ENVIROTRACE OF ORGANOTIN COMPOUNDS IN EASTERN CAPE HARBOUR WATERS

by

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Supervisor: Professor A. Sadimenko

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DECLARATION

I hereby declare that this thesis is my own work and has not been submitted previously for any degree at any University.

....

D. M. KATWIRE

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ACKNOWLEDGMENTS

There are many people I would like to thank for helping me to reach this goal. First and foremost, I would like to thank my wife Lutgard Katwire for her support throughout this process. Without her understanding, patience and perseverance, this work would have been impossible to complete. I wish to extend my appreciation to Prof. O. S. Fatoki who initiated this program to be part of my research project before he left the University of Fort Hare.

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Dedication

I dedicate this research work to my two fallen heroes – my parents who have gone to be with the Lord – Mr. Obadiah Ruhweza and Jeanette Basaasa – for because they were; I am. To my children: Pat, Mark, Rita, Machel, Bryan and Ilanga who had to bear long hours without me as I labored to put this work together.

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Abstract

Increasing public awareness of toxic moieties in our environment has put pressure on the regulatory agencies and the teaching institutions to improve testing methods so that these harmful agents can be identified and quantified at lower levels.

n-Butyltin (MBT), di-*n*-butyltin (DBT) and tri-*n*- butyltin (TBT) were determined in water and sediment samples of marine systems in the two major harbors located at East London and Port Elizabeth in the Eastern Cape Province. The organotin compounds were determined by optimized aqueous phase ethylation with sodium tetraethylborate method. The extracts were derivatized and the ethylated species were analyzed by gas chromatography. The instrument was fitted with a sensitive and selective flame photometric detector for Sn. The optimized method was verified by performing spiking experiments in natural water and seawater matrices and obtained good recoveries and reproducibility. Percentage recoveries in 50ng/L spiked river water ranged from 64.1 ± 2.0 for MBT, 91.1 ± 2.2 for DBT and 113 ± 5.1 for TBT. The limits of detection for the organotin compounds ranged from 4.19 ng/L to 12.5 ng/L. In water samples the content of MBT ranged from 5.05 ng/L – 49.95 ng/L, for DBT ranged from 6.0± 5ng/L – 22.35± 5 ng/L and for TBT ranged from 4.75ng/L to 18.85 ng/L. In sediment samples, it ranged from 0 – 56 ± 1.5 ng/g for MBT and 0 -118 ± 0.6 ng/g for DBT and 4.6 ± 0.01 – 1053 ± 0.32 ng/g for TBT. Moderate levels of organotin compounds were detectable at both harbor sites.

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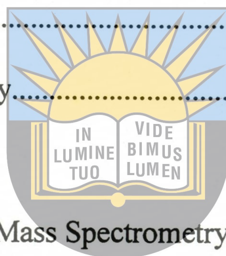

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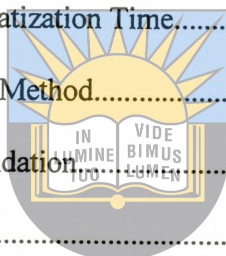

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ABBREVIATIONS AND NOTATIONS

Atm	atmosphere
AES	atomic emission spectrometry
BCF	bioconcentration factor
DBT	di-*n*-butyltin: $Sn(C_4H_9)_2^{2+}$
DCM	dichloromethane
DDTC	diethyl dithiocarbamate
EDC	endocrine disrupting chemicals
EEC	European Economic Commission
ELS	early life stage
EL	East London
ES	Electrospray
EQS	environmental quality standard
Et	ethyl
FPD	flame photometric detector
GC	gas chromatography
HOAc	acetic acid
HP-LC	high performance liquid chromatography
ICP-MS	inductively coupled plasma/mass spectrometry
IPCS	international program on chemical safety
ITMS	ion trap mass spectrometer
JICST	Japan information centre for science and technology
K_{∞}	partition coefficient

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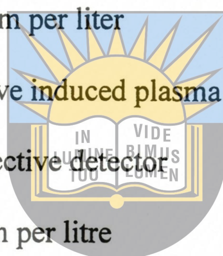
LC	liquid chromatography
LC_{50}	median lethal concentration
LEI	laser enhanced ionization
LLE	liquid - liquid extraction
MAFF	Ministry of Agriculture, Fisheries and Food
MBT	mono-*n*-butyltin: $Sn(C_4H_9)^{3+}$
MDL	method detection limit
µg / L	microgram per liter
MIP	microwave induced plasma
MSD	mass selective detector
ng / L	nanogram per litre
ng /g	nanogram per gram
NIOSH	National Institute for occupational safety and health
nmol	nanomoles
NOAEL	non- observed adverse effect level
ORTEPA	organotin environmental programme association
OTs	organotin compounds
PDMS	polydimethylsiloxane
PE	Port Elizabeth
PMT	photo multiplier tube
PRP	polymeric reversed phase
ppt	parts per trillion
PTFE	polytetrafluoroethylene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PVC	polyvinyl chloride
QF-AAS	quartz furnace atomic absorption spectrometry
RSD	relative standard deviation
SD	standard deviation
SFC	superfluid critical chromatography
SPE	solid phase extraction
SPME	solid phase microextraction
SRAJ	Shipbuilding Research Association of Japan
SRM	certified standard reference materials
STEB	sodium tetraethylborate
TBT	tri-*n*-butyltin: $Sn(C_4H_9)_3^+$
TBTO	tri-*n*-butyltin oxide
THF	tetrahydrofuran
TPT	tri-*n*-propyltin
TWG	Technical Work Group
US-EPA	United States Environmental Protection Agency
UV	ultraviolet
WHO	World Health Organisation

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Chapter 1

INTRODUCTION AND LITERATURE SURVEY

1.1. BACKGROUND INFORMATION

The increasing global use of organotin compounds in spite of their known toxicity potential has given rise to increasing concern about the environmental impact of these substances and especially on the aquatic ecosystem. The anthropogenic inputs of the derivatives of tin compounds to the aquatic environment are unquestionable (Blunden and Chapman, 1986). It is important to know that tin has the highest number of its organometallic derivatives in commercial use than any other element. Whereas tin in its inorganic form is considered to be non-toxic towards marine biota, however, the toxicological potential of all organotin species, in particular the triaryl compounds, is complex and of growing public concern (Champ and Pugh, 1987)

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Organotin compounds have four substituents on the tin atom with at least one of the substituents joined via a carbon atom to the tin site. Small linear alkyl groups (methyl, butyl or octyl groups) comprise the number of organic moieties classified as mono-, di-, tri-, or tetraorganotin compounds. The functional group ligands modify the functionality and performance of the compounds. These ligands can be small anions like chloride, or hydroxide used as intermediates in the formation of the larger molecules linked covalently through a heteroatom. The nature of the anion influences the physico-chemical properties, such as the relative solubility in water and non-polar solvents, vapor pressure but apparently has little influence on toxicity. It is, however well characterized and allows the prediction of the environmental fate.

1.2 OBJECTIVE

This study was intended to establish the occurrence of organotin compounds in the marine environment from some selected sites on the East London and Port Elizabeth harbors in the Eastern Cape. An attempt was made to assess the levels of tri-*n*-butyltin in water and sediment samples. To develop a simple but efficient optimized GC-FPD method for the analysis of organotin compounds using sodium tetraethyl borate. The optimized aqueous ethylation with sodium tetraethyl borate combined the sample extraction and derivatization to screening of the South African marine environment in the harbors.

1.3 USES AND APPLICATION OF ORGANOTIN COMPOUNDS

Some non-pesticidal organotin compounds such as dialkyl derivatives are widely used as stabilizers for polyvinyl chloride (PVC) plastics (DHHS-NIOSH, 1974) and as industrial catalysts. PVC stabilizers are essentially mono- and di- substituted organotin compounds. The trialkyl tin compounds are used as germicides for pears and onions mollusk eliminators, rodent repellent and as insecticides for wood, textile, paper, leather and glass products. Others uses include antifouling paint for boats, fishing nets, ship bottom and neoprene rubber, germicides for paper, wood, paint, leather, and textile work, as well as the reducing agents. These are also useful as catalysts for manufacture of fireproof polyester, as the hardening agents, waterproofing and antioxidant agents, corrosion inhibitors, and for medical purposes for control of schistosomiasis, and in the case of tri-*n*-phenyltin, as a fungicide. (Van der Kerk, 1978; Muller, 1987; Laughlin and Linden, 1987; Fent, 1996; Bosselmann, 1996). The commercial uses of organotin compounds expanded rapidly during the 1950s. Consequently, their wide application and subsequent spread in the environment need for a thorough knowledge of organotin induced toxicity, especially in depth knowledge of hepatoxicity, neurotoxicity, cutaneous toxicity, and immunotoxicity (Snoeij et al., 1987).

Some organotin compounds such as tri-*n*-butyltin and tri-*n*-phenyltin are the potent endocrine disruptors which affect reproductive hormone and hence lead to reproductive impairment of certain spe-

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cies of marine like mollusks at considerably low level of (1 ng / L) (Morcillo and Porte, *Environ. Pollut.*, 1999; Morcillo and Porte, *Environ. Res.*, 1999). The most pronounced effect studied was the shell malformation in oysters (Alzieu et al. 1989), imposex in gastropods (Bryan and Gibbs, 1991) and mortality of larva, this eventually cascaded into population decline or extinction of species of mollusks and oysters *Crassostrea gigas* (Kime, 1998; Nichols et al., 1999).

The most widely known compounds are tri-*n*-butyltin and triphenyltin (Evans, 1999; Fent et al., 1991), which are extensively used as antifouling boat bottom paints and as an active ingredient of many other products. Tri-*n*-butyltin retards fouling by marine organisms that clings to ship hulls. As a result, the barnacles significantly slow the ship's performance and durability (Bailey, 1986; Bohlander and Montemarano, 1997). The need to prevent fouling on ship's hulls was undisputedly and universally recognized as important for the efficient commerce, making it cost effective and minimizing the environmental impact on shipping. Since tri- *n*-butyltin started to be used as biocide in antifouling paints, it serves to keep barnacles and other organisms off the hulls. Environmental damage occurred when TBT became responsible for toxic effect at different aquatic trophic levels (Alzieu, et. al., 1991; Iwata et al., 1995; Moore et al., 1991).

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1.4 HISTORICAL PERSPECTIVEAND USES OF ORGANOTIN COMPOUNDS

Frankland prepared one of earliest synthetic organotin compound in 1849, which he later characterized as diethyltin diiodide (Frankland, 1849). The first commercial organotin compound was however registered in 1936. In the 1940's, several dialkyl compounds were patented as PVC stabilizers. Di-*n*-butyl tin is used in PVC plastics. It was in the early 1950s that researchers noted the biocidal properties of tri-*n*-butyltin compounds. Tri-*n*-butyltin was found to be highly effective against gram – positive bacteria but less toxic to mammals. This eventually led to the first registration of tri-*n*-butyltin as wood preservatives in 1958. Since then tri-*n*-butyltin have been used to protect paper, textiles, leather, feathers, and numerous types of polymers against bacteria and fungus. Tri-*n*-butyltin compounds are also

used as a biocide in cooling water, as a disinfectant and as a molluscide. In 1960, the use of tri-*n*-butyltin compounds as a molluscide, led to tri-*n*-butyltin oxide (TBTO) being used in antifouling paint for ships, which continues to be the largest use of tri-*n*-butyltin oxide (TBTO). Tri-*n*-butyltin compounds have also found uses in the health field. In tropical climates tri-*n*-butyltin oxide (TBTO) has been used as rubber pellets to control fresh water snail species that are responsible for diseases caused by schistosomiasis or blood flukes. The rubber pellets ensure a slow controlled release of tri-*n*-butyltin at a level, which do minimal damage to other aquatic life forms. Research has been done in using water-soluble forms of tri-*n*-butyltin compounds to combat the spread of diseases such as *Legionella pneumophilia* (Legionnaire's Disease) through air conditioning systems for treatment of cooling water.

Annual tri-*n*-butyltin use in the Netherlands in 1985 was reported to be 1.5×10^4 kg for wood preservation and 1×10^4 kg for antifouling paints (TWG, 1988). Use of organotin antifouling agent in Norway was 1.37×10^5 kg in 1986 for the treatment of nets and sea pens at approximately 600 fish farms (Linden, 1987). In Japan, the usage of TBT was estimated at 1 300 tones in 1987, of which two-third was used for antifouling paints on vessels and one-third for antifouling of nets in fish culture.

Tikkurila Coatings OY Company carried out a survey of total and retail sales of tri-*n*-butyltin containing paints and antifouling preparations for nets in Finland in 1987. Of a total of 42 000 L, 37 000 L were sold on retail in which the concentration of TBT in the antifouling paints was estimated to be between 4 and 18%. The use of TBT as a slimicide or fungicide previously estimated at 2.1 tons per year during the period 1968-1970 before it was discontinued. The estimated sale of wood preservatives containing tri-*n*-butyltin was 130 tons in 1987; these contained between 0.9 and 1.8% of tri-*n*-butyltin. Champ and Pugh (1987) reported that about 300 tons of tri-*n*-butyltin antifouling paints were registered in the USA in 1987, but only about 17 paints are now registered for the same use (EPA, 1988; MAFF / HSE, 1988) listed 345 different wood preservative formulations, 24 surface biocides and 215 antifouling paints containing tri-*n*-butyltin with registration approval for use in the United Kingdom under the

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Control of Pesticides Regulations. In 1989, the number of antifouling paints containing tri-*n*-butyltin registered for use in the United Kingdom had fallen to 148, with the number of wood preservatives and surface biocides remained at about the same 337 and 26 registered products, respectively (MAFF / HSE, 1989).

1.5. GLOBAL PRODUCTION AND DISTRIBUTION OF ORGANOTIN COMPOUNDS

The world consumption of tin in 1976 (Van der Kerk 1976) was estimated to be 2 $\times 10^5$ tons of which 2.8 $\times 10^4$ tons were organotin compounds (Zuckerman et al., 1978). This total is made up of:

- plastic stabilizers and catalysts, approximately 2.0 $\times 10^4$ tons;
- wood preservatives (tri-*n*-butyltin) 3-4 $\times 10^3$ tons;
- antifouling paints (Tri-*n*-butyltin) 2-3 $\times 10^3$ tons;
- other uses of di- and tri-*n*-butyltin, < 2 $\times 10$ tons. The global annual production of tri-*n*-butyltin compounds is estimated to be 4 $\times 10^3$ to 5 $\times 10^3$ tons (ORTEPA 1989).

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Data on global market share of tri-*n*-butyl producers and overall production of organotin compounds per annum are given in Tables 1.1 and 1.2, respectively.

Table 1.1. Global Market Share of Tri-*n*-butyltin Producers

Company	% World	% European Union
JOTUM (Norway)	18	18
HEMPEL (Denmark)	16	18
CHOGOKU (Japan)	14	10
SIGMA COATING (Netherlands)	10	12
AMERON (US)	4	1

Table 1.2. Overall ~ 60% of Production of Organotin Compounds per Annum

Year	Country	Tons	Reference
1971	USA	1×10^4	Luijten (1971)
1968 – 1970	Finland	2.1	Champ and Pugh (1987)
1985	Canada	1×10^3	Thompson et al. (1985)

1.6. GLOBAL REGULATIONS

In December 1979, the Japanese Government banned the use of tri-*n*-butyl tin compounds in certain products for household use e.g., paints, adhesive, wax, shoe polish, and textile products. Following the effects on the oyster industry in France in the late 1970s, and the subsequent correlation of the effects with tri-*n*-butyltin usage, the French government banned the use of tri-*n*-butyltin antifouling paints. In 1982, paints containing more than 3% TBT by mass were banned on boats of less than 25 m in length.

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In January 1986, the United Kingdom enforced regulations that prohibited the retail sale and supply of antifouling paints with a total tin concentration greater than 7.5 % by mass in co-polymer paints. These regulations were meant to control the use on small pleasure craft, ban the sale association paints containing high levels of organotin compounds and set upper limits on organotin compounds in co-polymer paints. The United Kingdom Department of the Environment took steps to determine the effectiveness of the legislation by setting up a monitoring program. Based on the results of this monitoring, a total ban on the use of tri-*n*-butyltin paints on small boats less than 25 m and fish-farming equipment was implemented in July 1987 (Abel et al., 1987). An environmental quality standard (EQS) of 20 ng / L for fresh water (covering both potable water and protection of sensitive aquatic biota) and 2 ng / L for seawater has been set (United Kingdom Department of the Environment, 1987) after presented in the British Parliament.

Global ban on the use of tri-*n*-butyltin in marine antifouling boat bottom paints on non-aluminum hulled vessels (less than 25 m in length) was institutionalized and also advocated limited use of tri-*n*-butyl tin on the smaller and recreational vessels that exist in shallow coastal waters where tri-*n*-butyl tin impacted on the growth oyster species. Regulatory approaches for the banning of the use of tri-*n*-butyltin based antifouling paints cited the International findings (de Mora et al., 1989) of higher levels of tri-*n*-butyltin in the surface waters of ports and open waters, affecting a larger number of snail species.

The concentration of tri-*n*-butyltin trends cannot yet be interpreted as definitive, given that relatively little time had passed since the controls were imposed. Nonetheless, such observations were indicative that the regulations restricting tri-*n*-butyltin paints have decreased the flux to the marine environment in some parts like New Zealand. France initiated controls on the use of tri-*n*-butyl tin in marine paints. In January 1982 such control resulted in the decrease of the aqueous tri-*n*-butyl tin concentration found in Arcachon Bay since 1981 (Sarradin, 1993; Abel, 1996). Overall, the regulations are summarized in Table 1.3.

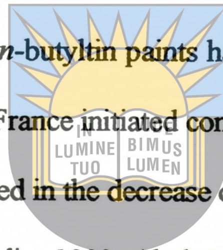
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Table 1.3. Current Worldwide TBT Regulation

Country	Year	Regulation
Austria	1989	Banned the use of tri-*n*-butyltin.
Australia	1989	Prohibited the use of tri-*n*-butyltin on vessels less than 25 m.
Canada	1989	Prohibited the use of tri-*n*-butyltin based paints on vessels less than 25 m, except for aluminum hulled vessels.
Commission of EEC	1992	Prohibited the use of tri-*n*-butyltin based paints on vessels less than 25 m, except for aluminum hulled vessels
Finland	1991	Prohibited the use of tri-*n*-butyltin based paints on boats less than 25m in length.
France	1982	Prohibited the use of tri-*n*-butyltin based paints on vessels less than 25 m in length
Germany	1990	Prohibited the use of tri-*n*-butyltin.
Hong Kong	1989	All tri-*n*-butyl tin antifoulants must have a valid permit for import / supply. All antifoulants must be registered.
Ireland	1991	Prohibited the use of tri-*n*-butyltin based paints on less than 25 m in length. TBT antifoulants available in 20 L containers.
Japan	1990	Tri-*n*-butyltin banned for all new vessels.
Netherlands	1990	Prohibited the use of tri-*n*-butyltin paints vessels.
New Zealand	1989	Application of tri-*n*-butyltin copolymer antifouling paint vessels greater 25 m in length was prohibited.
Norway	1989	The use of tri-*n*-butyltin based paints on vessels less than 25 m in length was prohibited.
South Africa	1991	Prohibited the use of tri-*n*-butyltin based paints. All antifoulants must be registered.
Sweden	1989	Prohibited the use of tri-*n*-butyltin based paints. All antifoulants must be registered.
Switzerland	1987	The use of tri-*n*-butyltin antifouling paints banned in fresh water lakes.
UK	1985	Restricted the sale of tri-*n*-butyltin based paints, effectively banning all TBTO free associated paints. All antifoulants must be registered as pesticides; advisory committee on pesticides must approve sale and use.
USA	1988	Prohibited the use of tri-*n*-butyltin based paints. All antifoulants must be registered.

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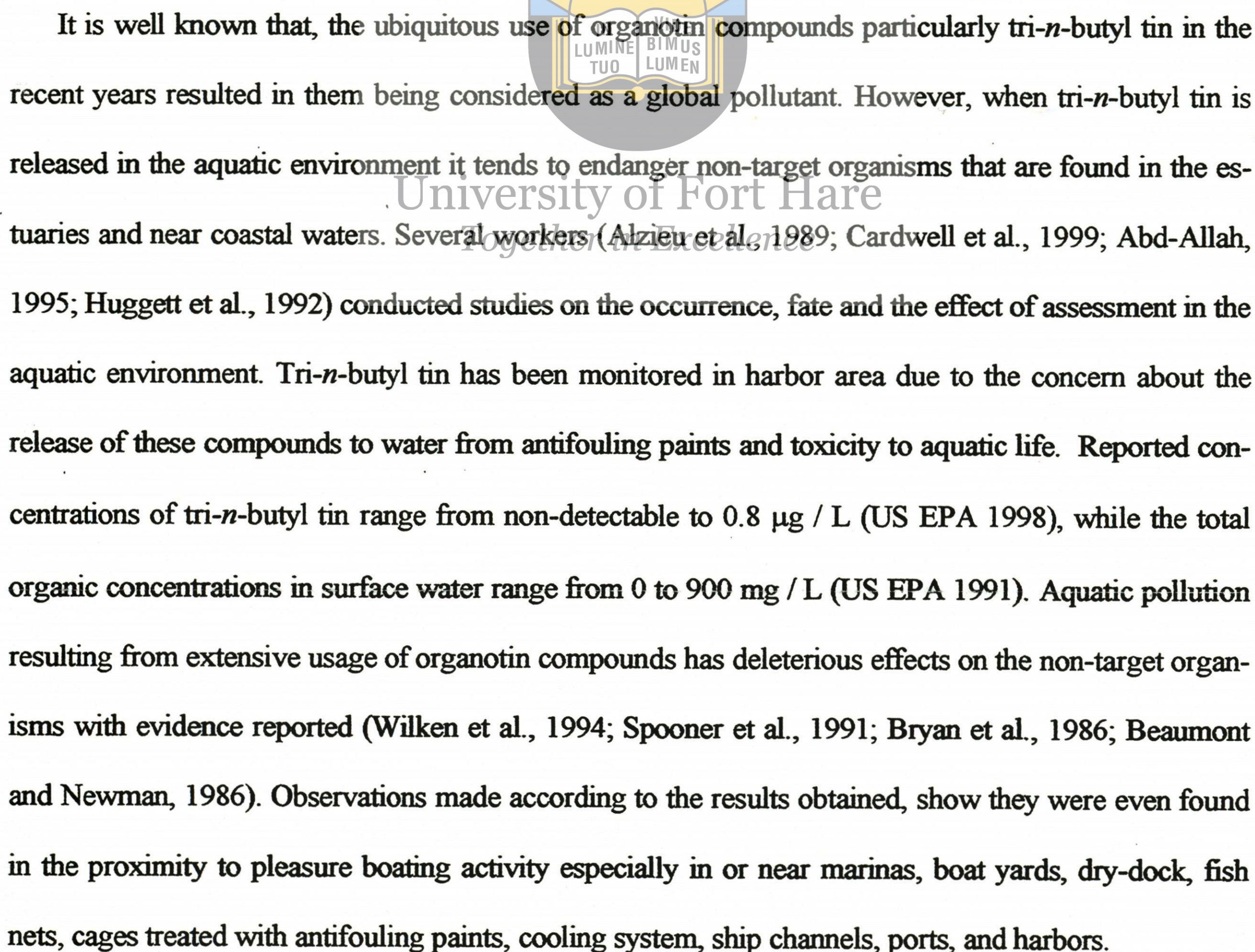
In USA, tri-*n*-butyl tin concentrations in marine sediments decreased within two years of tri-*n*-butyl tin restriction (Wuertz et al., 1991) and down trends have been observed for tri-*n*-butyl tin concentration in mussels (Uhler et al., 1993). Other authors (Irvine, 1980; Kilby and Bartley, 1993) observed

high concentration of TBT in sediments in Australia and observed similar trend in UK (Dowson et al., 1992). Marinas are known to show relatively high tri-*n*-butyltin concentration. A value of 518 ng / g tri-*n*-butyltin was reported in Boston Harbor USA (Makkar et. al., 1989) while 400 ng / g tri-*n*-butyltin was observed in Lake Lucerne Switzerland (Fent and Hunn, 1991) and up to 380 ng / L tri-*n*-butyltin was further reported in Puget Sound USA (Krone et al., 1989). The higher density of yachts and the more restricted water circulation characteristic of enclosed marine basins favored the enhanced accumulation of tri-*n*-butyl tin in marine sediments. The most obvious feature of the tri-*n*-butyltin distribution is that a hot spot of contamination is invariably found near wash-down facilities. Some countries that have enacted restriction on the use of organotin compounds, especially of tri-*n*-butyltin, are summarized in Table 1.3.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It is well known that, the ubiquitous use of organotin compounds particularly tri-*n*-butyl tin in the recent years resulted in them being considered as a global pollutant. However, when tri-*n*-butyl tin is released in the aquatic environment it tends to endanger non-target organisms that are found in the estuaries and near coastal waters. Several workers (Alzieu et al., 1989; Cardwell et al., 1999; Abd-Allah, 1995; Huggett et al., 1992) conducted studies on the occurrence, fate and the effect of assessment in the aquatic environment. Tri-*n*-butyl tin has been monitored in harbor area due to the concern about the release of these compounds to water from antifouling paints and toxicity to aquatic life. Reported concentrations of tri-*n*-butyl tin range from non-detectable to 0.8 μg / L (US EPA 1998), while the total organic concentrations in surface water range from 0 to 900 mg / L (US EPA 1991). Aquatic pollution resulting from extensive usage of organotin compounds has deleterious effects on the non-target organisms with evidence reported (Wilken et al., 1994; Spooner et al., 1991; Bryan et al., 1986; Beaumont and Newman, 1986). Observations made according to the results obtained, show they were even found in the proximity to pleasure boating activity especially in or near marinas, boat yards, dry-dock, fish nets, cages treated with antifouling paints, cooling system, ship channels, ports, and harbors.

The detected levels in river, seawater, and estuarities, in open coastal waters, bottom marine sediments, and biota (Waldock et al. 1988; Grovhoug et al 1986; Seligman et al., 1988; King et al., 1989) reported high levels of tri-*n*-butyl tin found to reach 1.58 μg / L in sea water and estuaries; 7.1 μg / L in fresh water, 2 300 μg / kg in coastal sediments, 3 700 μg / kg in fresh water sediment, 11 mg [kg dry wt] $^{-1}$ in fish and 6.39 mg [kg dry wt] $^{-1}$ in bivalves. These values are taken as representative because a number of factors may have given rise to anomalously high values (an example paint particles in water and sediment, these values are average of the values reported).

It was noted (Goldberg, 1986) that tri-*n*-butyltin was perhaps the most toxic substance ever introduced deliberately to the marine environment by mankind. The study (Huggett et al., 1992, 1996) is devoted to the ways of entering of tri-*n*-butyltin to the sea via dry-docks. Hull wash down is a 30 hours operation using 400 000 L of wash water resulting in tri-*n*-butyltin levels in wash down waste water ranging from 1.5×10^5 to 48.5×10^4 ng/L and thus are allowing massive amounts of tri-*n*-butyltin to be leached into the marine environment (Messing et al., 1997; Champ et al., 1999; Fox et al., 1999).

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It has been recognized for some time, that other tri-*n*-butyl tin stabilizers migrate from PVC products during the normal use; good example of this is the leaching of *n*-butyl tin (MBT) and di-*n*-butyl tin (DBT) into drinking water from PVC pipes (Forsyth and Jay 1997; Sadiki and Williams, 1999). It should be noted that the release of dibutyl tin compounds from consumer products as a metabolite from TBT, may also pose a risk to human health and environment. Despite this propensity, organotin compounds still are found as high concentrate additives in a variety of consumer products and children's toys. Kawamura et al. (2000) reported levels of this toxin up to 1 g / kg of *n*-octyl tin in PVC containers. A recent study in Germany raised much concern about the presence of comparatively high levels of TBT and other organotin compounds in PVC flooring (Oeko – Test 2000). The data of Allsopp et al. (2000; 2001) for both PVC flooring and carpets available for retail in the UK confirmed the ongoing use of these compounds in floor coverings, occasionally at very high concentrations (up to 0.57 g / kg

di-*n*-butyltin in treated carpet fiber). Assessment of the environmental impact from tri-*n*-butyltin has been well documented for public perception (Thain, 1983; Waldock and Miller, 1983; Waldock et al., 1983; Alzieu and Portman, 1984; Alzieu et al., 1981 – 1982).

Organotin compounds are known to cause toxicity at relatively low levels of exposure not only to marine invertebrates but also in mammalian systems. This could be in view of their lipophilicity, such that tri-*n*-butyltin is often more toxic than di-*n*-butyltin, which is in turn more toxic than *n*-butyltin (Cima et al., 1996). This is not always the case, as di-*n*-butyltin is more toxic than tri-*n*-butyltin to certain enzyme systems (Bouchard et al., 1999; Al–Ghais and Ahmad, 2000). In fish, di-*n*-butyltin is frequently a more potent immunotoxin than tri-*n*-butyltin (O'Halloran et al., 1998). Somewhat surprisingly, organotin compounds have differing toxicities depending upon the number and type of organic groups bound to the tin. In water, trisubstituted organotin compounds can be in steps decomposed to less substituted compounds down to inorganic tin. Monitoring studies in Japan found that biologically significant amounts of organotin compounds derived from antifouling paints had been released to the marine environment with high residues in fish which ranged from 0.06 to 0.75 ng / L tri-*n*-butyl tin and 0.03 to 2.6 ng /L triphenyltin. This gives some concern for future human health (Kakuno and Kimura 1987). A strong correlation has been found between imposex in neogastropods and organotin compounds (specifically tri-*n*-butyltin) concentrations. Imposex is the masculinization of female organisms; in this case the species development of a genital change and vas deferens. Studies of *Nucella* have shown that where appropriate population of gastropod occur, the presence of masculinized female snails (imposex) may be used as a bioindicator of tri-*n*-butyl tin contamination (Davies et al., 1987). No other xenobiotic compound has been found to cause imposex, but other factors may be involved. *N. emerginata*, found in the intertidal zone, was considered to be the most ideal species because of its short life span. Because imposex is irreversible, these species alone may be able to reflect remediation of tri-*n*-butyltin contamination. Imposex is measured in three ways: frequency of occurrence, female genital length, and the relative size of female genitals in relation to male penises. Tri-*n*-butyltin analysis

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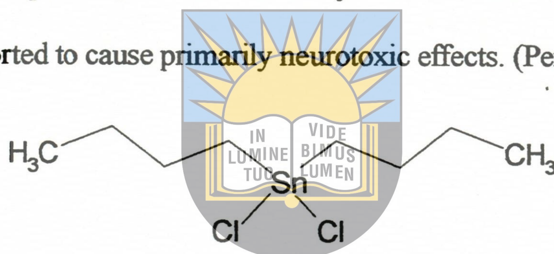
normally involves direct chemical analysis of sediments, seawater, and the surface micro layer. These methods are very expensive and time consuming; whereas the frequency of imposex is a fast and inexpensive measure.

1.7. STRUCTURE OF TRI-*n*-BUTYL TIN (TBT) COMPOUNDS

Two classes of compounds have been most extensively studied and documented. These are diakyltin homologues such di-*n*-butyltin dichloride DBTC and tri-*n*-butyl tin chloride TBTC. Typically, triakyl substituted tin compounds are more toxic in some ways than of the corresponding disubstituted tin. Some other organotin compounds, such as monoalkyl substituted derivatives, which have not been extensively studied, are reported to cause primarily neurotoxic effects. (Penninks and Seimen, 1980).



Formula: $C_8H_{18}Cl_2Sn$. Di-*n*-butyltin dichloride

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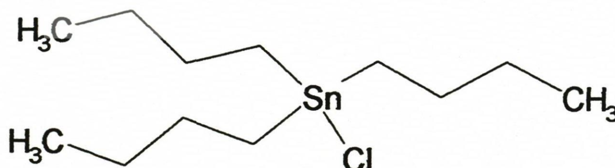

Formula: $C_{12}H_{27}ClSn$. Tri-n-butyltin chloride

1.8. CHEMICAL IDENTITY OF ORGANOTIN COMPOUNDS

Tri-*n*-butyltin compounds are organic derivatives of tin(IV) (Sn^{IV}) characterized by the presence of covalent bonds between carbon atoms and the tin atom, R_3SnX where R = *n*-C_4H_9. They conform to the following general formula: (*n*-C_4H_9)$_3$Sn–X where X is an anion or a group linked covalently to a heteroatom. The nature of X influences the physico-chemical properties of the compound. The molecular formulae of major tri-*n*-butyltin and di-*n*-butyltin compounds are as follows:

Di-*n*-butyl tin chloride	$C_8H_8Cl_2Sn$
Di-*n*-butyl tin diacetate	$C_{12}H_{24}O_4Sn$
Di-*n*-butyl tin oxide	$C_8H_{18}OSn$
Tri-*n*-butyltin benzoate:	$C_{19}H_{32}O_2Sn$
Tri-*n*-butyl tin chloride:	$C_{12}H_{27}ClSn$
Tri-*n*-butyl tin fluoride:	$C_{12}H_{27}FSn$
Tri-*n*-butyl tin linoleate:	$C_{30}H_{58}O_2Sn$
Tri-*n*-butyl tin methacrylate:	$C_{16}H_{32}O_2Sn$
Tri-*n*-butyl tin oxide:	$C_{24}H_{54}OSn_2$

1.8.1. MAJOR PROPERTIES OF ORGANOTIN COMPOUNDS

Some of the major properties of organotin compounds are summarized below in Table 1.4 compiled from World Health Organization (WHO, 1990).

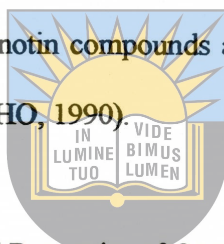

Table 1.4. Selected Physical and Chemical Properties of Organotin Compounds

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Property	Tri-n-Butyltin Chloride (TBTC)	Bis(Tri-*n*-Butyltin) Oxide (TBTO)	Tri-*n*-Butyltin Acetate (TBTA)
CAS No	146 – 22 – 9	56 – 35 – 9	56 – 36 – 0
Molecular Formula	$C_{12}H_{27}ClSn$	$C_{24}O_{54}OSn_2$	$C_{14}H_{30}O_2Sn$
Appearance	Colorless, transparent liquid; peculiar irritating odor.	Colorless, or slightly yellowish, transparent liquid, peculiar irritating odor	White powder
Density, g / mL	1.2 (20°)	1.17 – 1.18 (20°)	1.4942 (20°)
Vapor pressure, mm Hg	0.0092 (20°)	0.006 (25°)	
Melting Point, °C	-16	< -45	84.5 – 85
Water Vapor Pressure, mm Hg	0.00927	0.0016	-
Water Solubility, mg / L	0.7478	0.75	
log K_{∞}	4.76	3.19-3.84	3.24

1.8.2. ENVIRONMENTAL FATE OF ORGANOTIN COMPOUNDS

The earlier poor understanding of the complex environmental chemistry of organotin compounds and information on the potential for the organotin compounds to volatilize from water were limited. The indication that organotin compounds in water partitioned to the air during a 62 day period whereas 20% of the water evaporated appeared rather recently (Maguire et al., 1983). It had been speculated that the vapor pressures of some organotin compounds may be high enough such that they could partition to the atmosphere (WHO, 1980; Donard and Weber, 1988) but no actual measurements were available. The vapor pressure of tri-*n*-butyltin oxide was estimated to be

8.4×10^{10} atm, essentially non-volatile (Maguire et al., 1983).Direct entry of the extremely toxic tri-*n*-butyltin into the aquatic environment was primarily linked to extensive usage in antifouling paint biocide on ships. The lipophilicity of organotin compounds in 1-octanol / water partition coefficient was found to be at log K_{ow} 3.5 (Tsuda et al., 1988) and resulting into a high potency of bioaccumulation (Fent, 1991). Sediment – water partition coefficient for TBT fluctuates between 1 and 3.0×10^3 (Fent, 1996). Therefore these values determine the presence of tri-*n*-butyltin both in water and particulate matter. Tri-*n*-butyltin has low water solubility and other associated complexation and dissociation constants. It is readily adsorbed onto suspended particulates as minute organic material or inorganic sediments (Laughlin et al., 1986) the extent of binding depending on physico–chemical mechanism, location, organic content, and particle size. The major degradation pathways of organotin compounds are metabolism, hydrolysis and photolysis (Wania, 1998).

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1.8.2.1. PHOTODEGRADATION

Photodegradation of tri-*n*-butyltin by UV light is theoretically possible because UV light with wavelength longer than 290 nm possesses energy of 300 kJ / mol, while the energy required to break the carbon-tin bond is 190-220 kJ / mol. Simultaneously, bis(tri-*n*-butyltin) oxide absorbs in the UV region at 300 nm and less strongly at 350 nm. Laboratory measurements have shown that this route of degradation can occur and that it forms derivatives of di-*n*-butyltin. The di-*n*-butyltin seems to be resistant to photolysis, since very little *n*-butyltin is formed (Blunden and Chapman, 1986). While the phenomenon clearly exists, its importance varies considerably with different environmental conditions. Conditions of illumination, transmission of light and the presence of photosensitizing substances such as acetone and humic acids were considerably perceived to accelerate the process. According to Slesinger & Dresser (1978), the half-life of tri-*n*-butyltin in seawater subjected to UV light was 18.5 days, yet the half-life of tri-*n*-butyltin was 3.5 days. Seligman, et al. (1986) suggested that, under natural conditions, photodegradation was barely considered less important than biological action; consequently, the development of phytoplankton led to a partial degradation of tri-*n*-butyltin. Under these conditions, light caused no degradation over 144 days. According to Lee et al. (1987), degradation of low concentrations of tri-*n*-butyltin (less than 5 ng / L) in estuary water was increased when the assay was conducted in light. The half-life was between 6 and 12 days, and the presence of significant concentrations of phytoplankton increases the speed of degradation. According to Maguire et al. (1983), photolysis under natural light conditions in distilled or natural water was limited, leading to a tri-*n*-butyltin half-life in excess of 89 days. In these assays, it was possible to demonstrate the role of humic acids, particularly fulvic acid, which considerably augmented the speed of photolysis. Organotin compounds do not possess absorption maxima in the range of sunlight, do not undergo direct photochemical reaction in surface water, since the light spectrum and intensity is reduced by the water surface (Callahan et al., 1979).

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1.8.2.2. HYDROLYSIS

There is little reported on the hydrolysis of organotin compounds under normal environmental conditions to create a hydrophilic surface. According to the previous work (Maguire et al., 1983; Maguire et al., 1985), tri-*n*-butyltin remained stable for 11 months in distilled or natural water at 20°C, in the dark, and in a sterile medium. Under various conditions of pH, between 2.9 and 10.3, these authors found no change in tri-*n*-butyltin over 63 days. According to Seligman et al. (1986), slight degradation of tri-*n*-butyltin was apparent after 94 days in darkness in the presence of formalin as a sterilizing agent. It was, therefore, concluded that degradation does not occur or occurs only very slowly in normal environmental conditions of a sterilizing agent. The half-lives for hydrolysis of organotin compounds were in the range of 2 to 3 years (Maguire et al., 1983; Maguire et al., 1985; Waldock et al., 1990).

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1.8.2.3. VOLITALIZATION

The chemical behavior of most of the common organotin compounds in environmentally relevant media is not known. There is need to measure the solubility and vapor pressure of most important organotin compounds in order to provide a more reliable basis for the predicting their fate in environment. Maguire et al. (1985) observed that loss of organotin compounds by volatilization was limited and further noted that tri-*n*-butyltin have significantly lower volatility than that of water.

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1.8.2.4. ADSORPTION OF ORGANOTIN COMPOUNDS

Tri-*n*-butyltin and tri- *n*-phenyltin are sparingly soluble in water and easily adsorbed to particulate matter in the aquatic environment. When organotin compounds are released into water, they undergo pH dependent dissociation (TBTO to TBT + OH^-). The undissociated moieties have log K_{oc} on the order of 2.3 – 4.1 as a single physico-chemical descriptor and gets adsorbed onto suspended matter (Fent, 1996). The dissociated form can be adsorbed to particles seemingly by an unclear mecha-

nism (Hinga et al., 1987; Fent 1996). Similarly it is dependent on the nature of the sediment and the organic carbon of the sediment that influences bioavailability and toxicity of tri-*n*-butyltin (Meador, 1997). Many organotin compounds occur in water as cations, and are expected to partition to soil and sediments. A partition coefficient K_{∞} about 2.180 at 20°C was calculated (Maguire et al., 1985) to estimate the adsorption of tri-*n*-butyltin ions (as n-Bu_3Sn^+ in lake sediments). The investigations led to conclusion that the half-life of the desorption reaction was about 10 months, indicating that tri-*n*-butyltin can be strongly retained by sediments. Other studies were carried out (Strand, 1983; Cooney, 1988; EPA 1988) and speculated that organotin compounds are bound by soil texture.

1.8.2.5. BIODEGRADATION OF ORGANOTIN COMPOUNDS

Biodegradation is another major mechanism for transformation of organotin compounds in soil and water. It depends on the environmental conditions and the toxic effect of the available concentrations to the organisms involved (Stein and Kuster, 1982). As the degradation proceeds via hydroxylated intermediates, anaerobic degradation alone seems to be in particular at high tri-*n*-butyltin concentrations that may be toxic to microorganisms. For instance, in the literature aerobic and anaerobic organisms are reported both cause biodegradation, but the relative efficiency is highly complex and is not known conclusively. Degradation of tri-*n*-butyltin proceeds via splitting of the carbon-tin bond, which can result from various mechanisms occurring simultaneously in the environment and due to the variability of behavior in relation to physico-chemical properties and degradation pathways, specific modeling of the environment distribution constitutes a challenge. It includes knowledge of the physico-chemical mechanisms (hydrolysis and photodegradation and biological mechanisms (degradation by microorganisms and metabolism by higher organisms). While degradation definitely occurs as a result of these different mechanisms in laboratory studies, it is necessary to assess the relative importance of these different pathways to degradation of tri-*n*-butyltin in the field. Direct illumination of the cultures has been reported to lower the half- life, indicating the involvement of photosyn-

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thetic organisms. Literature on the environmental characteristics such as temperature, pH, light intensity, and microbial activity is in relationship to the physicochemical properties and degradation pathway (Fent, 1996) to confirm that organotin compounds are biodegradable. Tri-*n*-butyltin (TBT) is graded in the marine environment to di-*n*-butyltin (DBT), *n*-butyltin (MBT) and inorganic tin (Sn). A probable degradation scheme involving microorganisms is shown in Fig. 1.

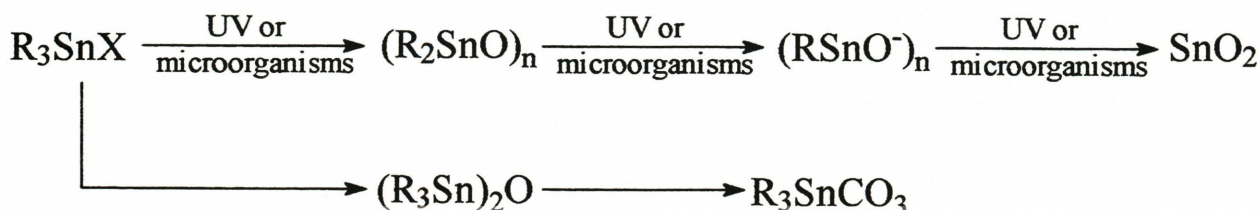

Fig. 1. A Probable Degradation Scheme Involving Microorganisms

Source: Elser and Paynter, 1989; Sheldon, 1975

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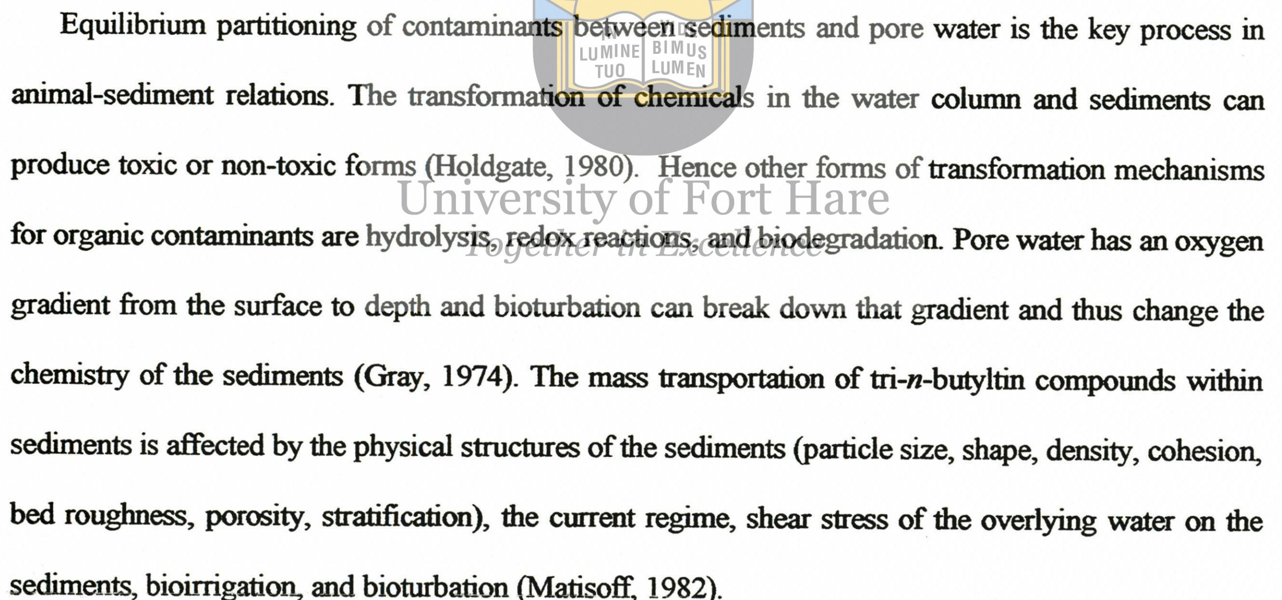
A mechanism of biodegradation also exists under anaerobic conditions (Maguire et al., 1985). Anaerobic degradation is considered to be very slow by some workers and more rapid than aerobic degradation by others. Slesinger and Dresser (1978) conducted studies in a Warburg respirometer under aerobic conditions and showed that microflora derived from activated sludge and soil was capable of partially degrading tri-*n*-butyltin. The half-life was 70 days, whereas under anaerobic contaminants, especially those with high equilibrium distribution (partition) coefficients (log $K_{\infty} > 106$), is determined by the behavior of particles. The size and nature of the particles and their associated contaminants are modified by physico-chemical processes (e.g., aggregation and dissolution) and biological processes (e.g., ingestion, metabolism, and defecation). Removal rates of particles and their contaminants from the water column (scavenging) are highest in estuaries and decreases with distance offshore. Dissolved contaminants are incorporated into particulate matter by adsorbing onto surfaces of inorganic or organic particles, and by incorporation into biological tissues. Physical dispersion in the water column due to advection, diffusion and transport of contaminated particles over long distances is not uncommon but non-conservative particle behavior, biological packaging, and uncertainties about processes

controlling particle resuspension from the bottom complicate dispersion are of interest. The primary removal mechanism of particles from the euphotic zone is their incorporation into bioorganic particles with large sinking rates. Once in the sediments, contaminants become chemically modified and released from the particles into the pore water. Organotin compound contaminants on particles and in pore waters re-enter the water column by resuspension. Contaminants on these aged particles may be less bioavailable than contaminants on younger particles. Resuspension can re-introduce contaminants on sediment particles and in pore water to the water column where the chemical environment is quite different.



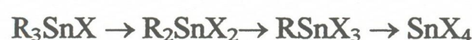
1.8.3. ANIMAL-SEDIMENT RELATIONS

Equilibrium partitioning of contaminants between sediments and pore water is the key process in animal-sediment relations. The transformation of chemicals in the water column and sediments can produce toxic or non-toxic forms (Holdgate, 1980). Hence other forms of transformation mechanisms for organic contaminants are hydrolysis, redox reactions, and biodegradation. Pore water has an oxygen gradient from the surface to depth and bioturbation can break down that gradient and thus change the chemistry of the sediments (Gray, 1974). The mass transportation of tri-*n*-butyltin compounds within sediments is affected by the physical structures of the sediments (particle size, shape, density, cohesion, bed roughness, porosity, stratification), the current regime, shear stress of the overlying water on the sediments, bioirrigation, and bioturbation (Matisoff, 1982).

The bioavailability of tri-*n*-butyltin contaminants depends on the nature of the contaminated particles, the specific contaminants, and the animal species in question. Bascietto et al. (1990) observed that once a contaminated particle is in the gut of an organism, availability depends on the type and strength of binding of the contaminants to the particle, and the presence or absence of a transfer mechanism from the particle to the cells of the organism. Contaminant materials not desorbed in the gut, or incorporated into the organism, are excreted in particulate (Iwata et al., 1997).

The process of adsorption and partition is a significant transport route of organotin compounds; in lakes and the particulates eventually settle to the sediments. Several workers (Valkirs et al, 1986; Maguire et al., 1986; Randall et al., 1986; Stang and Seligman 1987; Hinga et al., 1987) observed that tri-*n*-butyltin compounds remain in the aqueous layer, adsorb onto particulates and partition to biota, sediment, or surface layer.

A biotic degradation in many ways has shown that a degradation pathway for tri-*n*-butyltin compounds exists in the environment, through debutylation to di-*n*-butyltin theoretically completed with the liberation into water of the tin oxide (SnO_2).



$$R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4$$

Studies confirmed the evidence of tri-n-butyltin and its degradation pathways, the cause and mechanisms, and an understanding of the kinetics in different environmental conditions (Chapman and Price, 1972; Brinkman, 1981; Blunden et al., 1984; Maguire et al. 1985; Valkirs et al., 1986). Their influence on the importance of the adsorption mechanism depends on organic matter, suspended material and lipid containing materials. Several environmental characteristics like salinity, nature, and size of suspended particles, as well as temperature and organic matter influence the lifetime of organotin residues. An *n*-octanol / water partition coefficient (K_{ow}) describes the partitioning of an organic chemical between *n*-octanol and water. Likewise, *n*-octanol is believed to be best imitating the fatty structures in plant and animal tissues (Kenaga and Goring 1980). The log K_{ow} of tri-*n*-butyltin at pH 6.0 was reported to have bioconcentration factor of about 1,585 (Maguire et al., 1983) The most accurate log $K_{ow} > 3$ for tri-*n*-butyltin in seawater demonstrated the presence of potentially bioaccumulation concentration factor of 5500. It was reported (Laughlin and Linden, 1985; Tsuda et al., 1986) that log $K_{ow} > 3$ for seven organotin compounds whose concentration factors ranged from 9 to 4571. The magnitude of this value suggests that organotin compounds can partition to fat tissue significantly depending on the specific compounds. However, the desorption from sediments was

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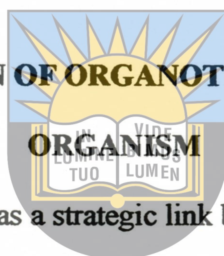
found to be low (Maguire et al., 1985) and resuspension of tri-*n*-butyltin was presumed to be responsible for the ongoing occurrence of imposex in marine gastropods (Salazar et al., 1987; Bryan et al., 1993) considered that the effect of the adsorbed TBT was partially masked and not availed to organisms; other documented evidence noted the adsorption is enhanced in the presence of salt. The sediments with high concentration of organic matter become the source and pathway of pollutants entry into an aquatic system (Langston et al., 1987). Anaerobic biodegradation occurs in water and sediment. Its half-life varies considerably around one and half months. In other words, tri-*n*-butyltin may portion from water to aquatic organisms assuming partitioning processes are responsible.



1.8.4. BIOACCUMULATION OF ORGANOTIN COMPOUNDS BY LIVING ORGANISM

Bioaccumulation has been identified as a strategic link between the external environment and the organisms (Laughlin et al., 1986). The available literature indicate that bioaccumulation of organotin compounds is affected by a variety of factors including organic carbon in sediment, pH, salinity, clay fraction, and the presence of inorganic constituents such as iron oxides (US EPA, 1991; Fent, 1996). These researchers noted that bioaccumulation is another transfer pathway of tri-*n*-butyltin via uptake, metabolic process, and elimination rates in sediment and water concentrations in a wide range of ecologically relevant species (Meador et al., 1993; 1996). Biological membranes are among the most important target sites for the toxic effects of hydrophobic pollutants on aquatic organisms. The uptake of persistent hydrophobic environmental pollutants occurs primarily into the more hydrophobic tissues, in particular into membranes and storage lipids of the organisms. Partition into membranes causes disturbances in their structure and functioning of the membrane (van Wezel and Opperhuizen. 1995; Chaisuksant et al., 1999; Antkowiak, 2000).

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The lipophilic properties of tri-*n*-butyltin and with a moderately high *n*-octanol-water partition coefficient (log K_{∞} > 3) contribute the inherent ability of chemical substance to bioaccumulate in water living organisms while constitutes minimal toxicity of hydrophobic pollutants. Evidence for such mechanisms and an evaluation of their importance is highly relevant for hazard assessment, both for the environment and for humans, since some of the organisms exposed to organotin compounds are human food items such as bivalve mollusk, crustaceans and fish. It was demonstrated (Alzieu et al., 1980) that in contaminated areas tin levels in the flesh of oysters were 100 times higher than concentrations in the water. It was reasoned (Kungolos et al., 1997) that the difference in bioaccumulation is responsible for the differences in the mode of interaction of the toxicants.

The bioconcentration factors varied between 100 and 3×10^4 in species resistant to the concentration of 20 mg / L of tri-*n*-butyltin (Blair et al., 1982). Other studies have shown that the possibility of bioaccumulation and biomagnifications in mollusk were as higher as 2 600.

It was conclusively indicated that, the bioconcentration factor (BCF) is pH-dependant, it decreased with increasing pH (Kishino and Kobayashi 1995). Numerous authors have postulated that bioaccumulation is another transfer pathway of tri-*n*-butyltin particularly significant for mussels. Further attempts reported that zebra mussels possessed an enhancement factor in the range of four orders of magnitude relative to the water column. However, bioconcentration factors in the organotin compounds are calculated accordingly as a function of the ratio between the concentration in organism and water. Concentration factors for tri-*n*-butyltin crucian carp (*Carassius Grandoculis*) are in the range between 360 and 3 400 exposed for 14 days to a varying of concentrations of tri-*n*-butyltin between 1.8 and 2.4 ng / L (Tsuda et al., 1987). Aquatic organisms (Laughlin et al., 1986) had concentration factor between 1 000 and 7 000 for mussels (*Mytilus Edulis*} that had been exposed for three and seven days to varying TBT concentrations of 23, 45, 63, 141 and 670 ng / L. Other researchers concluded that *in situ* bioaccumulation studies of the mussels would firmly be a good indicator or-

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ganism for monitoring marine pollution. Tin uptake and water concentration were monitored over a period of 51 days (Cheng and Jensen, 1989). They made a conclusion that the accumulation was found to increase with time for both total tin and organic tin. They believed that contamination via food was more important than via the water (Davies et al. 1987; Moore et al., 1991; Salazar and Salazar, 1992; 1995; Ward et al., 1981; Widdows et al., 1990). The researchers made further observation that tri-*n*-butyltin concentrations in the water were fairly undetected at less than 20 ng / mL as compared to average concentration in the bodies of microorganisms being 610 μg / kg in zooplankton, 3 091 μg / kg in the filter - feeding shellfish (mussels); 886 μg / kg in animal feeding small fish, 288 μg / kg in eels. The tri-*n*-butyltin bioaccumulation trigger was established at 219 μg / kg (as Sn), based on a multiple of the screening levels (PSDDA, 1989). All available literature has provided information about bioaccumulation as one of the important processes in determining the ultimate aquatic fate of organotin compounds (WHO, 1990). It was shown (Bouchard et al., 1999) that accumulation of tri-*n*-butyltin was via ingestion in marine organism, which may not be considered in the development of the action / cleanup goal, where other risks were underestimated (Kannan et al., 1997). Primarily, for the bioavailability characterization it is important to know different species' uptake of various chemicals or metal compounds directly from water, sediment, suspended particles, or food items under controlled combination of chemical properties, the ambient environment, morphological, biochemical and physiological attributes.

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Bioaccumulation of tri-*n*-butyltin was equally evident in fish specimens, a fact almost invariably ignored after exposure of the sheep head minnow *(Cyprinodon Variegatus)* for 58 days to concentrations of tri-*n*-butyltin varying between 0.96 and 2.07μg / L (Iwata et. al., 1995). In a whole body, bioconcentration factor of 2 600 was demonstrated (Ward et al., 1981). After returning the fish to clean water, loss of tri-*n*-butyltin was rapid over the first seven days then slower. After 20 days, some other authors reported a loss of 74 % from the muscle and 80 % from the viscera. Detection of di-*n*-

butyltin, *n*-butyltin, and inorganic tin suggested possible metabolism. The mullet (*Liza Aurata) was* exposed for 2 months to concentrations of 5 µg / L tri-*n*-butyltin and bioconcentration factors of 20 to 30 were reported in the liver and kidneys but no residues were found in the muscle (Bressa et al., 1984). After transfer to clean water, concentrations of tin decreased in all organs. Studies of bioaccumulation in salmon *(Oncorhynchus Tshawytscha)* exposed for 96 h to concentrations of 1.49 µg / L led to the bioconcentration factors of 4 300 in the liver, 1 300 in the brain, and 200 in the muscle (Short and Thrower 1986). Tri-*n*-butyltin was accumulated by carp *(Cyprinus Carpio)* exposed for 14 days to varying concentrations between 1.8 and 2.4 ng / L (Tsuda et al., 1987). Over 10 days they found a plateau in uptake and a concentration factor of 1 000; metabolism was evident pathway. A representative partition coefficient range between 360 and 3 400 for round crucian carp *(Carassius Grandoculis)* tissues exposed to tri-*n*-butyltin chloride (Tsuda et al., 1988). A bioconcentration factor (BCF) relates the concentration of a chemical in plants and animals to the concentration of the chemical in the medium in which they live. It was estimated that bioaccumulation factors of tri-*n*-butyltin were 100, 1 000, and 3 000 for marine and fresh water plant, invertebrates and fish respectively (Thompson et al., 1985). Marine algae can bioconcentrate tin (Sn^{4+}) by a factor of 1 900 (Seidel et al., 1980). The BCF of tri-*n*-butyltin was estimated to be 473 but measured BCF's were found a bit higher than normal (Laughlin and Linden, 1985). Tri-*n*-butyltin in marine oysters was measured as 2 300 to 11 400 (Waldock and Thain 1983). The highest observed levels were in dolphins, which lack metabolic capability and accumulated high levels of organotin compounds in their body through food chains (Lee, 1996; Iwata et al., 1997). Interestingly, relatively concentrations of butyltin compounds in liver and kidney were similar to those of heavy metals like methyl mercury, which implies that their distributions were less dependent on their affinity to lipids than other persistent organic pollutants (Tanabe et al., 1981).

The high bioaccumulation values for tri-*n*-butyltin are generally characteristic of species with a high rate of uptake metabolic conversion and elimination (Table 1.5).

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Generally, the kinetics of degradation of di-*n*-butyl and *n*-butyltin is less known. However, the degradation processes of tri-*n*-butyltin always result in the formation of metabolites less toxic than the parent compound. The degradation kinetics of tri-*n*-butyltin was lower than in water, the half-life being approximately 162 days. Naturally contaminated sediments were maintained in the laboratory, under flow-through conditions, at 12°C (Waldock and Thain, 1983). Degradation of sediment-bound TBT was found to be a slow process. In aerobic layers the half-life of TBT was between four and five months, but in deeper anaerobic layers, a half-life value was not obtained within 500 days (Thain, 1986). The relative importance of the compartmental distribution of organotin compounds and their overall persistence (Fig. 2) are difficult to study by means of generic model due to their complex bio-geochemistry (Fent, 1996)

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Table 1.5. Internal Fate Accumulation of Tri-n-Butyltin Compounds

Source: WHO (1990)

Object	Specific Name	Biological Accumulation Factor	Reference
Phytoplankton	Green algae	30 000 TBTO 20 µg / L	Maguire (1984)
	Haptophyceae	5 500	Laughlin et al. (1986)
Mollusk	Japanese Oyster	6 000 TBTO 15 µg / L	Waldock et al. (1983)
	Blue mussel	1 000 - 7 000 5 000 - 60 000	Laughlin et al. (1986)
Anthropoda	Crabs	60 TBTO 0.96 - 2.07 µg / L	Allen et al. (1980)
Fish	Sheephead minnow	2 600 TBTO 0.96 - 2.07 µg / L	Ward et al. (1981)
	Mullets (*Liza aurata*)	20 - 30 (liver, kidney) TBT 5 µg / L	Bressa et al. (1984)
	Crucian carp	1 000 360 - 3 400 TBTO 0.0018 - 0.0024 µg / L	Kakuno and Kimura (1987)
	Carp	1 000 TBTO 0.0018 - 0.0024 µg / L	

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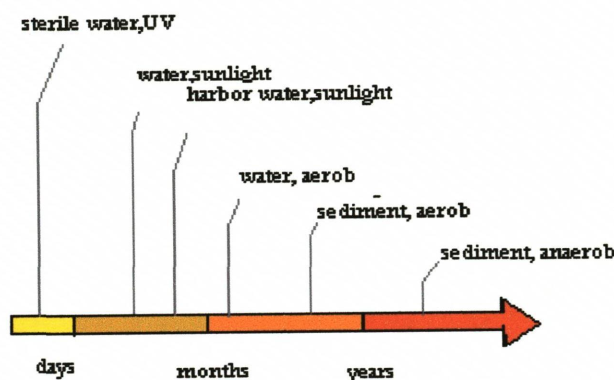

Fig. 2 Half-life of Organotin Compounds in Different Media

A half-life of one - three months has been reported for tri-n-butyltin in sandy and silt loam soils and 126 days in flooded silt loam (US EPA, 1987). The half-life of tri-*n*-butyltin in water was estimated to be several days in June and two-three weeks in November (Soderquist and Crosby, 1980). Half-lives of tri-*n*-butyltin in mussels (*Mytilus Edulis*) taken in the summer of 1989 in Yokohama (a busy port, heavily contaminated with tri-*n*-butyltin) and Urayasu (a river mouth, about 10 times less polluted than Yokohama) in Japan were estimated to be 139 and 127 days, respectively (Shiraishi and Soma, 1992). Biological half-lives of tri-*n*-butyltin in short-necked clams (*Tapes Amygdala Japonica*) and guppy (*Poecilia Reticulata*) were estimated to be approximately 30 days and 48 days, respectively (Takeuchi et al., 1989; Tas et al., 1990). The ecological half-life of tri-*n*-butyltin in gastropods was estimated to be 347 days (Mensink et al., 1996).

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1.9. KINETICS AND METABOLISM AND INTERNAL EXPOSURE

Body burdens of organotin compounds are highly variable, due to differences in external exposure and metabolism. The highest observed levels are in dolphins, which lack metabolic capability

and accumulate high levels of organotin compounds in their bodies through food chains (Iwata et al., 1997; Lee, 1996). In contrast, levels are generally lower in invertebrates and in fishes it is urged that because data are sparse, body burdens are high enough to suggest that there could be some risk to humans and certain marine mammals. Body burdens of tri-*n*-butyltin in the livers of Japanese males (taken from four people by autopsy in 1997 / 1998) were estimated to be 84 ng Sn /g (Takahashi et al, 1998; Takahashi, 1999). Interestingly, the relative concentrations of butyltin compounds in liver and kidney are similar to those of methyl mercury. This suggests that their distributions are less dependent on their affinity to lipid than other persistent organic pollutants such as PCB's and DDT (Tanabe et al., 1981). Tri-*n*-butyltin and triphenyltin also accumulate in livers and kidneys of non-human mammals. More than 10 ng Sn / g of *n*-butyltin ions, on a wet mass basis, were detected in the livers of dolphins collected in the semi-closed Seto Inland Sea, Japan, in 1985 (Iwata et al., 1997) and in the Gulf coast of the USA in 1989 (Kannan et al., 1997). They have also been found in the blubber and adrenal glands of coastal whales (Iwata et al., 1995), in the hair of sea lions and the feathers of common cormorants (Kim et al., 1996; Guruge et al., 1997). The lack of key metabolic enzymes in some cetaceans accounts for the differences observed in human and cetacean body burdens.

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Tri-*n*-butyltin is absorbed from the gut (20 - 50 % depending on the vehicle) and via the skin of mammals (about 10 %), and can be transferred across the blood-brain barrier and from the placenta to the foetus. Absorbed material is rapidly and widely distributed amongst tissues (principally liver and kidney). Metabolism in mammals is rapid; metabolites are detectable in blood within 3 h of tri-*n*-butyltin administration. Tri-*n*-butyltin as a substrate for mixed-function oxidizes *in vitro*, but these enzymes are inhibited by tri-*n*-butyltin *in vitro* at very high concentrations. Rate of loss differs with different tissues and estimates for biological half-lives in mammals range from 23 to about 30 days. Metabolism occurs in lower organisms but is slower, particularly in mollusks. The capacity for bio-accumulation is, therefore, much greater than in mammals. Tri-*n*-butyltin compounds inhibit oxida-

tive phosphorylation and alter mitochondrial structure and function. Tri-n-butyltin interferes with the calcification of the shell of oysters (*Crassostrea Gigas*) (Li et al., 1997). A number of workers have studied the absorption, metabolism, and elimination of organotin derivatives in various animal species, especially in mammals. Some studies were conducted *in vivo* and others *in vitro* using isolated liver microsomes (Matthiessen and Gibbs, 1998). The behavior of organotin compounds depends partly on their chemical structure and partly on speciation. The distribution of tri-*n*-butyltin in organisms is usually rapid, as is established that in a number of species such as rat, mouse, rabbit, and guinea pig (Bridge et al., 1967). It was found preferentially in the liver and kidney and, to a lesser extent, in the spleen, fat, lungs, brain, and muscle. Excretion is via the bile rather than the urine. In tissues, particularly the liver, there is a process of biotransformation characterized by progressive dealkylation leading to breakdown to inorganic tin (Cremer, 1957)

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In an *in vivo* study, Brown et al. (1977) administered ^{113}Sn labeled tri-*n*-butyltin to mice by injection. They reported an initial rapid elimination, followed by a slower phase, in the faeces. Part of the radiolabel was retained in the tissues but turnover occurred, with a biological half-life for elimination of 23 to 29 days. Evans et al. (1979), under similar conditions, administered ^{14}C-labelled tri-*n*-butyltin to mice in the drinking water, at low doses continuously for up to 30 days. There was adsorption from the intestine and accumulation in the liver, spleen, kidney, and fat (and to a lesser degree in muscle, lung, brain, and blood). In a second study, mice were similarly dosed for 31 days. On cessation of dosing with ^{14}C labeled tri-*n*-butyltin, examination of the animals for a further 15 days demonstrated loss of tri-*n*-butyltin retained in these tissues; the loss reached 97 % in liver, 73% in kidney, and 30 % in fat, and the tri-*n*-butyltin had disappeared completely from the blood. Studies in metabolism cages indicated that the principal route of loss was via the faeces; limited amounts of labeled CO_2 were exhaled. Iwai et al. (1981) studied the distribution and accumulation of tri-*n*-butyltin and its metabolites in areas of the brain of rabbits. After a single oral dose of tri-*n*-butyltin chloride,

high concentrations of tri-*n*-butyltin were found in the frontal and temporal lobes and in the cerebellum initially. Thereafter, there was a rapid decrease in tri-*n*-butyltin residues and an increase in levels of *n*-butyltin, which persisted for much longer. Persistence occurred preferentially in the gray matter rather than the white matter. The interpretation was that tri-*n*-butyltin which passes readily through the blood-brain barrier, is mainly dealkylated in the gray matter and that the metabolic product remains there. Humpel et al. (1986) administered bis(tri-*n* -butyl-^{113}tin) oxide orally to rats and found that the absorption varied between 20 % and 55 % depending on the vehicle used. High residues of tin were found in the liver and kidney (one to three days after dosing) of which only approximately 5% was unchanged tri-*n*-butyltin.

Other tissues showed lower concentrations of the label but the fraction of unchanged tri-*n*-butyltin was higher. The exact nature of the metabolites could not be identified by the analytical method used (HPLC), but the pattern was indicative of progressive debutylation. Daily administration of tri-*n*-butyltin oxide for fourteen days resulted in steadily increasing concentrations of label in all tissues. Steady-state levels were estimated after three to four weeks. When Snoeij et al. (1987) administered ^{14}C- labeled tri-*n*-butyltin acetate as a single oral dose to rats, about 20% adsorption occurred. The presence of di-*n*-butyltin (DBT) and *n*-butyltin (MBT) in plasma (after TLC separation) was demonstrated 3 h and 27 h after dosing. Tri-*n*-butyltin may cross the placenta to some extent, as was shown by the presence of label in rat foetuses after a single oral dose to the mother at day 18 of pregnancy. The concentration of the toxicant in foetal tissue was comparable to that of the mother's muscle tissue (Humpel et al., 1986).

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1.9.1. TOXICITY OF ORGANOTIN COMPOUNDS

According to Batley et al. (1989; 1992) toxicity is not only dependent upon the level of the actual element, but the actual species being present under extreme toxicity of tri-*n*-butyltin to mollusks and

its peculiar and highly specific effects have raised concerns about toxic effects on humans and other organisms. Although toxicological profiles of organotin compounds are complex in both aquatic organisms and laboratory mammals, organotin compounds are also used as fungicides or molluscicides in some countries, either applied directly or used to treat wood for piers and other materials. Toxicological profiles from conventional toxicity testing of aquatic organisms with tri-*n*-butyltin and triphenyltin are highly variable, median lethal concentration LC_{50} values range from 1.5 to 240 μg / L. It is unclear whether this is due to inherent differences in sensitivity or to differences in route of exposure (Matthiessen, 1974). The effects levels of oral toxicity are low, but the toxicological significance was remarkable for biocide and hence the mean lethal concentration LC_{50} is not quite particularly wide. However, some of the intra species variances in sensitivity are quite large. In particular, the mean lethal concentrations LC_{50} in larvae and adults of the Pacific oyster (*Crassostrea* Gigas) are 1.6 μg / L and 18μg / L for tri-*n*-butyltin while chronic larval mortality occurs at 0.05 μg / L and shell deformities occur at 0.02 μg / L (IPCS, 1990; US EPA, 1997; Short, 1987). The exposure to tri-*n*-butyltin resulting in detrimental effect such as imposex in sublittoral and littoral gastropods, occurs at as little as one ng Sn / L and affects at least 150 species (Swennen et al., 1997; de Fur et al., 1999)

Symptoms of tri-*n*-butyltin toxicity in fish include thymus reduction, decrease in numbers of lymphocytes and inhibition of gonad development (IPCS, 1999). These symptoms suggested that studies may reveal reproductive or other effects at lower concentrations than those reported from the subchronic tests of fishes conducted to date while effects on laboratory mammals observed that triphenyltin and tri-*n*-butyltin produce various health effects in laboratory mammals, including effects on the immune system, such as decreases in immunoglobulin concentrations, lymphopenia, and thymus or splenic atrophy in rats and mice, reproductive / developmental effects: lowest-observed-adverse-effect-levels are mostly in the several mg / kg range or lower), hyperplasia / adenomas on endocrine organs or decrease in white blood cells at 0.3 mg / kg body weight (bw) (IPCS, 1999).

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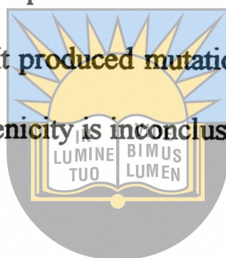
1.9.2 CARCINOGENICITY, MUTAGENICITY AND TETRAGENICITY

Carcinogenic effects were not evident in a screening study of triphenyltin acetate where mice received 19 daily oral doses of 0.464 mg / kg. Other studies have shown that animal species vary in their susceptibility to no carcinogenic effects of the organotin. Teratogenic effects have only occurred in experimental animals that caused overt maternal toxicity. The teratogenic potential of tri-*n*-butyltin is therefore, considered to be very low (IPCS No. 13 and 14, 1999). Comprehensive mutagenicity studies have shown that tributyltin compounds are not considered to have mutagenicity potential (IPCS, No. 13 and 14, 1999), but produced DNA nucleotide base pair substitutions (point mutations) in one bacterial strain tested. It produced mutations in Chinese hamster ovary cells. The result suggests that the evidence of mutagenicity is inconclusive.



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1.9.3. ENVIRONMENTAL TOXICITY

Diffuse input of tri-*n*-butyltin into the environment occurs predominantly from its use, and due to its physico chemical properties tri-*n*-butyltin concentrates in the surface microlayers and because of its persistence in sediment, there is still a concern about its impact on organisms. It is known that marine species exhibit rapid dose-intake responses when exposed to TBT in water (Cardwell and Meador, 1989; Meador, 1997; Fent 1996). However, their response to sediment-associated tri-*n*-butyltin has not been fully studied. Mortality responses for tri-*n*-butyltin in water exposures have been in sediment. The mode of toxic action in humans is unknown, but they are known to affect carbonate metabolism and other metabolic processes in the brain, liver, and muscle, as well as several enzymes and the oxidative activity mitochondria. It has been suggested that general sulfhydryl binding may be responsible for the effects seen in mammals. Mortality responses for tri-*n*-butyltin in water exposures have been reported from approximately 0.5 ng / mL to over 200 ng / mL, a range of about 400 fold (Cardwell and Meador 1989). Most of this variability is due to differences in the uptake and elimination kinetics between species, however, some of these values underestimate the toxic response because of insufficient time for exposure. It should be noted that many of the sublethal responses noted for tri-*n*-butyltin exposure would eventually lead to death of the organism in the environment. The data for adverse effects due to sediment exposure are much less abundant; however, a few studies indicate that sediment concentrations in the 100 - 1 000 ng / g range can have severe effects. For example Fent and Hunn (1991) noted that clams had disappeared in areas where sediment TBT exceeded 800 ng / g dry weight. Meador and Rice (2001) noted moderate to severe reductions in growth for the polychaete *Armandia Brevis* for sediment concentrations in this range (100 - 1 000 ng / g dry wt.). Bryan and Langston (1992) and Langston and Burt (1991) also suggested that some populations of bivalves (*Macoma Balthica* and *Scrobicularia Plana*) disappeared. In human health and aquatic toxicity liquid organotin compounds are assumed to be limited by the relative octanol / water partition coefficient, but this was not explored for ionizable organic compounds or organometallics, such as tri-*n*-butyltin. The sediment-

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water partition coefficient that has been normalized to the organic carbon content of sediment. This normalization greatly reduces the variability in the sediment-water partition coefficients observed among different sediment types. The documented reports relate organotin compounds with a relative log K_{oc} of 13.7, which still showed high toxicity towards fish and dolphins (Thomas and Paquin, 1991).

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Chapter 2

2.1. REVIEW OF ANALYTICAL METHODS APPLICABLE TO ORGANOTIN COMPOUNDS SPECIATION

The recognition of the speciation analysis has been established in connection with toxicology, environmental pollution and protection, as well as health. Different approaches to speciation gave rise of different analytical approaches. Speciation is understood as the occurrence of different forms, chemical and physical, of an element in the real sample. The speciation analysis is the process of identification and determination of different physico-chemical forms of organotin compounds in the real sample (Quevauviller, 1995).

To achieve a good assessment of the presence of organotin compounds, especially tri-*n*-butyltin and its degradation species in the marine environment, the accumulation rates in organisms and the possible effects on human health, a variety of analytical methods have been developed to differentiate ultra- trace levels of organotin species.

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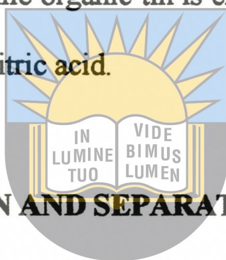
Various hyphenated techniques based on GC separations have been developed and used to determine organotin compounds in water, sediments, and biota. These techniques enable the sensitive and selective methods to determine organotin compounds at sub nanogram levels. Generally any procedure for speciation consists of five successive steps: (i) extraction of the analytes from the sample matrix, (ii) formation of volatile derivatives, (iii) pre-concentration, (iv) clean up, (v) detection, identification, and quantification (Boethling and Mackay, 2000). Sample extraction methods require significant amounts of manpower and time. Sample preparation has become a focus for improving laboratory efficiency as compared to instrumental analysis. There are strong trends to improve the analytical approaches by reducing the number of stages of sample analysis by combining some of them, such as a new technique of stir bar sorption – thermal desorption (Vercauteren et al., 2004) that has capabilities of combining extraction and pre-concentration. The current determination techniques

combine a separation technique such as gas chromatography (GC), liquid chromatography, super-critical fluid chromatography, HPLC, and capillary electrophoresis with fluorescence detection or indirect / direct UV absorbance, (Chang and Yeung, 1995). These efficient chromatographic separation methods utilize various types of detection techniques such as electrothermal atomization, atomic absorption spectrometry (ETA-AAS), quartz furnace atomic absorption spectrometry QF-AAS, IC-MS, ICP or MIP-AES, atomic emission spectrometry, and flame photometric detection. ITMS-GC remains the preferred separation technique owing to its high resolution and the detector versatility despite the more complex sample preparation procedure often required because of insufficient volatility of the ionic organic compounds. While organic tin is extracted with solvent, the inorganic tin is determined by AAS after digestion with nitric acid.



2.2. EXTRACTION AND SEPARATION METHODS

There are several methods, which are applicable for the extraction and separation of organotin compounds in water and sediments. Several of these protocols have been applied with varied quality of results.

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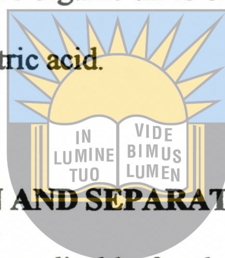
2.2.1. WATERS

The importance of protecting the integrity of water resources demands careful monitoring and evaluation of its quality. Organic pollutants enter the water disperse phase and ultimately contaminate the aquatic marine ecosystem. Earlier methods were based on acidification of the sample with hydrochloric (HCl), hydrobromic (HBr), or acetic acid (HOAc). They release alkyltin compounds from the suspended particulate matter and convert them into respective halides. These were then extracted into a variety of solvents of relatively high polarity. This approach usually succeeds with trisubstituted organotin compounds, tri-*n*-butyltin and triphenyltin, but fails for other species due to

combine a separation technique such as gas chromatography (GC), liquid chromatography, supercritical fluid chromatography, HPLC, and capillary electrophoresis with fluorescence detection or indirect / direct UV absorbance, (Chang and Yeung, 1995). These efficient chromatographic separation methods utilize various types of detection techniques such as electrothermal atomization, atomic absorption spectrometry (ETA-AAS), quartz furnace atomic absorption spectrometry QF-AAS, IC-MS, ICP or MIP-AES, atomic emission spectrometry, and flame photometric detection. ITMS-GC remains the preferred separation technique owing to its high resolution and the detector versatility despite the more complex sample preparation procedure often required because of insufficient volatility of the ionic organic compounds. While organic tin is extracted with solvent, the inorganic tin is determined by AAS after digestion with nitric acid.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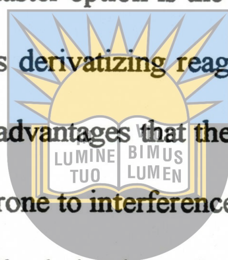
their high polarity. In addition, the more polar solvents are incompatible with Grignard reagents (*n*-pentyl magnesium bromide) used later for the derivatization and favor co-extraction of organic interference compounds. Therefore the recommended procedures are based on the extraction of low polar organotin complexes with tropolone or diethyldithiocarbamate using a non-protic a polar solvents, such as hexane (Rice et al., 1987; Uhler et al., 1993), toluene (Jiang et al., 1991), dichloromethane (Wade et al., 1990). Tropolone was preferred to diethyldithiocarbamate because under acidic condition it undergoes decomposition, giving rise to extractable interferences (Caricchia et al., 1993). An elegant approach which is rapidly gaining popularity is the extraction followed by an *in situ* alkylation step (Alzaga and Bayona, 1993). A faster option is the use of sodium tetraethylborate ($NaBEt_4$) in preference to the hydride generation as derivatizing reagents. Ethylation by $NaBEt_4$ and sodium borohydride ($NaBH_4$) offers a number of advantages that the species can be *in situ* derivatized (Rapsomanikis, 1994). Hydride generation is prone to interference and in case of monosubstituted organotin compounds, it leads to the very volatile derivatives. Organotin compounds are reactive and decompose when subjected to cleanup process.



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The conventional liquid-liquid extraction (LLE) method, which is widely used for extracting semi- and non-volatile organic pollutants from aqueous samples, is labor intensive and requires a large quantity of hazardous organic solvents. It utilizes the partitioning principle where the hydrophobic organic contaminant partitions itself between the two phases (organic and aqueous). It has been generally applied to separate different classes of compounds in chemical analyses including organotin compounds. The solid phase extraction technique is an alternative substitute for liquid-liquid extraction (Loconto, 1991 Part I; 1991 Part II; Markell et al., 1992). Solid phase extraction requires less organic solvent and less labor than liquid-liquid extraction, but extraction efficiencies are comparable. SPE also eliminates the emulsification that often occurs with liquid-liquid extraction. The advantages of SPE include a higher pre-concentration factor and ease of application in the field and in on line systems, while the drawback is that only filtered samples can be analyzed. Liquid-liquid

extraction methods often require a large amount of hazardous solvents and hence currently are not preferred to solid phase extraction procedures (Bayona and Cai, 1994). More recently solid-phase microextraction technique has been applied to organotin speciation (Morcillo et al. 1995; Jiang et al., 2000; Pawliszyn and Millan, 2000). In recent years, solid- phase micro extraction (SPME) is a newly developed inexpensive, relatively rapid and effective technique (Morcillo et al., 1995; Lespes et al., 1998). It offers an attractive alternative method, which minimizes some problems associated with other methods based on a higher enrichment factor, free of solvent and risk of contamination, and the ease of application to field sampling and automation.

2.2.2. SEDIMENTS

Aquatic sediments serve as sinks for many contaminants, prompting the need for the methods to assess the associated particulate matter and toxicity of contaminants associated with the sediments. As organotin compounds bind onto the surface of the sediment, the complete dissolution of the later prior to the analysis is not considered necessary. The basic approach to release organotin compounds from sediment involved by acid leaching (HCl, HBr, HOAc) in an aqueous or methanolic medium using sonification, stirring, shaking, or soxhlet extraction with an organic solvent. To increase the extraction yield, the addition of complexing agent tropolone is mandatory. Apparently no reliable and efficient method for extracting all organotin compounds from sediment has hitherto been developed. While the three and disubstituted compounds can be extracted quantitatively, only about 60 % or less of the monosubstituted compounds are recovered (Tutschku et al., 1994).

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2.3 EXTRACTION OF ORGANOTIN COMPOUNDS USING NON-POLAR SOLVENTS

Hexane (Rice et al., 1987) benzene, toluene (Jiang et al., 1991) dichloromethane (Wade et al., 1990), and ethyl acetate (Tsuda et al., 1987) are used as solvents, with a complexing agent (Stab et

al., 1993). Vortexing, shaking, or sonication are the modes of enhancing through mixing and serve to improve extraction efficiency. The Soxhlet extraction is applied with volatile solvents, without complexing agent. The efficiency with which *n*-butyltin compounds are extracted from spiked sediment with non-polar solvents in presence of complexing agent is arguably satisfactory. In contrast, very poor recovery is obtained with *n*-butyltin tin and di-*n*-butyl tin with dichloromethane without complexing agent.

2.3.1. USE OF NON-POLAR SOLVENTS PLUS ACIDS FOR EXTRACTION OF ORGANOTIN COMPOUNDS

The sample is treated with hydrochloric acid with shaking or sonication, followed by sequential solvent extraction. Hydrobromic acid, or acetic acid, or their mixtures are used. This improved the extraction efficiency of *n*-butyltin. Sonication has become the most widely used stirring method for sediment matrix, whereas energy-mixing methods are used for biotic materials (Milde et al., 1997). Mixtures of solvents have been used to increase the polarity of the medium, e.g. hexane with ethyl acetate, hexane with diethyl ether, and chloroform with ethyl acetate. The salting out effect or ion-pairing effect is used to increase the efficiency of extraction of organotin compounds from aqueous phase to the organic phase, when HCl is used.

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2.3.2 EXTRACTION OF ORGANOTIN COMPOUNDS WITH THE USE OF POLAR SOLVENTS

Extraction of organotin compounds using polar solvents could be performed for the entire range of the aqueous HCl or HCl and HOAc in polar organic solvent (methanol, acetone, acetic acid, and methanol with dichloromethane, as well as *n*-butanol, methanol and acetic acid). The goal for this process is to extract all compounds of interest as completely as possible while preserving their

chemical structure for analysis. Polar solvents are necessary to extract polar (acid and base) compounds and to aid in removing water from the sediment matrix, which can interfere with the proper extraction of non-polar compounds.

Liquid extraction is used to convert organotin compounds from the extract. The tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one) (Wade et. al., 1990) is used with salting to increase the solubility of organotin compounds in the organic solvent. Alternative simple extraction method is based on sonication when no acid is used (Gomez-Ariza et al 1995). The polar solvent extracts tri-*n*-butyltin more efficiently, whilst di-*n*-butyltin and *n*-butyltin are easily extracted in methanolic HCl.

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2.4 SUPERCRITICAL FLUID EXTRACTION (SFE)

Supercritical fluid extraction is a favorable analytical technique for speciation studies because sample preparation and applications take short extraction times. Supercritical fluid extraction using CO_2 with a methanol modifier is an efficient way of extracting organotin compounds from soil and sediments (Alzaga and Bayona 1993; Cai et al., 1994; Dachs et al., 1994). However, it doesn't always work the best for all chemicals. It is non polar, so in case where the extracted chemical is polar, a substance called a modifier is used to increase the polarity of the solvent. Methanol is the most commonly used modifier because it is the least toxic of the polar solvents that is used (Hawthorne, 1990; Hedrick, et al., 1992). No organic solvent involved and no cleanup steps employed since the SFE with neat CO_2 gives cleaner extracts which can directly be analyzed (Bayona, 1993). Although most organotin compounds are soluble in carbon dioxide, an organic modifier methanol is mandatory to extract native organotin compounds from soils and sediments. Two approaches have been evaluated, to improve the extraction efficiency of mono and di-*n*-butyltin species: the addition of complex-

ing agents e.g. diethyl ammonium diethylthiocarbamate and alkylation in the reaction cell with Grignard reagent prior to extraction. Recall that Grignard reagents such as *n*-pentyl magnesium bromide are often contaminated with tri-*n*-butyltin. Therefore a careful blank evaluation is required (Cai et al., 1994; Koskinen, et al., 1995). Recoveries obtained by the first approach are satisfactory for di and triorganotin species but a cleanup step is usually needed. On the other hand, the second method yields satisfactory recoveries for tri-*n*-butyltin and triphenyltin.

2.5. SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

Supercritical fluid chromatography is a relatively recent chromatographic technique, very attractive for speciation since volatile derivatives of organotin compounds do not have to be prepared prior to their determination in SFC (Sadoun et al., 1993). The sample is carried through a separating column by a supercritical fluid (typically carbon dioxide) where the mixture is divided into unique bands based on the amount of interaction between the individual analytes and the stationary phase in the column. As these bands leave the column a detector determines their identities and quantities. Supercritical fluid chromatography has several main advantages over other conventional chromatographic techniques (GC and HPLC). Compared with HPLC, SFC provides rapid separations without the use of organic solvents. With the desire for environmentally conscious technology, the use of organic chemicals as used in HPLC could be reduced with the use of SFC. Because SFC generally uses carbon dioxide collected as a by-product of other chemical reactions or is collected directly from the atmosphere, it contributes no new chemicals to the environment. In addition, SFC separations can be done faster than HPLC separations because the diffusion of solutes in supercritical fluids is about ten times greater than that in liquids (and about three times less than in gases). This results in a decrease in resistance to mass transfer in the column and allows for fast high-resolution separations. Compared with GC, capillary SFC can provide high resolution chromatography at much lower tem-

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peratures because of the added co-solvents or modifiers to the supercritical solvent at low percentage, make a significant change to the solvent properties during analysis.

2.6. SOLID PHASE MICROEXTRACTION (SPME)

Solid phase microextraction is based on simplification of the sample preparation procedure. It is evident that SPME has replaced the solvent consuming and labor intensive liquid - liquid extraction with a GC coupled to an atomic emission detector (GC AED), which allows simultaneous determination of the derivatized organotin compounds (Cai et al., 1994). Extraction time profiles of analytes by headspace SPME are characterized by a three-part curve: first, by a rapid increase in mass sorbed within the first minute of exposure of the SPME phase to the headspace,. followed by a much slower increase, then no change at equilibrium. The initial increase corresponds to the sorption of analyte initially present in the headspace, and the slower increase is due to the mass transfer of analytes from the aqueous phase (Pawliszyn, 1995).

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Headspace SPME is a filament coated with adsorbent material for the extraction from liquid matrix in the determination of tin species. The technique enables different derivatization methods (ethylation, phenylation, hydride generation) *in situ* followed by GC separation (Mester et al., 2000). A possibility of special interface between SPME and ICP / MS for the direct introduction of volatile metal species into the plasma has been developed. Using the SPME-ICP / MS interface direct speciation of methyl mercury with no chromatographic separation has been carried out (Vercauteren et al., 2000; Mester et al., 2000).

For sampling, cleanup and pre-concentration of non-volatile metal species, the in-tube SPME technique can be applied. This is essentially the use of tailor-made fused silica capillaries coated on the inside with a suitable polysilicone acryl ate polymer, connected to the HPLC system in place of a sample introduction loop. The capillary is washed several times with the sample solution until equi-

librium is reached. The main advantage of in tube SPME combined with HPLC is that it can be fully automated. This extraction method was used to sample different organotin compounds (Wu et al., 2000). SPME with *in situ* derivatization by sodium tetraethylborate offers excellent detection limit (1 - 10 ng / L) with a minimum sample consumption (2 - 20 mL) and handling time (ca. 10 min). A clear advantage of SPME is the possibility of desorption directly into the analytical instrument, the only drawback of SPME is a small volume of the polydimethylsiloxane (PDMS) coating used (≤ 0.5 μL) (Arthur and Pawliszyn, 1990).

2.7. DERIVAZITATION TECHNIQUES

Gas chromatography procedures require derivatization reactions to produce volatile organotin compounds for separation and it is often necessary to determine their concentrations accurately at low levels. However, derivatization is time-consuming and yields may vary, depending on the organotin species. The extracted organotin compounds undergo derivatization to a volatile form to be separated by GC. Derivatization methods include the formation of alkyl (methyl or *n*-pentyl derivatives using a Grignard reagent, formation of ethyl derivatives using sodium tetraethylborate or formation of hydrides (R_nSnH_{4-n}) using sodium borohydride The procedure involved in the derivatization of organotin compounds is usually lengthy, entails a great deal of sample handling which leads to errors. Separation of these derivatives are readily analyzed by gas chromatography (Szpunar-Lobinska et al., 1995)

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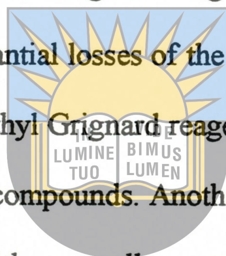
2.7.1 ALKYLATION WITH GRIGNARD REAGENTS

More universal character of derivatization by formation of a tetra-substituted derivative via a Grignard reagent has been employed in many research reports. Alkylation with a variety of Grignard reagents followed by the formation of derivatives: methyl (Avila et al., 1997; Milde et al., 1997), ethyl (Tolosa et al., 1992; Alzaga and Bayona, 1993), di- and tri-*n*-butyl (Szpunar-Lobinska et al.,

1994) *n*-propyl (Nagase et al., 1995) *n*-pentyl (Avila et al., 1995); cyclohexyl (Cai et al., 1994; Reader and Pelletier, 1992; Bouchard et al., 1999) derivatives. However, the method is time consuming and requires strict anhydrous conditions and non-protic solvents, which necessitate solvent exchange when polar solvents are used as extracting agents. Furthermore, liquid- liquid step becomes necessary to isolate the derivatized organotin compounds. Cai et al., 1994 found the formation of dialkylmono- and disulfides when the derivatization is performed *in situ* on a sediment sample before the supercritical fluid extraction, which necessitates a large excess of Grignard reagents. Similar side reactions occur when the derivatization is performed on the extracts. A wide range of extractions was reported, but too long exposure of phenyl to Grignard reagent can lead to disproportionation reactions. Several authors have reported substantial losses of the most volatile tin species when the derivatization is performed with methyl and ethyl Grignard reagents. It is thus advisable to avoid evaporation to dryness of derivatized organotin compounds. Another limitation of the methyl derivatives is that they do not allow the determination of the naturally occurring methyl-*n*-butyltin.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2.7.2 ETHYLATION WITH SODIUM TETRAETHYL BORATE REAGENT

Another procedure of derivatization consists of ethylation and implies usage of sodium tetraethylborate ($NaBEt_4$) (Rapsomanikis et al. 1986; Cai et al., 2000) as an alternative to the hydride. $NaBEt_4$ is the only reagent, which is able to produce quantitative ethylation in aqueous or polar solvents. Sodium tetraethylborate has been successfully applied in determination of organotin compounds in the environment samples (Rapsomanikis et. al., 1986). The ethylation procedure minimizes analyzing time, provides more reproducible results. Furthermore, the ethylation can take place in aqueous phase and the extraction can be performed simultaneously. Tolosa et al. (1996) developed single-step extractions and ethylation procedures for aqueous samples followed by GC with FPD, MS or MIP-AES. In spiked river sediments, the derivatization yield of MBT using $NaBEt_4$ is lower than that given by hybridization methods, but matrix effects are reduced (Cai et al., 1994). The

method is particularly successful for aqueous samples, but lower derivatization yields than those given by the Grignard reaction are observed in complex matrix containing large amounts of co-extracted compounds (Ceulemans et al., 1994). The $NaBEt_4$ procedure allows a simultaneous extraction-derivatization in a buffered medium. The ethylated derivatives are recovered with non-polar solvents (Wilken et al., 1994; Kuballa et al.,1995). SPME technique has recently been used for speciation analysis of ethyl organotin derivatives (Millan and Pawliszyn 2000).

2.7.3 HYDRIDE GENERATION WITH SODIUM BOROHYDRIDE ($NaBH_4$)

The inorganic forms of elements such as tin react with sodium borohydride with formation of a simple hydride and for alkyltin compounds a series of volatile species is obtained by the hydride formation method followed by purge and trap. The advantage of hydride generation is related to their pre-concentration in liquid nitrogen trap (cryotrap), where from they may be released by slow rising of temperature and determination in atomic absorption. Liquid-liquid extraction becomes convenient, but the signal could be easily suppressed in the presence of interferences, which may occur in organic matrices (Martin et al.,1994; Quevauviller et al., 1996).

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Hydride generation with $NaBH_4$ has seldom been used in off-line methods, owing to the lack of hydride stability. However, this derivatization procedure combined with the use of cry focusing quartz furnace atomic absorption spectrophotometer (CF-QFAAS) allows the determination of *n*-butyltin and highly volatile organotin compounds (i.e. methyltin), which is not possibly determined by most off-line methods. However, phenyltin cannot be analyzed by the methods mentioned above. For the on-line hydride generation, cry focusing coupled to quartz furnace atomic absorption spectroscopy (HG-CF-QFAAS) methodology allows reducing the sample handling steps to a minimum, which makes this approach to be one of the most rapid alternatives for the analysis of organotin compounds (Cai et al., 1994).

The amount of derivatization reagent needs to be optimized according to the matrix characteristics, since the matrix is able to inhibit the hydridization reaction. In this regard, the uncomplexed tropolone suppresses the hydride generation reaction. Recently the solid phase micro extraction technique was introduced as an elegant and practicable extraction technique for volatile organotin compounds (Vercauteren et al 2000) and also for semi-volatile organic compounds for speciation analysis of hydride derivatives (Jiang et al., 2000).

2.8. CLEAN-UP OF EXTRACTS OF ORGANOTIN COMPOUNDS

Exhaustive extraction of sediment or tissue samples brings into the sample extract organic and inorganic constituents other than those of interest. These constituents can interfere with the analysis being performed, but often can be removed or minimized through a subsequent clean-up step. However, most of the analytical procedures based on GC determination require a clean-up, usually after the derivatization step. Silica is the adsorbent most used, other adsorbents are: florisil (Harino et al., 1992), alumina (Stab et al., 1993), alumina - silica, amino and C18 cartridges, florisil - alumina, and florisil - silica. In most of the methods applied to sediments that use GC-MS or GC-FPD, a desulfurization with activated copper following the clean-up is performed. However, alkylsulfides generated during the Grignard derivatization from elemental sulfur occurring in the sediment are not removed by this procedure. Alternatively, other desulfurization reagents such as tetra-*n*-butyl ammonium hydrogen sulfate and sodium sulfide have been successfully applied (Lalere, 1995). Florisil is a preferred adsorbent for biotic matrix with high lipid content. Hexane or hexane-ethanol mixtures are the most widely used eluents during the clean-up step because they allow GC determination without evaporation to dryness. More volatile solvent such as *n*-pentane is used to minimize the evaporation losses of the most volatile species. Other analytical procedures perform the cleanup before derivatization. Since underivatized organotin compounds have strong adsorbate - adsorbent interaction

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strength, polar eluents are needed to achieve quantitative recovery, which leads to poor clean-up efficiency. Tropolone in hexane as eluent was preferred.

2.9. DETERMINATION TECHNIQUES

Usually after the protocol of extraction and clean-up of organotin compounds from water and sediments samples, the extracts after derivatization are subjected to instrumental determination. Methods employed in such determination include the following techniques.

2.9.1 ATOMIC ABSORPTION SPECTROMETRY

In most analytical methods developed, organotin compounds separation is based on chromatographic separation prior to the detection. However, some analytical techniques like atomic absorption spectrometry (AAS) allow tri-*n*-butyltin determination by hydride generation AAS and graphite furnace atomic absorption spectrometry (GF-AAS) after hydridization and selective extraction in sediment as well as water and biological samples (Mickie, 1987). The analytical techniques provide accurate and precise and reliable data, with adequate sensitivity to meet the required detection time.

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While GF-AAS uses the stabilized temperature platform furnace with Ni matrix modifier, enables the chromatographic properties of organotin compounds to be detected with fewer errors caused during the sample preparation.

2.9.2. GAS CHROMATOGRAPHY

The chromatographic separation is often a prerequisite for speciation analyses, particularly when coupled to the specific element detection (Maguire and Huneault, 1981). For low molar mass species, gas chromatography has been the preferred technique, although it often requires derivatization due to the ionic or involatile nature of the investigated species. Depending on the compounds to be ana-

lyzed, a wide range of derivatization or sample preparation techniques have been developed which have not only their particular range of applicability, but their merits and shortcomings as well

GC based speciation methods have been widely used since they give higher resolution than LC methods, which allow simultaneous determination of *n*-butyl, phenyl, cyclohexyl, methyl, ethyl and *n*-octyltin (Muller, 1984). Another advantage of GC over LC is the possibility of using several internal standards and surrogates standards that have properties similar to the target analytes. This allows the recovery steps in the analytical procedure to be traced. The main disadvantage of GC methods is that they usually require production of volatile organotin derivatives to perform their separation.

Packed columns are used exclusively in cryogenic trapping when hydride derivatization is carried out. The hydrides are purged with helium stream and trapped in a U shaped packed column cooled in liquid nitrogen. The column is then heated rapidly until the purging step is completed. This method is only successful for the determination of methyl and *n*-butyltin. Low levels of tri-*n*-butyltin (0.1 to 1 ppt) in water by hydride generation technique were determined using the gas chromatography coupled to the atomic absorption spectrometer (GC-AAS) (Ritsema, 1992).

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On the other hand, capillary columns gained acceptance during the 1990's and nowadays they are commonly used rather packed or megabore columns. A sample is usually introduced into the column by splitless injection because the non-volatile co-injected compound is retained in the liner. The limitation is the low sample capacity (up to 2 µL) and the discrimination of low volatile organotin compounds against the high volatile tin species. Cold on column and temperature programmable injectors avoid some of the limitations of the splitless mode and then allow up to 5 µL to be injected. In order to prevent column contamination GC Tenax packing in the injection port or uncoated deactivated tubing has been used (Müller, 1987).

The high efficiency achieved by the capillary gas column 30 mm long and 0.25 mm ID, 0.25 micron thickness or equivalent that allows satisfactory resolution of organotin compounds according to carbon number even with non-polar, non-selective stationary phase, such as dimethylpolysiloxane or

5 % diphenyldimethylpolysiloxane. Organotin compounds with equal number of carbon co-elute. Hence use of the mid-polarity stationary-phase such as 50 % diphenyldimethylsiloxane or 14% cyanopropylphenyl and 86% dimethyl siloxane leads to improved resolution between specific organotin compounds containing phenyl and cyclohexyl moieties (Kuballa et al., 1995). According to Müller (1987), temperature programming in which temperature was varied improved the course of separation allowing the resolution of all the components run. From the detection point of view, GC is highly flexible can be coupled with selective detectors. In this respect, the following detectors have been used for organotin compounds speciation: flame ionization detector, tin-selective flame photometric detector (Unger et al., 1986; Alzaga and Bayona, 1993; Cai et al 1994; Nagase et al., 1995); microliquid chromatography electrospray ion trap mass spectrometry (Jones et al., 1999); electron capture detector atomic absorption spectrometry (Andrease and Byrd, 1984; Dirkx et al., 1994); atomic emission detector (Liu, et al., 1995; Ceulemans et. al., 1994; Chau et al., 1996), and mass spectrometer (Jackson et al., 1982); inductively coupled plasma mass spectrometry (ICP - MS) (Jiang et al., 2000); and microwave-induced plasma atomic emission spectrometry (Tutschku et. al., 1994). ECD and FID detectors were used in the earlier speciation studies but seldom since the late nineties. The lack of selectivity and or sensitivity of these detection systems led to their replacement by more sensitive low cost detectors such as mass spectrometer (MS) in the electron impact mode and flame photometer detector (FPD) equipped with an interference filter at 610 nm or AAS.

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Unfortunately, the low molar masses of diagnostic ions in the electron impact or chemical ionization modes impair moderate selectivity in case of complex matrices. Similarly, FPD suffers some interference associated with co-extracted sulfur species (Cai et al., 1994). AED and MS are most valid, sensitive, and selective detection systems coupled to GC used in organotin speciation (Dowling and Uden 1993). The combination of capillary gas chromatography (CGC) and inductively coupled plasma mass spectrometry (ICP-MS) has become a powerful technique for the speciation of organotin compounds in complex environmental samples, owing to the high resolving power of CGC and to

the high sensitivity of ICP-MS. However, the high cost and maintenance of operation of the GC-microwave induced plasma atomic emission spectrometer (MIP-AES) system precludes its application to monitoring studies involving a large number of samples.

2.9.3. LIQUID CHROMATOGRAPHY

Most of published methods have been tested on standards, few on environmental samples. In spite of the advantage to avoid derivatization step, LC has some limitation arising from the insufficient sensitivity of the most common detector compared to the level found in environmental samples. Filtered water for solid–phase extraction with liquid chromatography /mass spectrometry positive ion electrospray ionization (LC/MS-ESI) has been used (Betowski and Jones, 1996; Lindsey et al., 2001). *n*-Butyltin compounds are the most species considered, in some other instances preference is given to phenyltin. Ion exchange chromatography is most applied mode compared to reverse phase normal phase, and ion pair chromatography.

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Ion exchange chromatography is performed in silica based cation-exchange column, it has been the most applied, (Compano et al., 1995; Rivaro et al., 1995). Mobile phases consist of a mixture of methanol or sometimes, acetonitrile and water, containing ammonium acetate or citrate. The separation of tri-*n*-butyltin and di-*n*-butyltin among the other organotin compounds is achieved at the same pH. Meanwhile, in other separations of di- and triorganotin compounds based on normal phase type 78 % cyanopropyl - methylpolysiloxane has been used. The mobile phase consisted of high percentage of *n*- hexane together with polar solvent such as ethylacetate, tetrahydrofuran (THF) and HOAc. A mobile phase consists of tropolone in toluene. Reverse phase with octadecylsilane stationary phase (C18) have been used with polar mobile phase containing complexing agent such as tropolone.

On the other hand, reverse phase ion pair approach has been applied in the separation of tri- organotin compounds. Polymeric based column (PRP-1) or an octylsilane column (Capcell Pak C8, 4.6

× 150 mm) were utilized, whereas pentane sulfonate or hexane sulfonate are used as an ion-pair (Kumar et al., 1993).

Several detectors or hyphenated techniques have been used in LC: AAS, ICP-MS, fluorimetry, MS, laser-enhanced ionization (LEI), and ICP-AES. Among different AAS modes, flame AAS with pulse nebulization and off-line GFAAS were the earliest. When ICP-MS is coupled to LC, pneumatic nebulizers and spray chamber are the common systems for sample introduction. ICP methods suffer from incompatibility of most of mobile phases. When fluorimetric detection is used, derivatization with fluoregenic reagent such as flavone derivative is mandatory. The reaction is performed after chromatographic separation (Compano, 1995).

2.9.4. GC / MS SYSTEMS

Mass spectrometry is an established analytical technique that identifies compounds by the mass (more correctly, mass to charge ratio) of the analyte molecule. Mass spectrometry is especially noteworthy among analytical techniques because the signals produced by a spectrometer are the direct result of chemical reactions such as ionization and fragmentation, rather than energy state changes of most other spectroscopic techniques. Because of this distinction, mass spectrometry is considered as the only definite analytical technique and detector. In the environmental field, mass spectrometers are typically used as detectors for gas chromatographs. Coupling mass spectrometer with gas chromatographic systems allows separations and subsequent determination of components of highly complex mixture with a high degree of certainty. Because of the increased durability of modern instruments, field gas chromatographs / mass spectrometers (GC/MS) are capable of the same analyses as fixed laboratory instruments (Borman, 1998).

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Chapter 3

EXPERIMENTAL

3.1. MATERIALS

All chemicals used in this study were of analytical grade and of good quality. In view of solvents used, care was demonstrated to use those free of analytes. All reagents were of analytical grade and were used without any purification.

Table 3.1. List of Reagents Used

Reagent	Density, g / mL	Grade	Supplier
Acetic acid	1.048	Analytical	Aldrich
Dichloromethane	1.3	HPLC	Merck
Ethyl magnesium bromide	NA	Analytical	Aldrich
Methanol	0.79	HPLC	Merck
Sodium acetate	1.52	Analytical	Aldrich
Sodium chloride	2.17	Analytical	Merck
Sodium sulfate	2.7	Analytical	Merck
Tri-*n*-butyl tin	1.25	Analytical	Sigma
Tropolone	NA	HPLC	Aldrich
Tri-*n*-butyltin dichloride	1.4	Analytical	Sigma
n-Butyltin	NA	Analytical	Sigma
Tetraphenyltin	1.06	Analytical	Sigma
Tri-*n*-propyltin chloride	1.29	Analytical	Sigma
Sodium tetraethylborate	NA	Analytical	Strem Chemical

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3.1.1 CONTAMINATION SOURCES

Contamination of laboratory apparatus with organotin compounds arises from the use of materials containing diorganotin stabilizing compounds, e.g. as in many plastics (PVC) may yield discrete artifacts, causing misinterpretation of chromatograms of the analytes and use of such materials was avoided. However, organotin compounds are readily adsorbed onto glassware, and losses may occur

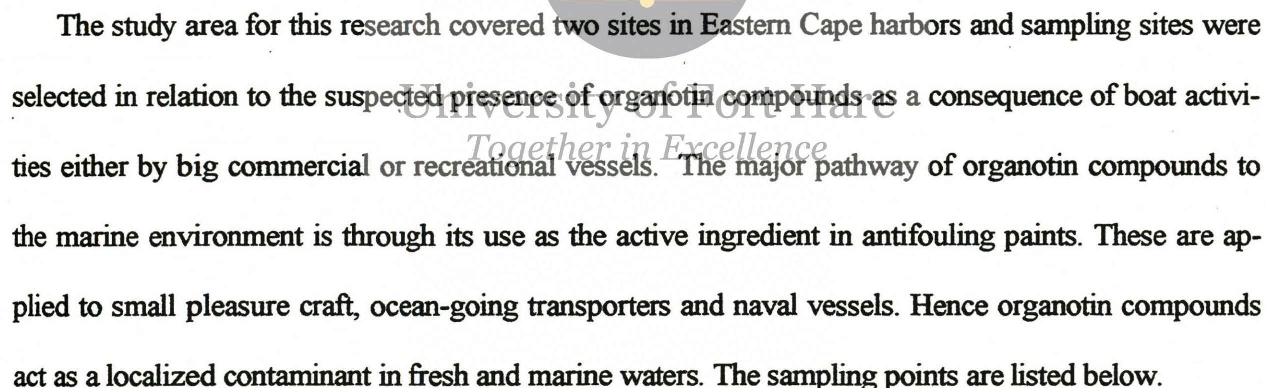
during the use of glass equipment. As polyethylene flasks are known to release trace amounts of organotin compounds, only glass flasks were used.

3.1.2. CLEANING AND PREPARATION OF GLASSWARE

The organotin compounds have an affinity for bonding with glass. In order to make sure of the absence of background contamination, all glassware was soaked in conventional liquid detergent for 8 hours, then rinsed with tap water and soaked for further 8 hours in 12 N hydrochloric acid. Finally the glassware was rinsed thoroughly in distilled water and dried in an oven at 50°C.

3.2. STUDY SITES AND SAMPLING PROTOCOL

3.2.1 DESCRIPTION OF THE STUDY AREA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The study area for this research covered two sites in Eastern Cape harbors and sampling sites were selected in relation to the suspected presence of organotin compounds as a consequence of boat activities either by big commercial or recreational vessels. The major pathway of organotin compounds to the marine environment is through its use as the active ingredient in antifouling paints. These are applied to small pleasure craft, ocean-going transporters and naval vessels. Hence organotin compounds act as a localized contaminant in fresh and marine waters. The sampling points are listed below.

3.2.2. SAMPLING POINTS OF EAST LONDON (EL) HARBOR

EL1. This is a dry dock area designed for pointing and stripping of ships. It is at the entrance mouth of Buffalo River.

EL4. This is a motor scrap area and is also the main dock area. This site is at the entrance mouth of Buffalo River and is closest to the industrial discharged effluents entrance channel from the nearby industries such as Mercedes Benz, candle industry, and wood factories.

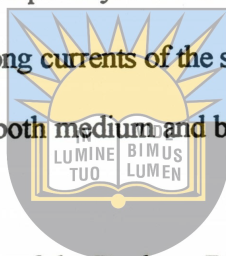
EL10. This is a fishing site with less anthropogenic activities. This site is closer to oil storage and transport system.

EL11. This is a docking activity site. The harbor workshop area is nearby and there is a lot of transportation involving cars and trains.

EL14. This is an entrance channel site of the harbor from the side of the Indian Ocean. It is one of the fishing sites with no anthropogenic activities taking place.

3.2.3. SAMPLING SITE OF PORT ELIZABETH (PE) HARBOUR

PE1. This is a docking site for the big ships only. It is situated on the open side of the harbor where there is a lot of water movement due to strong currents of the sea.



PE2. This is also a docking site for both medium and big ships. The site is closer to some of the structural construction build in the harbor.

PE3. This site is at the entrance mouth of the Baakens River and is also a docking site. Lot of the structural constructions is located next to this site.

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PE4. This site is also at the entrance mouth of the Baakens River with docking activities.

PE5. This site is next to the docking area of big ships carrying ore. The ships also release some wastes into the water.

3.2.4. SAMPLING PROCEDURE

Water samples were collected into a clean Winchester bottles from the sampling sites. The water samples were immediately preserved by adding 5 mL of the concentrated HNO_3 and stored in ice packed cooler box. After sampling, they were transported to the laboratory where they were stored at 4°C in a refrigerator until analyzed.

Sediment samples were collected from about 0-5 cm below surface from the same location where water samples were taken. The samples were drawn into clean 1 L wide mouth Nalgene plastic containers and covered immediately after sampling. In the laboratory, they were kept frozen at −18°C prior to sample preparation and analysis.

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3.3. METHODS

3.3.1. METHOD PERFORMANCE APPROACH

The performance of a method is determined by two sets of criteria: qualitative and quantitative. The strategy was to study those reliable parameters in detail to take full advantage of the FPD sensitivity and selectivity in monitoring organotin compounds. Uncertainty challenge arose in the initial stages of this study. There were many physical parameters, which needed to be carefully optimized in order to take full control of operating parameters, which were interrelated. The practical implementation was cumbersome, as a variety of parameters such as detector temperature, oven temperature, injector temperature, and hydrogen : air gas ratio needed a detailed optimization. From a practical point of view, after the operating conditions were set right, this improved the sensitivity and detectability of the organotin compounds. The calibration curves (peak areas *vs.* organotin standard injected) obtained was based on an average of the FPD response. It was further noted that the FPD detection system with respect to the tin selectivity depended much on the air : hydrogen gas mixture ratios.

3.3.2. OPTIMIZATION OF INSTRUMENTAL PARAMETERS

The GC temperature program presents a compromise between conditions, which yielded maximum separation, and a minimum total analysis. It was observed that poor programmed temperatures produced poor separations. Configuration of the oven temperature is very crucial in the chromatographic work and entails adequate temperature setting of the oven to obtain resolution of the analytes. Several oven temperature programs were tried in an effort to obtain good peak resolution with relatively fast analysis time .

Table 3.2. Ramped Oven Temperature Optimized for the GC (Injector 250°C; Carrier Velocity 50 cm / s)

Oven Temperature Program	Analysis Time, min
80°C (1 min) to 220°C @ 10°C to 250°C @ 15°C (5 min)	23.0
100°C (2 min) to 200°C @ 20°C to 260°C @ 15°C (15 min)	26.6
50°C (5 min) to 200°C @ 20°C to 250°C @ 10°C (2 min)	19.5
70°C (1 min) to 190°C @ 20°C to 270°C @ 15°C (5 min)	17.3

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3.3.3. THE CARRIER GAS

Sample transfer to the column is most effective with the use of helium as the carrier gas. Helium exhibits a flat Van Dempter profile. It was established that it minimizes the critical impurities and instrument downtime; in addition it enhanced the separation of low boiling compounds as it allowed more interaction with the stationary phase at higher average linear gas velocity. When the carrier gas flow rates were appropriately optimized, the sample detectability improved.

able 3.3. GC-FPD Operational Parameters

erkin Elmer auto system XL gas chromatograph fitted with flame photometric detector (FPD) with a ıt off tin filter at 610 nm. The GC was typically fitted with one column; Perkin- Elmer PE-5MS Elite series: 30 m ×0.25 mm (i. d.) fused silica capillary column with a 0.25 μm film thickness (Perkin Elmer Life and Analytical Science, Inc.)

Parameter	Optimized Instrument Conditions
Injection temperature	250°C
Detection temperature	250°C
Initial temperature	70°C
Initial hold	0°C
Ramp 1 rate	20°C min to 190°C
Ramp 2 rate	15°C min to 270°C
Final hold	5 min
Carrier Gas Flow (Linear Velocity)	50 cm / s
Flame	Hydrogen : air
Hydrogen flow rate	185 cm^3 / min
Air flow rate	250 cm^3 / min
Split vent	35 mL / min
Detector	FPD at 300°C
Run time	17.3 min
Data processing	TotalChrom Workstation

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3.3.4. DETECTION OF GAS FLOW RATIO.

Hydrogen : air flow rates. The performance of the FPD to detect organotin compounds heavily depended upon the hydrogen rich fuel, in which both the carrier and the combustion gases exit through the detector. The air gas carries out a critical role, such as that if adjusted too low, the detector response diminishes, and in fact when the flow rates were varied strong effects of the lack of sensitivity on the detector was noticed. However, raising the hydrogen flow rate to above 350 $cm^3\ min^{-1}$ caused a higher detector signal background that significantly decreased sensitivity and meanwhile caused peak distortion. On lowering the flow rate to below 60 $cm^3\ min^{-1}$, a small flame was observed which not only re-

sulted in a slight decrease in the detector signal background, but also caused flame instability and even flamed out. The sensitivity of the detector improved when the ratio of both hydrogen and air flow rates were optimized at 185 cm^3 min^{-1} and 250 cm^3 min^{-1} respectively. It was observed that in the complexity of optimizing the hydrogen and air, dramatic sensitivity was attained virtually maximum output on the PMT of the detector. Apparent high signal intensity signified the attainment of a hydrogen rich flame (fig. 3).

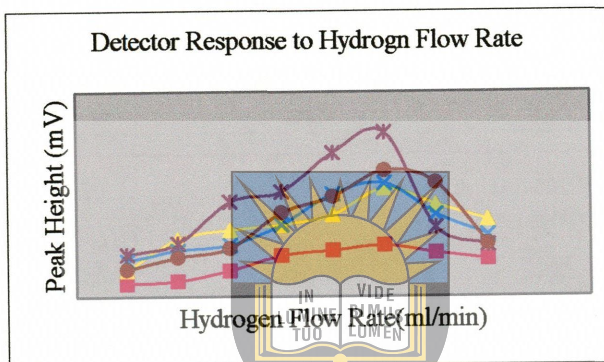

Fig. 3. **Plot of H_2 Flow Rate *vs.* Detector Response**

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Optimizing and maintaining the set values of flow rate of both hydrogen and air appeared to be important for the optimum FPD sensitivity (fig. 4).

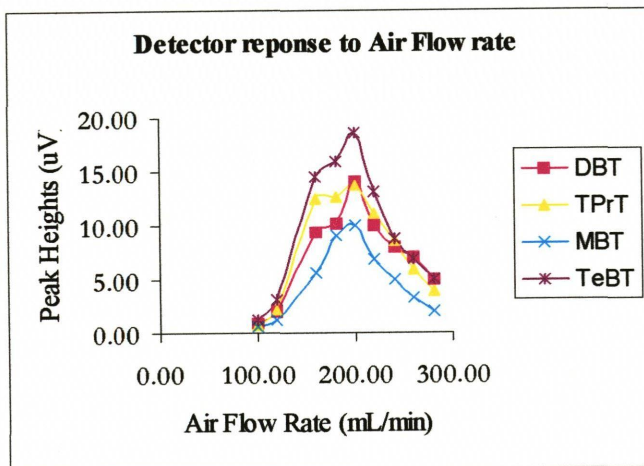

Fig. 4. **Plot of Air Flow Rate *vs.* Detector Response**

3.3.5. INJECTION TEMPERATURE

The injection port had to be maintained at hot temperatures in order to ensure adequate and fast volatilization of the analyte devoid of decomposition. The maximum sensitivity was found at drastically higher temperature range of 250°C, which under these conditions was needed despite the initial slow temperature gradient (fig. 5)..

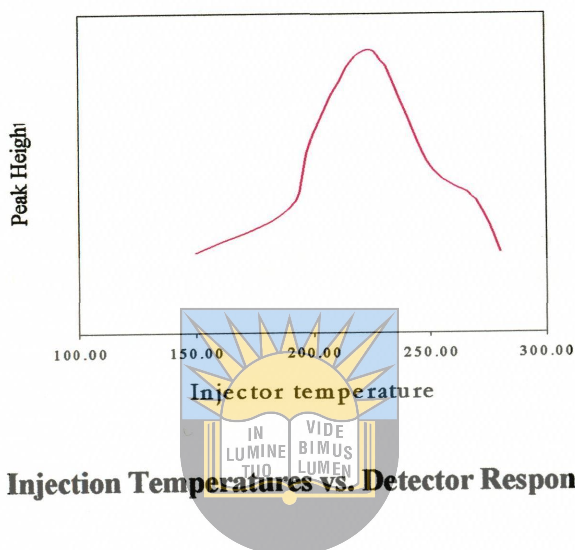

Fig. 5. **Injection Temperatures vs. Detector Response**

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3.3.6. SIGNAL AND NOISE OF THE DETECTOR

The detector noise is the ultimate limit to sensitivity. The noise signal was set to the maximum usable sensitivity during the optimization. Typical FPD detector temperature was found suitable at 250°C while the voltage was kept constant at 70 % of PMT range in relation to the signal to nose ratio. As can be seen from Fig. 6, 250°C appears to be the optimal FPD detector temperature.

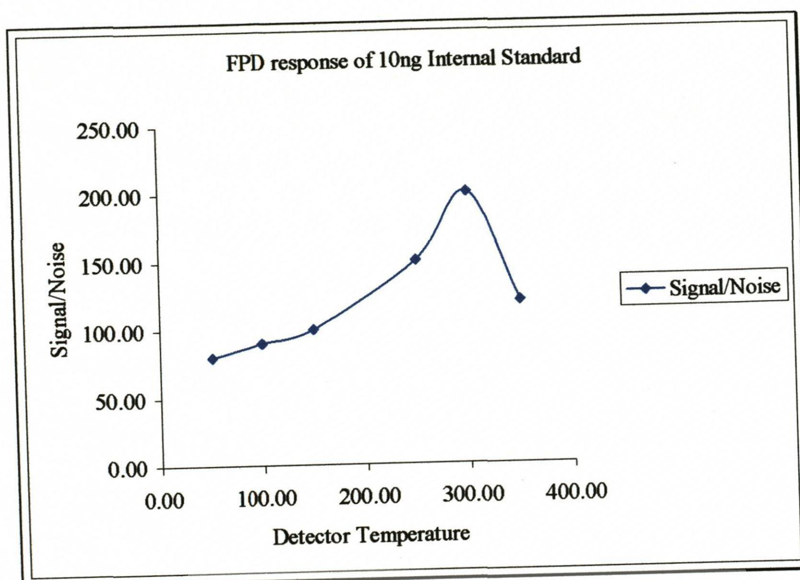

Fig. 6. **Detector Temperature *vs.* Signal Response**

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3.3.7 PREPARATION OF STOCK STANDARD

A stock solution of 1 000 mg / L of the standard pure *n*-butyltin chloride was prepared in distilled methylene chloride. The required volume of the organotin compounds was calculated from the density of each of the species. Tri-*n*-propyltin in methylene chloride was used as an internal standard. Working concentrations were prepared by serial dilution of the stock standard solution. All stock solutions were stored in the dark at 4°C. The stock solution containing the internal standard was run on the GC. The retention time and response factor of each component was noted.

3.3.8. DETERMINATION OF RETENTION TIMES (RT) OF THE ORGANOTIN COMPOUNDS.

The retention times of the organotin compounds were determined by injecting 4 µL of each organotin compounds seven times in the GC under the optimized instrumental conditions.

3.3.9. DETERMINATIONOF RESPONSE FACTORS (RF) OF THE ORGANOTIN COMPOUNDS.

The RF of the organotin compounds relative to the internal standard (I.S), tri-*n*-propyltin or tetra-*n*-propyltin was carried out by injecting 4 μL of a mixture of the organotin compounds together with the internal standard in the GC. Response factor was calculated based on the following equation:

$$RF = \frac{\text{Peak area of the analyte (organotin compounds)}}{\text{Peak area of the internal standard}}$$

Peaks identified through retention time by injection of a standard organotin mix under identical chromatographic conditions are presented in fig: 7.

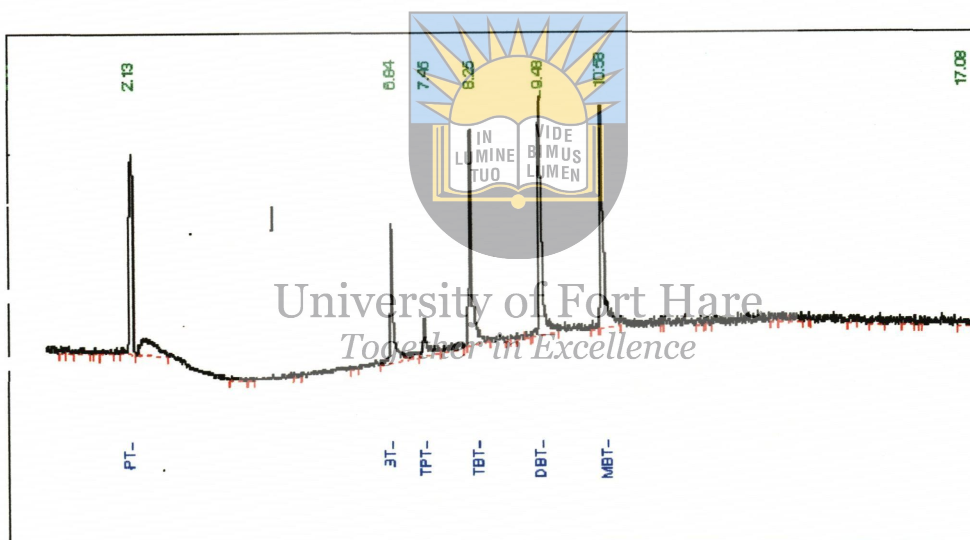

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Fig. 7. **The Chromatogram of the Retention Times of Organotin Standards**

Run Time: 17.33 min; concentration (80 ng/L as Sn). Column: PE-5MS Elite 25 m × 0.25 mm × 0.25 μm. Carrier: He. Oven; Temperature program in the Table 3.2. Injector splitless (35 s). Detector FPD. 4 μL injected.

3.4. METHOD DETECTION LIMIT (MDL) OF ORGANOTIN COMPOUNDS

EPA defines the method detection limit as, "the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte" (Glaser et al., 1981). The approach is based on a single concentration design, and has thus become heavily scrutinized by

many in the environmental field for its inaccurate assumptions (i.e. uniform variance). Although results obtained from single concentration design estimators are rarely mathematically justified, they are much less complex. The steps taken to arrive at the MDL are outlined as follows:

(1) Measurements are taken on at least seven samples of the prepared solution. Results are tabulated and the standard deviation of the data set is taken:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} X_i\right)^2}{n}\right] \quad S = \left(S^2\right)^{\frac{1}{2}}$$

(2) Using the degrees of freedom from the data set and the appropriate confidence level (usually 99%), the critical t-value was looked up using reference tables:

$$t_{v,\alpha} = (\text{look-up value})$$

(3) The MDL was computed as the product of the standard deviation and the critical t-value:

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$$MDL = t_{v,\alpha} \times s$$

(4) To test for uniform variance, another solution is spiked with a slightly different concentration of the analyte. Measurements are taken on at least 7 samples of this new solution and results are tabulated as with the first solution.

(5) An F-test for two sample variance is performed on the two data sets to ensure that the difference between the variances are "statistically insignificant" ($F_{stat} < F_{crit}$).

(6) If determined insignificant, the procedure continues by pooling the two sample variances as follows:

$$s^2_{pooled} = \frac{v_1 s_1^2 + v_2 s_2^2}{v_1 + v_2}$$

3.4.1. QUALITY ASSURANCE STUDIES

Since no certified standard reference materials were available, the spiking method was applied in the quality assurance process for validation of the analytical methods. The appropriate spiking level was ten times the determination of the detection limit.

3.4.2. EXTRACTION / DERIVATIZATION OF ORGANOTIN COMPOUNDS

3.4.2.1. WATER

2 mL of concentrated HCl, 5 g of NaCl, and a mixture of standards consisting of TBT, DBT, and MPT were added to 250 mL of distilled water in a 250 mL Pyrex glass vessel. 2 mL of 0.25 % tropolone in hexane and 2 mL of dichloromethane were added, and the solution was stirred vigorously for 30 min using a magnetic stirrer. The combined organic layers were dried by adding 1 g of anhydrous Na_2SO_4. The solution was filtered using Whatman filter paper No.1, and the residue was washed with 2 mL *n*-hexane. The solution was transferred into a 12 mL screwed cap tube and the organic solvents evaporated to dryness using gentle stream of argon gas at 25°C. 1 mL of *n*-hexane was added to dissolve the residue and 0.3 mL of Grignard reagent (*n*-hexyl magnesium bromide in diethyl ether) was added to the solution. The mixture was shaken for about 30 seconds using Vortex and then sonicated for 30 minutes at room temperature. The tubes were then cooled in ice and 2 mL of 1 M H_2SO_4 was gradually added to decompose the unreacted Grignard reagent. The aqueous phase was removed and 2 mL of deionized water was added and shaken for 15 sec using Vortex. The deionized water was added then to force the organic phase into the narrow neck to facilitate its recovery. The organic phase was transferred in a 4 mL vial and dried with 0.5 g of anhydrous Na_2SO_4. After filtration, 4 μL of the extract was injected into the GC.

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3.4.2.2. SEDIMENT SAMPLES

About 20 g of wet sediment was placed in a 250 mL Pyrex flask with PTFE cap, and 2 g of NaCl and 2 mL of concentrated HCl were added. The mixture was sonicated for 1 hour to remove hydrogen sulfide and then extracted with 25 mL of 0.25% tropolone solution in hexane : dichloromethane (1: 1) for two hours using a magnetic stirrer. The organic phase was recovered with Pasteur pipette after addition of about 2 mL of distilled water. 5 mL of the organic phase was transferred into the 12 mL screw cap and dried with 1 g of anhydrous sodium sulfate. The solution was filtered using Whatman filter No.1 and the residue washed with 2 mL hexane. The solution was collected in 12 mL screwed cap tube and the organic solvents evaporated to dryness using a gentle stream of argon gas at 25°C. The residue was dissolved with 1 mL of *n*-hexane.

3.4.3. DERIVATIZATION OF ORGANOTIN COMPOUNDS

3.4.3.1. GRIGNARD REAGENT

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The derivatization process using Grignard reagent involved the formation of alkyl, methyl, *n*-pentyl or *n*-hexyl derivatives of the organotin compounds. This was performed by adding 300 µL of the Grignard reagent (*n*-hexyl magnesium bromide) to the concentrated water and sediment extracts, and then shaken for 1 min. This was allowed to stand for about 20 minutes. Excess Grignard reagent was removed by adding 2 mL of 1 M H_2SO_4 and 2 mL of deionized water. The derivatized extract was recovered and the aqueous phase was LLE twice with 2 mL *n*-hexane, and finally a cleanup on a silica gel was performed.

3.4.3.2. SODIUM TETRABOROHYDRIDE

The organotin compounds were converted to their hydride form by the use of sodium borohydride, the performance reduced the alkyltin compounds to the hydrides (R_nSnH_{4-n}). When a solution of sodium borohydride was added to the vessel, a vigorous reaction occurred due to the hydrogen evolution. Concentration of $NaBH_4$ was optimized for 1 mL volume addition in the range of 0.5 to 6 % at a pH value of 3.3 of acetic and sodium acetate buffer solution.

3.4.3.3. USING SODIUM TETRAETHYLENE BORATE ($NaBEt_4$)

The organotin compounds were transformed into sufficiently volatile compounds by the formation of ethyl derivatives using sodium tetraethylborate. A 0.5 % (w/v) of aqueous solution of $NaBEt_4$ was prepared in double distilled water. In addition, acetate buffer solution with a pH value ranging from 3 to 5 was prepared by taking 50 mL of dilute acetic acid of varying concentration combined with 27.5 mL of 2M sodium acetate trihydrate. 1 mL of 0.5 % aqueous $NaBEt_4$ solution was added to the extract; 10 mL of buffer solution and 1 mL of *n*-hexane containing 300 μL of an internal standard (tri-*n*-propyltin) were finally added. The mixture was shaken manually for five minutes. After the phase separation, the *n*-hexane phase was collected by means of Pasteur pipette and transferred into a clean vial.

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3.4.4. SILICA GEL COLUMN CHROMATOGRAPHY

The concentrated extract in 2 mL of hexane was purified with silica column chromatography. The chromatographic glass column (30 cm ×10 mm) was slurry packed with 10 g of the activated silica gel (fluorisil), which was made into slurry with 1 % water-adsorbent using distilled water. About 0.5 gm of anhydrous sodium sulfate was placed at the top of the column to absorb any water in the sample extract or the solvent. The concentrated sample extracts were transferred onto the col-

umn using a disposable Pasteur pipettes. Organotin compounds were eluted twice with 10 mL dichloromethane. The eluted extracts were collected, combined and dried with anhydrous sodium sulfate and then evaporated using a moderate stream of argon. 300 µL internal standard tri-*n*-propyltin was added and the residues were reconstituted with 1 mL *n*-hexane.

3.4.5. DETERMINATION OF BLANK LEVELS

Blank extraction of unspiked distilled water was extracted with methanol / NaOH and care was taken that it did not contain any target of organotin compounds. Internal standard was added and the dried extract and reconstituted with 1 mL of *n*-hexane for GC analysis. The results obtained in fig. 8 indicate that no organotin species were detected than the internal standard added. Derivatization methods are generalized in Table 3.4.

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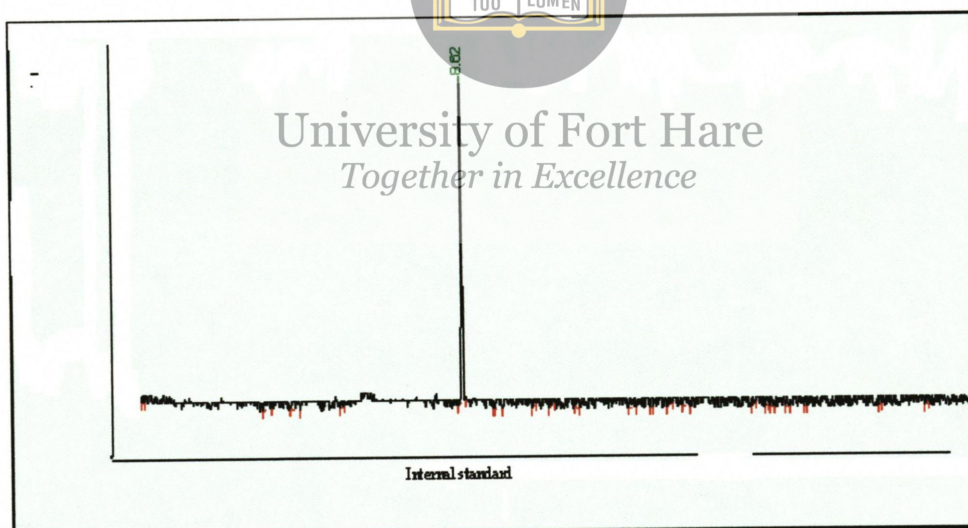
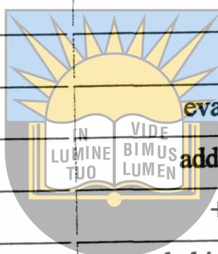


Fig. 8. Chromatogram of a Blank Sample with Clean Background

Table: 3.4 Generalization of the Derivatization Methods

Water Samples	Sediment Samples
250 mL of water in 250 mL flask	10-20 g wet sediment in 250 ml flask
+ 2 mL conc. HCl	+50 mL of distilled water
+ 4 g NaCl	+ 5 mL conc. HCl + 2 g Na Cl
+ 4 mL of 0.25% tropolone in n-hexane and CH_2Cl_2	+ 25 mL of 0.25% tropolone in *n*-hexane and CH_2Cl_2
Magnetic stirrer 2 hrs	magnetic stirrer 2 hrs
centrifugation, separation	centrifugation, separation of solids and aqueous phase
organic phase	5 mL of organic phase
drying with 1 g Na_2SO_4	drying with 1 g Na_2SO_4
filtration	filtration
evaporation to dryness with N_2 or Ar	evaporation to dryness with N_2 or Ar
add 1 mL *n*-hexane (shaking 0.5 min)	add 1 mL *n*-hexane (shaking 0.5 min)
+ 0.3 mL EtMgBr (1 M in THF)	+ 0.3 mL EtMgBr (1 M in THF)
shaking (Vortex 1 min), sonication 30 min	shaking (Vortex 1 min), sonication 30 min
cooling with ice, add 2 mL H_2SO_4 1 M	cooling with ice, add 2 Ml H_2SO_4 1 M
separation of aqueous phase	separation of aqueous phase
add 2 mL distilled water, Vortex 1 min	add 2 mL distilled water, Vortex 1 min
separation of aqueous phase	separation of aqueous phase
drying with Na_2SO_4	drying with Na_2SO_4
filtration	Filtration
GC / FPD analysis	GC / FPD analysis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CHAPTER 4

RESULTS AND DISCUSSION

4.1. OPTIMIZATION OF THE GC CONDITIONS

Optimization of the gas chromatographic conditions / parameters is very crucial and is an important step in the chromatographic work. This is necessary if high sensitivity, fast analysis and good peaks resolution are desired. With the conditions optimized, the gas chromatography (GC) yielded high sensitivity, fast analysis and good resolution of the peaks of organotin compounds. In this work, good optimization permitted detection limit to the range of 4.19 ng / L -12.50 ng / L to be achieved within the analysis time of 17.3 minutes.

Helium gas was found suitable in this work and was used as the carrier gas at a flow rate of 1.1 mL / min. Additional GC parameters, which consisted of the column temperature programming, were optimized to achieve acceptable peaks resolution. The injector temperature of 250°C was found suitable in contributing to the chromatographic resolution.

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The temperature of the detector played a significant role in the instrumental optimization. The detector temperature needed to be optimally higher than that of the analyzed compound to obtain the maximum response. It was found to be the case as the detection response of organotin compounds increased proportionally to the detector temperature as the sensitivity depended on the amount of tin present. Flame photometric detector being a selective detector was used and the temperature gradient was optimized at 250°C to give good detector performance to identify and quantify organotin compounds down to the nanogram level.

4.1.1. RETENTION TIMES AND RESPONSE FACTORS OF THE ORGANOTIN COMPOUNDS

The retention times and the response factors of the organotin compounds are presented in Table 4.1. A more important observation was that the oven program set at 270°C, allowed complete separation of all organotin compounds of interest within 17.3 min. The retention times ranged from 6.7 min for tetra-*n*-butyltin to 10.6 min for *n*-butyltin. The separation was found reproducible where the percentage difference of retention times and peak areas were less than 3 % in most cases (n = 3).

Table 4.1. Response Factors ± SD and Retention Times ± SD [a]

Compound	Response Factor	Retention Time
MBT	0.96 ± 0.03	10.60 ± 0.19
DBT	0.91 ± 0.03	9.48 ± 0.08
TBT	0.78 ± 0.03	8.33 ± 0.08
TPT	1.36 ± 0.06	7.47 ± 0.13
TeBT	0.63 ± 0.03	6.74 ± 0.04

[a] Data ± standard deviation for the seven replicate injections of standard organotin compounds.

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4.2. EFFECTIVENESS OF SEPARATION AND DETERMINATION OF THE LIMIT OF DETECTION

Some GC methods for the determination of organotin compounds have used a variety of capillary columns. In this work, fused silica capillary column, PE-5MS Elite series (30 m length × 0.25 mm ID × 0.25 μm film thickness) gave the best separation after derivatization within the three methods investigated, namely hydridization with sodium borohydride, $NaBH_4$ alkylation with Grignard reagents, and ethylation with sodium tetraethylborate $NaBEt_4$. The detection limits of the organotin compounds ranged from 4.19 ng / L to 12.50 ng / L for TBT, DBT, and MBT. The detection limit is a statistical concept, based on the ability of a measurement method to determine an analyte in a sample matrix, regardless of its source of origin. There is no actual scientific meaning for the detection

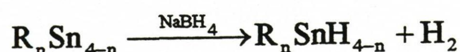
limit until it is defined in terms of a measurement process and a statistical method for analyzing the data produced. It was calculated from the linear regression equation of the calibration curve of the organotin compounds Table 4.2) as generated by the TotalChrom software coupled to the gas chromatograph (Miller and Miller ,1998)

Table 4.2. **Detection Limits of the Organotin Standards**

Organotin Compound	Detection Limit (ng / L)	Correlation Coefficient
Tri-n-butyltin chloride	4.19	0.9997
Di-n-butyltin chloride	6.02	0.9993
n-Butyltin trichloride	12.5	0.9971

4.3 OPTIMIZATION OF EXTRACTION AND ANALYTICAL PERFORMANCE

In selecting an appropriate extraction procedure for the organotin compounds, the following three liquid-liquid extraction methods were applied using tropolone, HCl, *n*-hexane, and ethylation, extract simultaneously by $NaBEt_4$ and by hydridization using $NaBH_4$. The extraction results were evaluated and compared to find the most suitable extraction method for this study that could recover all the organotin compounds close to 100 % from water and sediment samples. The Grignard reaction has been commonly used for organotin alkylation. The method involves a number of steps such as extraction, drying, alkylation, and concentration. Hence the method was found to be time consuming and expensive. From the results, it may be concluded that there was a possibility of degradation of organotin compounds during extraction because the derivatization reaction proceeded in organic media. Hydride generation of volatile organotin derivatives has been performed directly in aqueous phase using sodium tetrahydroborate ($NaBH_4$). Alkyltin compounds react with $NaBH_4$ under acidic condition to yield the corresponding hydrides according to:



$$R_nSn_{4-n} \xrightarrow{NaBH_4} R_nSnH_{4-n} + H_2$$

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However, organotin hydride derivatives were found to be unstable during the liquid – liquid extraction and required cryogenic protection of hydride species as it was observed the method was vulnerable to contamination. The *in situ* technique of ethylation by sodium tetraethylborate ($NaBEt_4$) was able to produce quantitative results and did not suffer from drawbacks that accompany hydridization reaction and Grignard alkylation (Fig. 9).

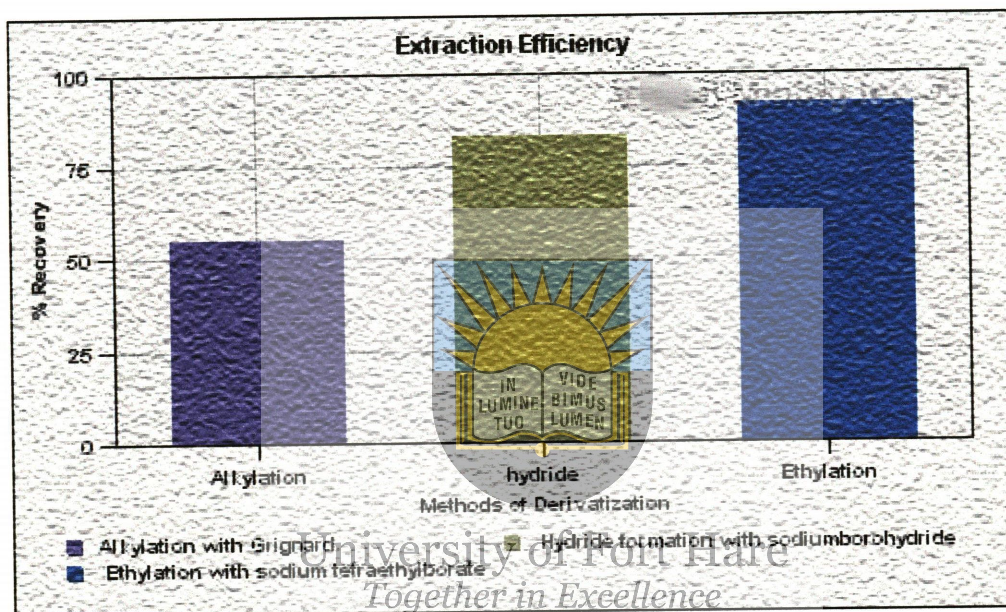

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Fig. 9. Evaluation of Derivatization Methods

It is suggested there are other variables that play a key role in achieving high and reproducible recovery of organotin compounds, which needed prior optimization. These variables are extraction time, temperature, pH and precision of extraction efficiency.

4.4.1. EFFECT OF EXTRACTION TIME IN THE LIQIUD–LIQUID METHODOLOGY

It has been observed that time is a critical factor in any extraction procedure. Many of the variables that determine exhaustiveness of an extraction technique are governed by time. The influence of time on the extraction recovery of organotin compounds was tested to shorten the extraction time, the reaction and sampling time of an organotin compound with sodium tetraethylborate ($NaBEt_4$). Organotin compounds were complexed by tropolone ligand, which was used to improve the extractability of mono- and disubstituted organotin compounds into dichloromethane solvent, which is of low polarity, before Grignard derivatization was done. Besides this procedure was considered to be more time consuming, cost ineffective for routine speciation and also presumed the possibility of degradation of organotin compounds during the extraction time. The fifteen minutes extraction time proved to be sufficient for the organotin compounds as shown in Fig 10.

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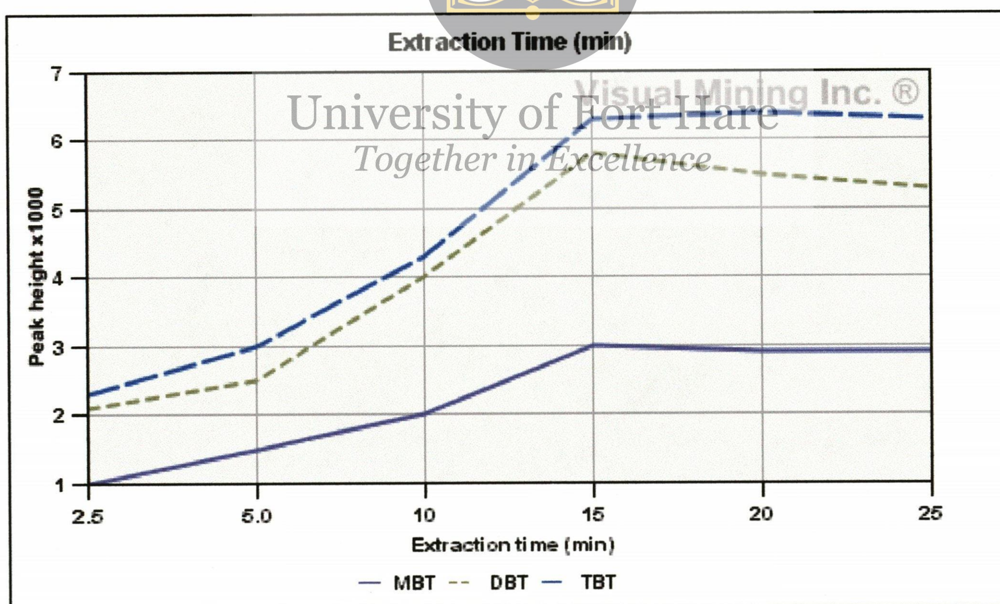

Fig: 10 Effect of Liquid Solvent Extraction Time

Other studies (Yang and Lam, 2001) have also shown that tri-*n*-butyltin and di-*n*-butyltin can be extracted completely within several minutes. The recovery rates of MBT, DBT and TBT using liq-

uid-liquid extraction *n*-hexane, tropolone derivatized by the Grignard alkylation procedure were in the range of 73-100 % for the analyzed organotin compounds.

4.4.2. THE ROLE OF pH ON THE ETHYLATION DERIVATIZATION

As in many previously reported studies, the acidity of the solution plays a key role in the extraction of various organotin species from different matrices (Dirkx et al. 1994). After extraction, derivatization was carried out by the one step ethylation / extraction using $NaBEt_4$. The effect of pH was tested over the range of 5 - 12. Acetate buffer and internal standards were added to the samples and thorough ethylation was done with aqueous $NaBEt_4$ in the presence of *n*-hexane as an extractant. However, addition of the alkaline sodium of tetraethylborate increased the pH of the samples. It was necessary to check the true pH effect on the ethylation reaction, which takes place in aqueous media. The effect of pH on the extractability is shown graphically in Fig.11.

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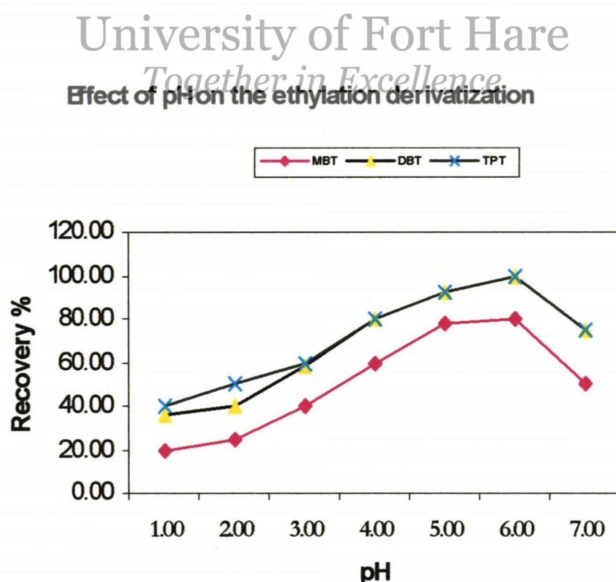

Fig. 11. Effect of pH on the Efficiency of $NaBEt_4$

From Fig.11, it is obvious that there exists a strong influence of pH, and a similar shape for all compounds is observed. Optimal MBT response was observed between pH 5.0 and 6.0 with a sharp decline greater than these values. The poor response at low and high pH was attributed to the acidic

degradation of sodium tetraethylborate and possibly due to formation of stable butyltin hydroxide species. DBT response was found to be in the pH range of 3.0 to 6.4 with a decline in response below pH 3.0. TBT response was optimal in the pH range of 3.0 to 7.0. In order to ensure the best response for all the organotin compounds, the optimum desirable pH was found in the narrow range of 5.0 and 6.0 with the best response at just above 6.0.

4.4.3 EFFECT OF DERIVATIZATION TIME.

Organotin compounds were extracted into *n*-hexane, and then these were transferred to the aqueous layer and the pH was adjusted to 5.0, reacted with $NaBEt_4$ and finally extracted back into *n*-hexane. The effect of shaking time was set from 2 to 10 minutes to test the derivatization efficiency at a constant value of 1.0 mL of 0.5 % $NaBEt_4$. The results suggested that the ethylation and extraction took place more rapidly than expected and the derivatization was set at 10 min. reaction time to achieve quantitative derivatization (Fig. 12).

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4.4.4 PERFORMANCE OF THE METHOD

Determination of organotin compounds requires significant sample preparation to ensure reproducible extraction of the organotin compounds without decomposition, as well as derivatization of the analyte prior to detection. More attention was paid to minimize uncertainties of extraction efficiency and the reproducibility of the results. However, methodologies and performance characteristics for numerous current approaches for organotin compounds have proved a difficult task because of their instability and very low concentration levels of mono-, di-, and tri-*n*-butyltin. Questions have been raised concerning the selection of appropriate extraction efficiency methods. Other studies indirectly noted that no single methodology could be recommended for consistent use (Pellegrino, 2000; Quevauviller et al., 2000).

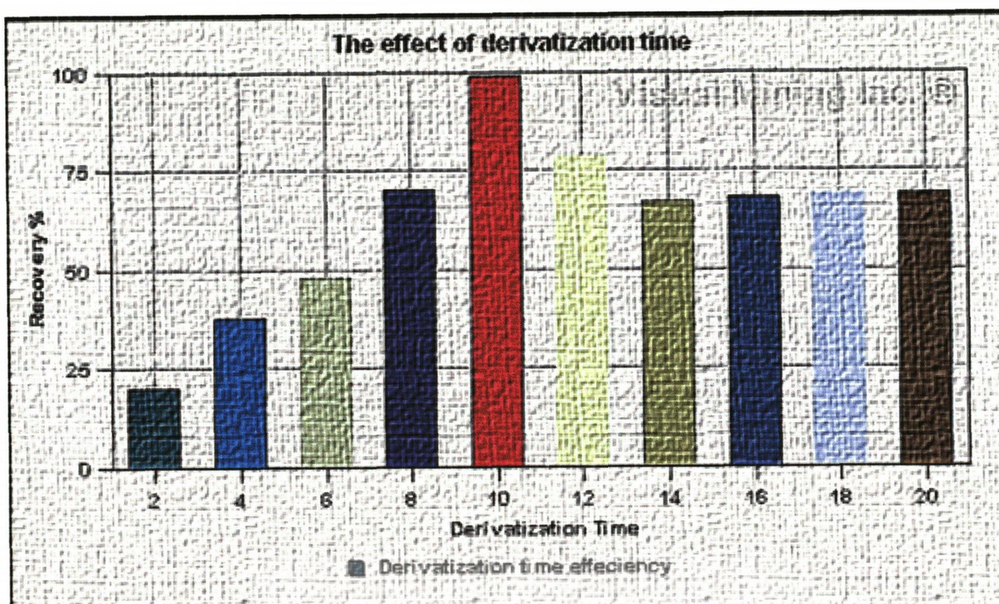

Fig. 12. Effect of Derivatization Time

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This work was framed in a series of studies devoted to exploring the efficiency of extraction and derivatization methods for the determination of the organotin compounds in water and sediment samples. The extraction efficiency of organotin compounds from spiked distilled water by liquid – liquid extraction with benzene, ethyl acetate, toluene, *iso*-octane, and hexane followed by column chromatographic cleanup was performed and the results are given in Table 4.3. From the results, the efficiency of extraction of benzene as extracting solvent ranged from 51.6 ± 5.5 to 64.7 ± 5.5 %, that of *iso*-octane ranged from 71.4 ± 5.0 to 97.2 ± 7.6 %, that of toluene ranged from 74.5 ± 5.0 to 98.0 ± 1.6 %, that of ethyl acetate ranged from 85.00 ± 4.17 to 96.0 ± 5.1 %, and *n*- hexane ranged from 97.90 ± 5.54 to 101.0 ± 3.6 %.

Table 4.3. Mean Percentage Recovery ± S.D of Organotin Compounds Standards from Spiked Distilled Water at Fortified Levels 0-500 (ng / L) by LLE Using Five Extracting Solvents

Organotin Compound	Benzene	Ethyl Acetate	Toluene	*Iso*-octane	*n*-Hexane
MBT	51.62 ± 5.52	85.00 ± 4.17	74.0 ± 5.5	85.0 ± 4.1	97.9 ± 5.5
DBT	64.70 ± 5.54	91.6 ± 4.6	83.0 ± 2.3	71.4 ± 5.5	97.20 ± 5.97
TBT	86.60 ± 8.46	96.1 ± 5.1	98.0 ± 1.7	97.2 ± 7.6	101.0 ± 4.6

It appears from the results that hexane is the best solvent among the sets because the amount of analytes recovered from it was higher than in the other solvents with acceptable reproducibility of the relative standard deviation (RSD) from 4.5 to 10 %. It is known that the recovery of analytes from the environmental waters is influenced greatly by matrix effect. Therefore, in order to estimate the accuracy and precision of the present method and hence to confirm its applicability, recovery testing was applied to water samples (distilled water, tap water and seawater). They were placed in 100 mL capped vials, spiked with the internal standard solution, with different volumes (from 0 to 500 μL) of the standard mixture containing MBT, DBT, and TBT (50 –500 ng / L as tin for each compound in dichloromethane). Results are given in Table 4.4.

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For spiked river water samples, the recovery of tri-*n*-butyltin ranged from 94.9 to 113 %, which was spiked with standards ranging from 50 to 500 ng / L. Similarly, the recovery for the other organotin compounds, tended to be satisfactory 63 to 100% when spiked at same levels. These compounds also showed similar patterns in a seawater matrix, where the recovery was found consistent, which could be judged excellent. Fig. 13 shows a gas chromatogram of an extract of a spiked seawater sample from the harbor.

Table 4.4. Mean % Recoveries of Organotin Compounds in River Water; Tap Water and Seawater [a] (*n*= *4*)

Matrix	Spiked Level (ng / L)	MBT	DBT	TBT
River Water	50 100 200 500	64.1 ± 2.0 78.1 ± 4.8 77.7 ± 2.2 75.0 ± 1.7	91.1 ± 2.7 86.6 ± 1.1 88.5 ± 4.7 86.6 ± 1.1	113.0 ± 5.1 97.7 ± 2.0 94.9 ± 5.8 106.0 ± 3.9
Tap Water	50 100 200 500	63.9 ± 5.2 62.6± 5.2 60.4± 9.2 63.9± 0.7	86.3 ± 0.3 86.9 ± 0.3 80.6 ± 4.0 78.8 ± 3.5	92.9 ± 3.2 91.5 ± 3.2 91.5 ± 3.1 91.4 ± 3.1
Sea Water	50 100 20 500	84.0 ± 2.2 75.8 ± 4.0 78.8 ± 3.5 77.9 ± 10.0	79.0 ± 5.9 61.7 ± 0.5 68.8 ± 5.8 63.3 ± 3.3	100.0 ± 6.2 93.5 ± 1.3 96.4 ± 1.3 93.4 ± 6.6

[a] Conditions: seawater pH 7.9; river water pH 7.8; tap water pH 7.1.

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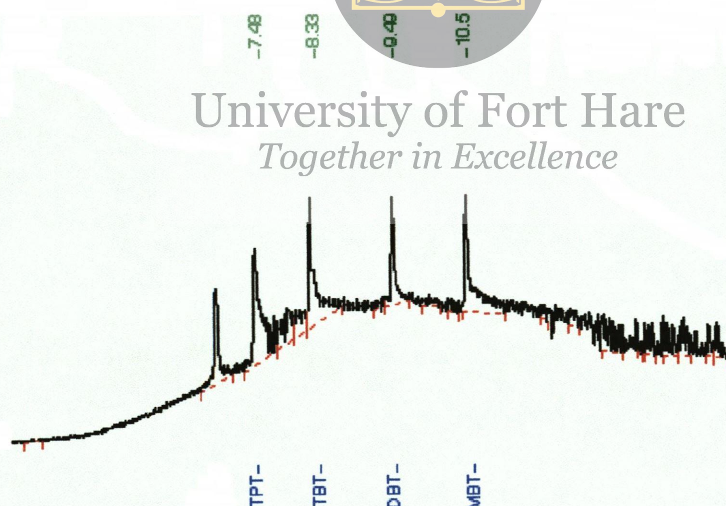

Fig. 13. *Representative Chromatogram of Spiked Sea Water Extract*

A chromatogram of seawater after extraction and the sample was analyzed in fourfold and good concordance was obtained. The results therefore demonstrate that the organotin compounds can be simultaneously separated and determined from seawater and river water samples by the $NaBEt_4$ ethy-

lation, and the whole procedure can be accomplished in set time thereby significantly diminishing the risk of losses during the sample preparation.

Table 4.5. Average Recovery Percentage (R %) of Organotin Compounds in Spiked Sediment Sample at Fortification Level of 25-500 ng / g by LLE Using *n*-Hexane

Compound	Spiked Concentration	No of Replicates	Average Recovery	% Recovery	RSD
Tri-*n*-propyltin	500	7	470	94	15
Tri-*n*-butyltin	500	7	450	90	8
Di-n-butyltin	500	7	450	90	8
Mono-*n*-butyltin	500	7	460	92	8

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The average recoveries using hexane as the extracting solvent ranged from 86.0 % to 94 % with the relative standard deviation ranging from 8 % to 15 % when the sediments were spiked with a mixture of organotin standards at 500 ng/g of each (Table 4.5). Therefore the recoveries of analytes in the sediment matrix (80 % - 94%) were acceptable. The results for the analyzed concentrations of TBT, DBT, MBT, and total organotin compounds (sum of TBT, DBT, and MBT) for the East London Harbor water samples are presented in Table 4.6 and those for the sediment sample from the same site are presented in Table 4.7.

The organotin compounds concentrations are reported as nanogram of tin (Sn) per L (ng / L of Sn) or nanogram of tin (Sn) per gram (ng / g Sn) of dry mass sediment to enable direct comparison of the three-organotin species. The organotin compounds were detected in all water and sediment samples analyzed. The result of the organotin compounds in water ranged from 11.70 ± 0.45 ng / L – 32.20 ± 0.50 ng / L (MBT), 6.50 ± 0.12 ng / L – 33.20 ± 2.50 (DBT), and 4.20 ± 0.10 - 25.7 ± 0.30 ng / L (TBT). The highest concentration of total organotin compounds obtained in water samples was 24.90 ± 0.30 ng / L to 69.50 ± 0.55 ng / L (∑OTs = MBT + DBT + TBT). The concentration of organotin com-

pounds in the sediments varied widely depending on the site location, ranging from less than 0.1 to 56 ng / g for MBT, less than 0.1 to 118 ng / g for DBT and 4.6 ng / g to 1053.7 ng / g (Table 4.7).

High concentration of organotin compounds in the sediments from Slipway (E10 and E11) sites was probably the result of tri-*n*-butyltin leaching from paints on the hulls of the large vessels docked close to wharfs. It could also be attributed to the harbor activities such as stripping and painting of ship, which significantly contaminate the harbor environment. Orient pier exhibits presence of organotin compounds most probably attributed to tri-*n*-butyltin leaching from the sites adjacent to the vessel repair facilities including hull repainting operations which are potential source of tri-*n*-butyltin to the environment.

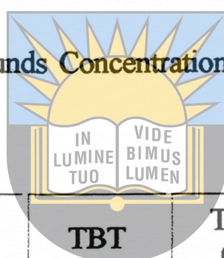

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Table 4.6. Variation of Organotin Compounds Concentration (ng / L) in East London Harbor Water Samples [a]

Sample	Date	MBT	DBT	TBT	Total OT	MBT / OT	TBT / DBT	% TBT
EL14	15/05/01	22.60 ± 0.28	7.20 ± 0.06	4.20 ± 0.15	34.1 ± 0.5	0.67	0.58	12
EL14	16/07/01	31.70 ± 0.43	15.50 ± 0.52	15.30 ± 0.30	62.50 ± 1.25	0.50	0.87	24
EL14	16/07/01	16.70 ± 0.27	7.50 ± 0.15	7.5 ± 0.4	31.70 ± 0.41	0.52	0.99	24
EL14	14/08/01	18.80 ± 0.70	7.90 ± 0.23	6.6 ± 0.2	33.5 ± 0.6	0.56	0.83	20
EL14	11.09.01	12.60 ± 0.30	7.70 ± 0.06	8.20 ± 0.01	28.6 ± 0.4	0.44	1.06	29
EL11	15/05/01	12.90 ± 0.05	30.80 ± 0.20	25.7 ± 0.3	69.50 ± 0.55	0.19	0.83	37
EL11	12/06/01	12.70 ± 0.28	22.10 ± 0.50	12.80 ± 0.2	47.60 ± 1.03	0.26	0.57	36
EL11	16/07/01	32.20 ± 0.50	11.90 ± 0.07	15.40 ± 0.10	59.50 ± 0.62	0.54	1.31	26

EL11	14/08/01	12.80 ± 0.57	13.70 ± 0.20	15.00 ± 0.06	41.50 ± 0.90	0.31	0.90	31
EL4	15/05/01	22.40 ± 0.52	16.80 ± 0.20	11.60 ± 0.20	50.80 ± 0.96	0.43	0.69	23
EL4	12/06/01	12.80 ± 0.18	11.80 ± 0.10	11.00 ± 0.39	35.20 ± 0.69	0.36	0.89	30
EL4	16/07/01	18.70 ± 0.32	15.50 ± 0.40	11.70 ± 0.20	45.90 ± 0.96	0.40	0.76	26
EL4	14/08/01	19.10 ± 0.10	16.40 ± 0.70	11.70 ± 0.20	47.30 ± 1.04	0.40	0.70	25
EL4	11/09/01	20.70 ± 0.43	10.60 ± 0.43	9.80 ± 0.17	41.10 ± 1.03	0.50	0.97	25
EL14	10/01/02	20.40 ± 0.50	7.30 ± 0.40	4.20 ± 0.10	31.90 ± 0.50	0.64	0.58	13
EL14	08/06/02	22.30 ± 0.55	8.30 ± 0.40	15.80 ± 0.10	46.40 ± 0.70	0.48	1.92	34
EL14	07/11/02	16.40 ± 0.68	8.01 ± 0.12	11.10 ± 0.10	35.60 ± 0.80	0.46	1.40	31
EL14	10/01/02	11.90 ± 0.06	7.70 ± 0.42	12.40 ± 0.50	31.0 ± 0.9	0.38	0.13	39
EL14	30/01/03	11.90 ± 0.45	7.70 ± 0.35	12.50 ± 0.20	32.0 ± 1.0	0.37	0.20	39
EL11	10/01/02	21.30 ± 0.76	33.20 ± 2.50	13.90 ± 0.10	68.40 ± 3.33	0.32	0/43	21
EL 11	08/06/02	18.50 ± 0.15	14.60 ± 0.60	10.0	42.90 ± 0.80	0.42	0.67	23
EL11	07/11/02	18.70 ± 0.31	19.60 ± 0.30	13.0	51.30 ± 0.60	0.52	0.84	24
EL11	10/01/03	19.40 ± 0.90	15.70 ± 1.20	7.80 ± 0.30	42.93 ± 2.40	0./45	0.50	18
EL11	30/01/03	15.60 ± 0.78	11.09 ± 0.60	7.20 ± 0.50	35.10 ± 1.80	0.44	0.65	20
EL4	10/01/02	12.90 ± 0.12	6.50 ± 0.12	5.50 ± 0.05	24.90 ± 0.30	0.52	0.85	22

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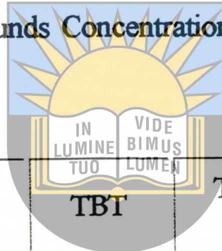
EL4	11/09/01	42 ± 1.0	119 ± 1.00	1019 ± 0.64	1180 ± 2.64	0.50	0.97	86
EL14	10/01/02	nd	nd	5.9 ± 0.06	5.9 ± 0.06	0.64	0.58	na
EL14	08/06/02	nd	nd	4.6 ± 0.01	4.6 ± 0.01	0.48	1.92	na
EL14	07/11/02	nd	nd	5.25 ± 0.05	5.25 ± 0.05	0.46	1.40	na
EL14	10/01/02	nd	nd	6.15 ± 0.05	6.15 ± 0.05	0.38	0.13	na

[a] Data: (EL14) , (EL11) and (EL4) are samples taken at Orient Pier, Dockyard and West Quay sites,

Table 4.7. Variation of Organotin Compounds Concentration (ng / L) in East London Harbor sediments Samples [a]



Sample	Date	MBT	DBT	TBT	Total OT	MBT / OT	TBT / DBT	% TBT
EL11	15/05/01	1.93 ± 0.06	6.0 ± 0.01	13.3 ± 0.02	21.23 ± 0.09	0.09	2.22	63
EL11	12/06/01	2.96 ± 0.60	6.0 ± 0.10	14.80 ± 0.12	24.46 ± 0.19	0.26	0.57	60
EL11	16/07/01	3.70 ± 0.10	6.90 ± 0.07	11.0 ± 0.01	21.60 ± 0.03	0.54	1.31	51
EL11	14/08/01	2.17 ± 0.17	6.86 ± 0.05	11.4 ± 0.06	20.23 ± 0.25	0.31	0.90	56
EL4	15/05/01	2.94 ± 0.05	5.93 ± 0.03	11.2 ± 0.18	20.27 ± 0.12	0.43	0.69	55
EL4	12/06/01	55 ± 1.0	113.6 ± 0.52	1053.7 ± 0.32	1222.3 ± 1.84	0.36	0.89	86
EL4	16/07/01	56 ± 1.5	118 ± 0.06	1033 ± 1.76	1205.0 ± 3.32	0.40	0.76	86
EL4	14/08/01	36 ± 1.0	102 ± 0.06	1150.4 ± 0.55	1188.4 ± 1.61	0.40	0.70	96

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EL4	11/09/01	42 ± 1.0	119 ± 1.00	1019 ± 0.64	1180 ± 2.64	0.50	0.97	86
EL14	10/01/02	nd	nd	5.9 ± 0.06	5.9 ± 0.06	0.64	0.58	na
EL14	08/06/02	nd	nd	4.6 ± 0.01	4.6 ± 0.01	0.48	1.92	na
EL14	07/11/02	nd	nd	5.25 ± 0.05	5.25 ± 0.05	0.46	1.40	na
EL14	10/01/02	nd	nd	6.15 ± 0.05	6.15 ± 0.05	0.38	0.13	na

[a] Data: (EL14) , (EL11) and (EL4) are samples taken at Orient Pier, Dockyard and West Quay sites,

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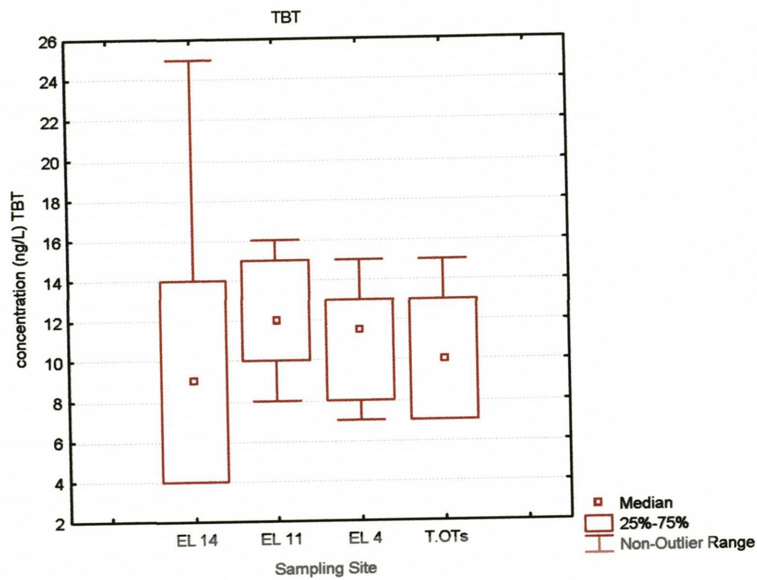

Fig. 14. Box and Whiskers Plot of concentration *vs.* TBT for the Water of East London Harbor

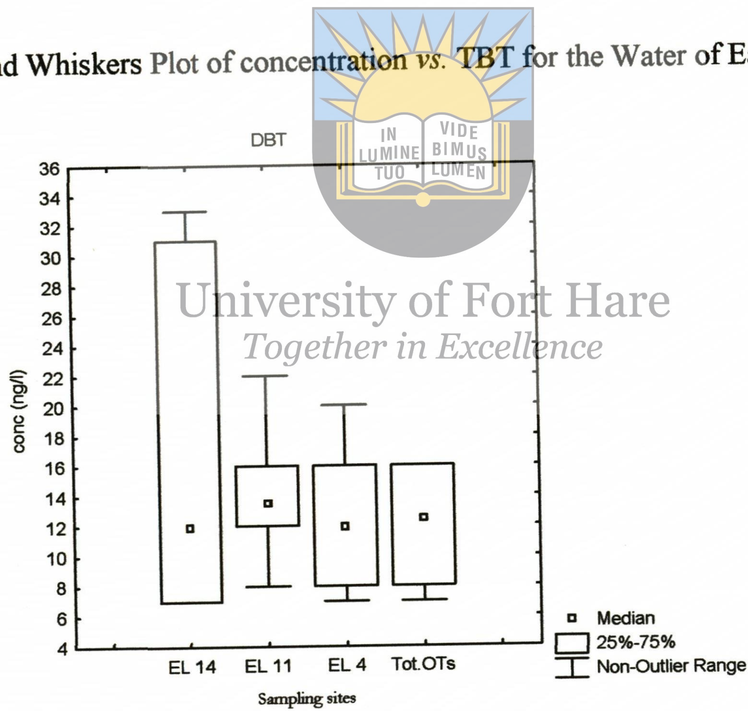

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Fig.15. Box and Whiskers Plot of concentration *vs.* DBT for the Water of East London Harbor

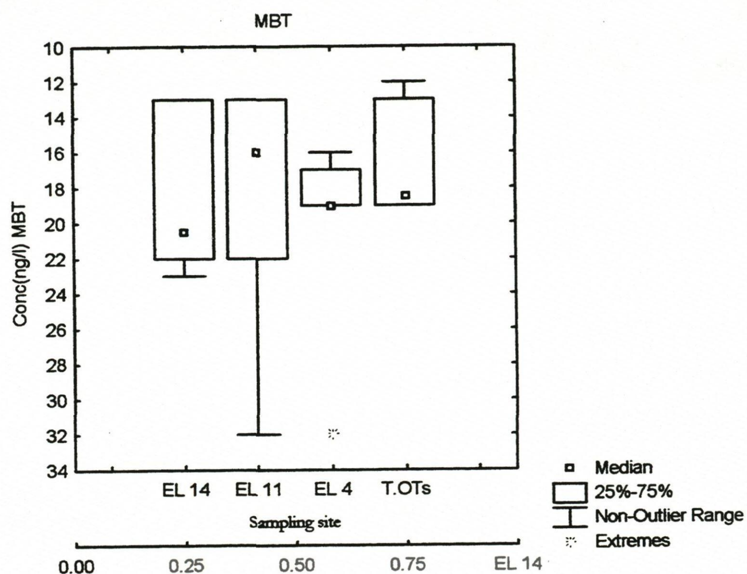

Fig. 16. Box and Whiskers Plot of concentration *vs.* MBT for the Water of East London Harbor

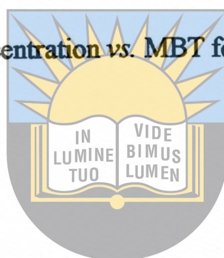
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Table 4.8. Variation of Organotin Compounds Concentration (ng / L) [a] in Port Elizabeth Harbor Water Samples

Sample	Date	MBT	DBT	TBT	Total OT	MBT / OT	TBT / DBT	% TBT
PE1	15/05/01	29.20 ± 0.70	8.70 ± 0.22	4.73 ± 0.05	42.63 ± 1.00	0.69	0.54	10
PE1	12/06/01	18.10 ± 0.10	15.30 ± 0.23	5.33 ± 0.60	38.73 ± 1.00	1.47	0.34	13
PE1	16/07/01	49.90 ± 0.50	19.00 ± 0.20	5.64 ± 0.05	74.54 ± 0.70	0.67	0.30	8
PE1	14/08/01	20.30 ± 0.40	19.6 ± 0.3	8.98 ± 0.26	48.85 ± 0.70	0.42	0.46	18
PE1	11/09/01	12.50 ± 0.10	6.07 ± 0.11	9.89 ± 0.10	28.48 ± 0.30	0.44	0.63	35
PE2	15/05/01	14.60 ± 0.50	22.29 ± 0.27	11.8 ± 0.2	48.69 ± 1.00	0.30	0.52	24
PE2	12.06.01	24.80 ± 0.10	19.80 ± 0.15	18.90 ± 0.11	63.50 ± 0.40	0.39	0.95	30
PE2	16/07/01	22.70 ± 0.40	18.80 ± 0.15	12.85 ± 0.10	54.35 ± 0.70	0.42	0.68	23
PE2	14/08/01	39.70 ± 1.07	18.50 ± 0.45	16.2 ± 0.5	74.41 ± 2.00	0.54	0.88	22
PE4	15/05/01	23.60 ± 0.60	11.5 ± 1.1	6.51 ± 0.11	41.61 ± 1.80	0.56	0.54	15
PE4	12/06/01	14.08 ± 0.10	8.30 ± 0.19	4.97 ± 0.05	28.00 ± 0.40	0.53	0.60	17
PE4	16/07/01	16.60 ± 1.90	9.80 ± 0.15	4.71 ± 0.29	31.11 ± 2.30	0.54	0.49	15
PE4	14/08/01	12.60 ± 0.40	8.30 ± 0.65	4.85 ± 0.20	25.75 ± 1.10	0.49	0.60	19
PE4	11/09/01	10.74 ± 0.30	9.79 ± 1.00	5.90 ± 0.08	26.43 ± 1.30	0.40	0.60	22

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PE1	11/01/02	10.79 ± 0.40	9.84 ± 2.30	5.12 ± 0.60	25.75 ± 3.20	0.42	0.52	20
PE1	08/06/02	12.00 ± 0.15	8.60 ± 0.60	4.69 ± 2.04	25.29 ± 2.80	0.47	0.54	19
PE1	07/11/02	14.90 ± 0.38	9.15 ± 0.95	6.40 ± 0.57	30.45 ± 1.90	0.49	0.70	21
PE1	10/01/02	11.30 ± 0.62	9.09 ± 1.50	7.24 ± 0.50	27.63 ± 2.60	0.41	0.79	26
PE1	30/01/03	9.85 ± 0.08	7.00 ± 0.24	5.84 ± 1.70	22.69 ± 2.00	0.43	0.84	26
PE2	10/01/02	14.50 ± 1.47	23.50 ± 1.30	12.50 ± 1.30	50.50 ± 2.90	0.29	0.53	24
PE2	08/06/02	18.9 ± 1.7	25.50 ± 0.80	13.60 ± 1.60	58.0 ± 4.1	0.32	0.53	23
PE2	07/11/02	22.4 ± 3.2	26.36 ± 3.30	12.70 ± 2.14	61.46 ± 8.60	0.36	0.48	21
PE2	10/01/03	27.0 ± 2.3	19.26 ± 0.40	13.0 ± 1.9	59.26 ± 4.60	0.46	0.67	22
PE2	30/01/03	28.90 ± 6.10	18.40 ± 0.91	10.50 ± 0.50	57.80 ± 7.51	0.50	0.57	18
PE4	10/01/02	16.60 ± 3.80	7.90 ± 0.38	6.00 ± 0.66	30.50 ± 4.84	0.54	0.75	20
PE4	08/06/02	12.70 ± 0.40	10.90 ± 0.27	7.05 ± 1.80	30.65 ± 2.50	0.41	0.65	23
PE4	07/11/02	17.70 ± 0.50	7.00 ± 0.28	5.55 ± 0.90	30.25 ± 1.70	0.59	0.79	18
PE4	10/01/03	16.35 ± 0.70	7.43 ± 0.68	6.70 ± 3.60	30.48 ± 4.90	0.53	0.90	22
PE4	30/01/03	11.74 ± 1.10	7.90 ± 0.55	9.30 ± 1.10	28.94 ± 2.70	0.40	1.20	32

[a] Data (PE 1), (PE 2), and (PE 4) are samples taken at 103 Berth, Slipway and Ore Berth sites respectively. At the Port Elizabeth harbor, the results of the analyzed organotin compounds are presented in Table 4.8.

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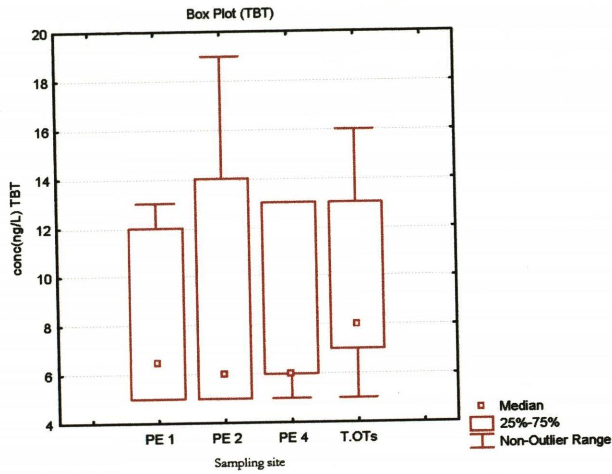

Fig.17. Box and Whiskers Plot of concentration *vs.* TBT for the Water of Port Elizabeth Harbor

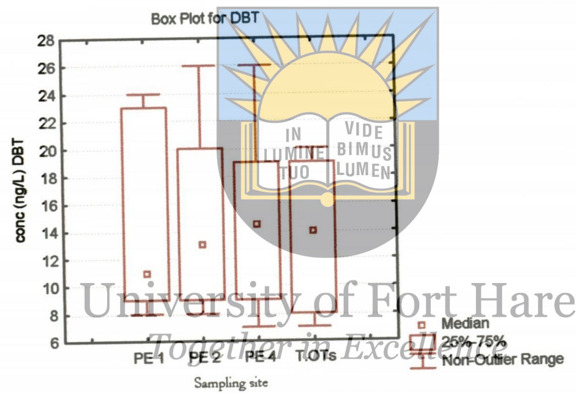

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Fig. 18. Box and Whiskers Plot of Concentration *vs.* DBT for the Water of Port Elizabeth Harbor

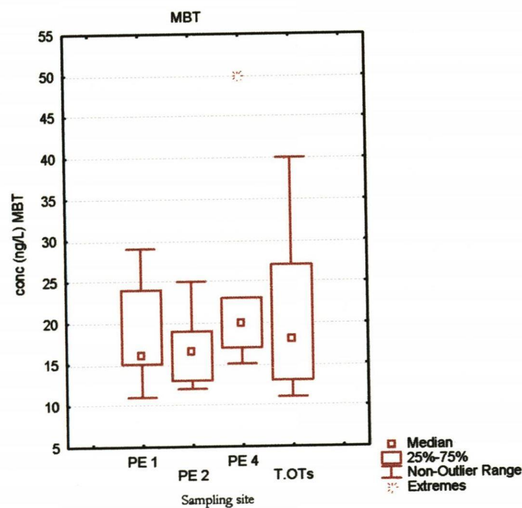

Fig. 19. Box and Whiskers Plot of Concentration *vs.* MBT for the Water of Port Elizabeth Harbor

For the Port Elizabeth harbor, the content of the organotin compounds in water ranged from 10.74 ± 0.30 ng / L to 49.90 ± 0.50 ng / L (MBT), 6.07 ± 0.11 – 26.36 ± 3.30 ng / L (DBT), and 4.69 ± 2.04 ng / L - 18.9 ± 0.11 (TBT). The highest concentration of total organotin compounds obtained in water samples was 22.69 ± 2.00 ng / L to 74.41 ± 2.00 ng/L (∑OTs = MBT + DBT + TBT). The concentration of organotin compounds in the sediments varied widely depending on the site location, ranging from 6.10 ± 0.05 to 12.50 ± 0.30 ng / g for MBT, 2.00 ± 0.02 to 12.57 ± 0.46 ng / g for DBT and 4.53 ± 0.33 ng / g to 21.1 ng /g TBT (Table 4.8).

Table 4.9 Mean Concentration (ng /g) [a] of Organotin Compounds in Sediment Samples Collected from Port Elizabeth (PE) Harbor

Sample	Date	MBT	DBT	TBT	Total OT
PE1	15/05/01	8.40 ± 0.55	12.50 ± 0.46	9.82 ± 0.02	30.18 ± 1.03
PE1	12/06/01	6.60 ± 0.29	12.20 ± 0.23	6.48 ± 0.02	25.18 ± 0.43
PE1	16/07/01	6.90 ± 0.10	11.70 ± 0.08	4.53 ± 0.33	23.30 ± 0.51
PE1	14/08/01	6.90 ± 0.10	11.75 ± 0.50	4.57 ± 0.70	23.20 ± 1.30
PE1	11/09/01	6.54 ± 0.45	11.09 ± 0.60	6.30 ± 0.14	23.93 ± 1.19
PE2	15/05/01	8.90 ± 0.10	2.00 ± 0.02	19.90 ± 0.06	30.80 ± 0.18
PE2	12/06/01	8.87 ± 0.28	3.50 ± 0.12	15.50 ± 0.30	27.87 ± 0.70
PE2	16/07/01	12.50 ± 0.30	5.29 ± 0.11	21.06 ± 0.06	38.85 ± 0.47
PE2	14/08/01	11.70 ± 0.50	6.54 ± 0.45	23.02 ± 0.60	41.26 ± 1.55
PE4	15/05/01	6.16 ± 0.05	11.80 ± 0.27	17.45 ± 0.08	35.41 ± 0.40
PE4	12/06/01	10.90 ± 0.06	11.60 ± 0.62	17.62 ± 0.05	30.12 ± 0.73
PE4	16/07/01	9.0 ± 0.1	12.57 ± 0.59	13.15 ± 1.60	30.87 ± 0.74
PE4	14/08/01	9.87 ± 0.76	11.10 ± 0.65	16.09 ± 0.34	37.06 ± 1.78

[a] PE1, PE2, and PE4 are sediment samples taken from Berth, Slipway and Ore Berth sites respectively.

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Generally, the levels of organotin compounds including the metabolites of *n*-butyltin in both harbors are relatively high. TBT degrades to other lesser toxic organotin compounds DBT and MBT in water and sediment. The ratio MBT / total organotin compounds were used in this study to determine

whether the discharge was recent or not. Based on comparison ranges, low ratios of 0.03 to 0.1 indicate recent discharges of tri-*n*-butyltin and the ratios as low as 0.03 show TBT hot spots. Ratio of MBT / total organotin compounds for East London and Port Elizabeth range 0.39- 0.69 and 0.30 – 0.69, respectively, above the benchmarks indicated. Studies, carried out in Hong Kong, measured ratio correlated with the recent release of TBT into sediments in marinas and shipyards, which were heavily contaminated (Ko et al., 1995).

The ratio [TBT] / [DBT] has also been used previously as a first estimation of the stability of the organotin compounds (Tolosa et al., 1992). This is because the organotin compound degradation occurs via a successive cleavage of the alkyl substituents (Maguire et al., 1986). In this study the ratio [TBT] / [DBT] obtained was 0.44 – 1.31, hence reflecting slow degradation process of TBT. High ratios of 4.3 ± 2.9 were reported in highly contaminated Spanish marinas (Krone et al., 1989). Ratios of [TBT] / [DBT] as shown in Tables 4.5, 4.6, 4.7, 4.8 and 4.9 appear moderately uniform and signify similar microbiological activities pertaining to both sampled sites. It is obvious from the data available that these sites have low values of the ratio of [TBT] / [DBT] implying instability of TBT in the areas sampled. This is no surprise as pollution levels of tri-*n*-butyltin are low at both ports because both are situated on the open coast where the oceanic currents disperse and transport contaminants from one place to another without allowing TBT pollutants to settle down into sediments (Monteiro et al., 1991; Week et al., 1991).

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5. CONCLUSION AND RECOMMENDATION

The presence of organotin compounds TBT and some of its degradation residues in the water system is an indication of potential gross contamination in the South African water environment. There is paucity of data on the occurrence and established levels of organotin compounds in aquatic environment. A comparison with earlier data (CSIR, 1979; Bailey, 1996) shows that the South African coastal zone was relatively unpolluted. The results from two harbors displayed inevitable problems associated with active harbors. However, organotin compounds as pollutants were not documented. In view of the levels of the organotin compounds observed, they are endocrine disrupting chemicals damaging neurological, behavioral, and hereditary development. This loss of potential in humans may be expressed as reduced intellectual capacity and social adaptability as impaired responsiveness to environmental demands, while in the wildlife the presence of organotin compounds may destabilize wildlife population.

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The results for both harbors of Eastern Cape are lower than those reported elsewhere for the similar toxic species (Ko et al., 1995). Both harbors illustrate profound effects for the reason that economic and social consequences emerge from small shifts in functional potential at the population level, it is of paramount importance to monitor levels of contaminations in South African water environment. Good methods for translating the findings of the concentrations of organotin compounds in the sampled site areas should be explicit in order to provide useful information for the decision makers and the public. Stringent legislative measures should be taken to curb the uncontrolled usage of these contaminants in marine paints. Enhanced regular monitoring of organotin compounds in the aquatic environment should be undertaken basing on the fact that East London and Port Elizabeth harbors are currently undergoing tremendous coastline expansion that will consequently lead to pollution problems common in many developed nations.

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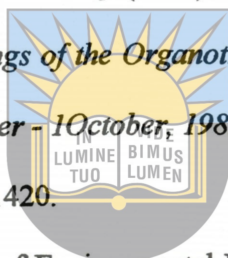

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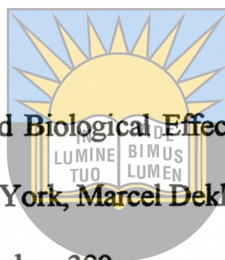
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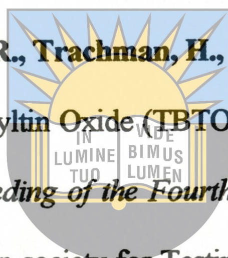

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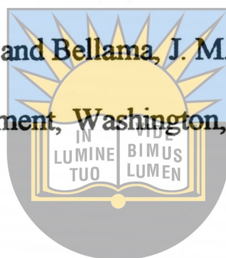

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