER 23, 2011	11-12	ND	*0.010*	March 10,2012	11-12	**ND**	**0.010**	June, 20, 2012	11-12	**ND**	ND
SEPTEMBER 23, 2011	11-13	ND	*0.010*	March 10,2012	11-13	**ND**	0.010	June, 20, 2012	11-13	**ND**	ND
SEPTEMBER 23, 2011	12-11	**NF**	*0.010*	March 10,2012	12-11	**NF**	0.010	June, 20, 2012	12-11	NF	NF
SEPTEMBER 23, 2011	12-12	**NF**	*0.010*	March 10,2012	12-12	NF	0.010	June, 20, 2012	12-12	NF	NF
SEPTEMBER 23, 2011	12-13	**NF**	*0.010*	March 10,2012	12-13	**NF**	0.010	June, 20, 2012	12-13	NF	NF

ND: Not detected

NF: Not found