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SPECIATION OF ORGANOMETALLIC OF TIN, LEAD AND MERCURY IN ENVIRONMENTAL SAMPLES

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A thesis submitted to the Faculty of Sciences, University of the Witwatersrand, Johannesburg in fulfilment of the requirement for the degree of Doctor of Philosophy

Johannesburg, 2007

Declaration

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.

……

(Signature of candidate)

……day of ……..2007

Abstract

Organic derivatives of tin, lead, mercury are the most widely distributed organometallic ecotoxicants in the environment. While some of these organometallic compounds exist in the environment as a result of direct discharge, anthropogenic emissions alone cannot explain the ubiquity, for example, of the organomercury and organolead compounds in marine and fresh waters, sediments and biota. It is known that some organometallic compounds are formed via a bioconversion from the inorganic contaminants.

Depending on the source, they can enter the environment in varied forms inorganic species of different stability. These can be bio-converted further by environmental biota; for instance metals such as tin and lead can be discharged into the environment in the form of organometallic species which can undergo further transformation.

Determination of organometallics in environmental and biological samples is difficult due to matrix effect and their low concentrations. Separation and preconcentration is necessary to enhance final determination. Speciation studies are even more complicated. Speciation is an important aspect and gives information about bioavailability of the metal thus their toxicity.

This work focused on the development of a method for speciation of organo-species of tin, lead and mercury. A new derivatisation agent has been synthesised

and used successfully. SLM probe extraction has been exposed to a new matrix. Different environmental samples have been analysed for organo-species of tin and lead and their pathways predicted.

The SLM probe extraction gives the advantage of carrying out several extractions, reduction of the amount of solvent used and avoidance of emulsion problems. A simple system has been developed and applied successfully on organotin and organolead extraction from aqueous environmental sample. For reproducibility of the results, pH, salinity, stirring rate and extraction time were optimized.

An analytical method for simultaneous in situ ethylation, using new derivatisation agent bromomagnesium tetraethylborate (Et_4BMgBr), of organotin and organomercury compounds in sediment samples was developed. The determination of mercury and tin compounds is achieved by species-specific isotope dilution, derivatisation and gas chromatography – inductively coupled plasma mass spectrometry (GC-ICP-MS). In derivatisation, pH and the amount of derivatisation agent were studied. Percentage recovery and accuracy of the method was confirmed by comparison of experimental results with sediment and plant certified reference material (IAEA 405 for sediment and CRM 279 for plant).

Although organolead compounds as a gasoline additive are banned in most countries, in some regions, lead is still added to gasoline in varying proportions of

different tetraalkyllead compounds and contamination by organolead compounds is still present at different places, e.g.: lead alkyl manufactures

The use of both tetraalkyllead and butyltin is banned (tetraalkyllead as gasoline additive and butyltin in antifouling pints and PVC materials). This work focussed on their conversion in water and soil. This should provide an insight into their presence in the environment and an understanding of their degradation in the environment.

A method for full speciation and determination of alkyl lead and inorganic lead (II) after the tetramethyllead degradation in aqueous samples has been developed. This was accomplished by in situ derivatisation with sodium tetraphenyllead borate $NaB(Ph)_4$ derivative. The derivatisation was carried out directly in the aqueous sample and the derivatives were extracted using the supported liquid membrane probe extraction (SLMPE). The extracted analytes were then transferred to a GC/MS for separation and detection. This study focused on the transformation of tetramethyllead in aqueous media, at different concentration of major elements, K^+,Na^+,Ca^{++}, Mg^{++},Cl^-,SO_4^{--}. Adsorption / desorption on soil of ionic organolead and organotin were also studied.

As South Africa is one of the world's major producers of coal, mercury should be monitored as it is a side product in coal combustion. The trend of inorganic

mercury and methylmercury in sediment found in this work indicated a possible methylation of inorganic mercury to methylmercury in Klipriver sediments.

Dedication

To my wife Céline Niwemahoro and my late mother Agenesta Bashemera

Acknowledgement

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ABBREVIATIONS

AAS - Atomic Absorption Spectrometry

ADME - Absorption, distribution, metabolism and excretion

ATSDR - Agency for Toxic Substances and Disease Registry

BC - Before Christ

BCF - Bioconcentration factor

BET - Brunauer Emmet Teller

CAA - Clean Air Act

CAAA - Clean Air Act Amendments

CE - Capillary electrophoresis

CEC - Cation exchange capacity

CEE - Communauté Economique Européenne

CHNS - Carbone Hydrogen Nitrogen and Sulphur analyser

CRM - Certified Reference Material

CT SPE - Cold trap solid phase extraction

CSIR - Council for Scientific and Industrial Research

CZE – Capillary Zone Electrophoresis

DBT - Dibutyltin

DCE – Division of Chemistry and the Environment

DEL - Diethyllead

DML - Dimethyllead

EC - External calibration

EEC - European Economical Community

EC ICP OES - External Calibration Inductively Coupled Plasma Optical Emission Spectroscopy

EIA - Environmental Impact Assessment

USEPA - United State Environmental Protection Agency

EU - European Union

FAO - Food and Agriculture Organization

Fd - Formate dehydrogenase

FID - Flame Ionisation detector

GC-FID - Gas Chromatography Flame Ionisation Detector

GC ICP MS – Gas Chromatography Inductively Coupled Plasma Mass Spectroscopy

GC MS - Gas Chromatography Mass Spectrometry

GPS - Global positioning system

HPLC - High Performance Liquid Chromatography

IAEA - International Atomic Energy Agency

IC - Ion Chromatography

ICCM - International Council on Mining and Metals

INERIS – Institut National de l-Environment Industriel et des Risques

IUPAC - International Union of Pure and Applied Chemistry

LCABIE - Laboratoire de Chimie Analytique, bioinorganic et environement

LLE – Liquid Liquid Extraction

LOD - Limit of detection

LOQ - Limit of quantitation

MAE - Microwave assisted extraction

MBT - Monobutyltin

MeTr - Methyltransferase

MIP AED – Microwave Induced Plasma Atomic Emission Detector

MS - Mass Spectrometry

NADP - Nicotinamide Adenine Dinucleotide Phosphate

NMR - Nuclear Magnetic Resonance

NRF - National Research Foundation

OLC - Organolead Compounds

OM - Organic matter

OMI - Organisation Maritime Internationale

ORP – Oxidation Reduction Potential

OSHA – Occupational Safety and Health Administration

OTC – Organotin compounds

PEL - Permissible exposure limit

PTFE - polytetrafluoroethylene

PVC - Polyvinyl Chloride

RSD - Relatie Standard Deviation

SADC – Southern Africa Development Community

SAMA - South African Mercury Assessment

SDSU - San Diego State University

SLME - Supported liquid membrane extraction

SLMPE - Supported liquid membrane probe extraction

SPE - Solid Phase Extraction

TBT - Tributyltin

TEL - Tetraethyllead

THF - Tetrahydrofuran

THF -Tetrahydrofolate

TiEL - Triethyllead

TiML - Trimethyllead chloride

TMAH - Tetramethylammonium hydroxide

TML - Tetramethyllead

TPhT - Triphenyltin chloride

TWA - Time weighted average

U.K - United Kingdom

UNEP - United Nation Environmental Program

NUR - national University of Rwanda

USA - United State of America

UV - Ultraviololet

WHO - World Health Organization

XRF – X -Ray fluorescence

CHAPTER 1

INTRODUCTION

Tin, lead and mercury are heavy metals considered as dangerous pollutants due to their toxicity towards the environment. They can be found in all components of the environment (air, water, and soil), but in general at very low concentrations. Their analysis becomes even more difficult due to their occurrence in a numbers of different species.

The species with metal carbon bond are defined as organometallics. In this context the compounds with Sn-C bond are called organotin, the ones with Pb-C are organolead and those with Hg-C bond are organomercury. These organometallics besides those of arsenic are the most widely distributed in the environment.

Each species has its specific biogeochemical properties and its toxicity. Thus the total concentration determination is not sufficient for the understanding of their effect on the environment. Only the chemical speciation of the compounds can elucidate the problem.

The definition of speciation has been a subject of discussion for many years. IUPAC has now defined it as the analytical activity of identifying and measuring the quantities of one or more individual chemical species, where the latter are specific forms of an element defined as molecular, complex, nuclear structure, or

oxidation state in order to understand the transformations between forms which can occur, and to infer from such information the likely environmental consequences [IUPAC, DCE division].

1.1. Tin, lead and mercury species

Generally tin exist in oxidation states two and four. The Sn (IV) forms organotin. The organotin compounds are generally represented as R_nSnX_{4-n} (n=1, 2, 3, 4; R=alkyl or aryl; X=H, OR, halogen etc.) [Fent, 1996; Hoch, 2001; Kent, 1996].

Lead exists as inorganic lead Pb (II) but it can also exist in oxidation state four present in organolead. The general representation of organolead compounds is R_nPbX_{4-n} (n=1, 2, 3, 4; R=alkyl or aryl; X=H, OR, halogen etc) [Manceau et al, 1996].

Mercury can exist in four principal chemical species in environment; the elemental mercury Hg°, inorganic mercury (Hg^{2+}), monomethylmercury (MeHgX, X=halogen) and dimethylmercury Me_2Hg [Stein et al., 1996].

1.2. Sources of pollution by organometallic compounds of tin, lead and mercury

Some pollutants occur naturally, others are anthropogenic. Human activities are the source of increasing emission of pollutants to the environment. Some of the organo-species of tin, lead and mercury can be discharged into the environment

directly as organic species or converted from inorganic forms. Depending on the media, the inorganic species (Sn, Pb, Hg) can be bioconverted by the environmental biota [Förstner et al., 1993; Harrison et al., 1980; Alzieu et al., 1989; Reuther et al 1999, Gilmour et al., 1998; Mason and Sullivan, 1999; Cossa et al., 1994; Tessier, 2004, Monperrus et al, 2003a, Monperrus et al 2004].

1.2.1. Use and source of organotin pollution

Whereas the metal tin has been utilized since the Bronze Age, the organotin applications came into use in the early 19th century. Due to tin hardening effect on copper, tin was used in bronze implements as early as 3,500 BC. However, the use of the pure metal started in about 600 BC. Tin mining is believed to have started in Cornwall and Devon in the southwest of England [Wikipedia, 2006a, Tin]. The first commercial application of organotin compounds was the use as an additive to PVC (polyvinylchloride) in the 1940s [Blunden et al., 1984].

The PVC polymer becomes unstable under the influence of heat and light, resulting in discolouration and embrittlement. The incorporation of organotin compounds in their synthesis can prevent this kind of degradation [Hoch, 2001]. The organotin compounds mainly used in this plastic industry are the monosubstituted ($RSnX_3$) and disubstituted (R_2SnX_2).

Due to different tin derivatives physicochemicals properties, tin is the element used intensively in industrial applications [Blunden and Chapman, 1986].

Trisubstituted organotin compounds (R_3SnX) are used as biocide. The biocidal properties are strongly influenced by the R-groups [Hoch, 2001]. The most important of these compounds are the tributyl-, triphenyl-, and tricyclohexyltin compounds, which are used as agricultural and general fungicides, bactericides, antihelminthics, miticides, herbicides, molluscicides, insecticides, nematocides, ovicides, rodent repellents and antifoulants in boat paints [Fent and Hunn, 1996; Kannan et al., 1995 & 1997a; Michel and Averty, 1999; Negri et al., 2004, Fromme et al., 2005]. The tetrasubstituted organotin compounds (R_4Sn) are mainly used as intermediates in the preparation of other organotin compounds [Ikonomou et al., 2002].

The application in PVC represents about 70% of application of organotin compounds and 20% are the biocidal application in agriculture or in paint industry. In 1992 the worldwide production of organotin compounds was estimated to 50,000 tones [Hoch, 2001], the current estimations of worldwide production is not available. This might be the consequence of the acknowledgement of organotin toxicity.

Many studies have been done on organotin compounds in marine environment. Looking at every day application of PVC in our life, a good example would be the study of organotin compounds in terrestrial water system. The overview of organotin in environment done by Hoch (2001) indicates a concentration of 35 mg (Sn) m^{-3} in leachate from PVC pipe for the first use, and 1 mg (Sn) m^{-3} as

subsequent release. The concentration depends on the pH of the leaching conditions, the pipe length and the length of the alkyl chain on organotin.

The presence of PVC in environment becomes magnified due to its recycling problem. Each PVC product contains a unique mix of additives. In Europe, where PVC recycling is more advanced than the rest of the world, less than 3 percent of post-consumer PVC is recycled. It is expected that by 2020 only 9 percent of all post-consumer waste in Europe would be recycled [Thornton, 2002, Environmental Impacts of Polyvinyl Chloride (PVC) Building Materials, Health building network].

1.2.2. Use and source of pollution of organolead

Lead was considered by the alchemists as the oldest metal. It has been used by man for at least 7000 years, because it is widespread, easy to extract and easy to work with. It is highly malleable and ductile as well as easy to melt [Wikipedia, 2006b, Lead]. Whereas lead was known a long time ago, the chemistry of organolead compound started in the 19^{th} century. Seyferth (2003), in his review on the rise and fall of tetraethyllead indicates that Carl Jacob Lőwig (1803-1890) was the first person to report the preparation of the first alkyllead compounds in 1853. By far the most important organolead compound is tetraethyllead. It was first prepared as a pure compound in 1859.

Tetraethyllead found its application in automobile as antiknocking agent in gasoline. The knocking in engines occurs when the mixture of fuel and air in the cylinder is ignited by the spark plug and the smooth burning is interrupted by the unburned mixture in the combustion chamber exploding before the flame front can reach it. The consequences of knock, besides the irritating noise, include overheating, loss of power output, waste of gasoline, and, when extreme, damage to the engine. The research on antiknocking agent started in 1916. The effectiveness of tetraethyllead as antiknocking agent was discovered by Thomas Midgley in 1922. Later, tetramethyllead was also used. At the beginning 0.79 ml L^{-1} of tetraethyllead and later 1.05 ml L^{-1} was found to be much more effective [Seyferth, 2003b]. The use of these compounds increased steadily up to 1973. A decline in consumption started as more cars fitted with catalysts requiring lead-free gasoline came into use [WHO, 1977]. Organolead is also used as solvents for fatty materials and rubber [Budavari , 1989; Sax and Lewis, 1989].

The R_4Pb can be released into environment by gasoline spillage, evaporation or incomplete combustion in motor engine. This results in about two percent loss of alkyllead to the atmosphere [Tessier and Turner, 1996]. Depending on the environmental conditions, tetralkyllead can be degraded to ionic alkyllead (R_3PbX, R_2PbX_2) till inorganic lead (Pb II).

A global atmospheric deposition of approximately 410 000 tonnes of lead per year was calculated by USEPA in 1986. Note that the estimations take into account the

wind speed, surface area and texture [UNEP, 2003, Inclusion of the chemicals tetraethyl lead and tetramethyllead and adoption of the draft decision guidance document].

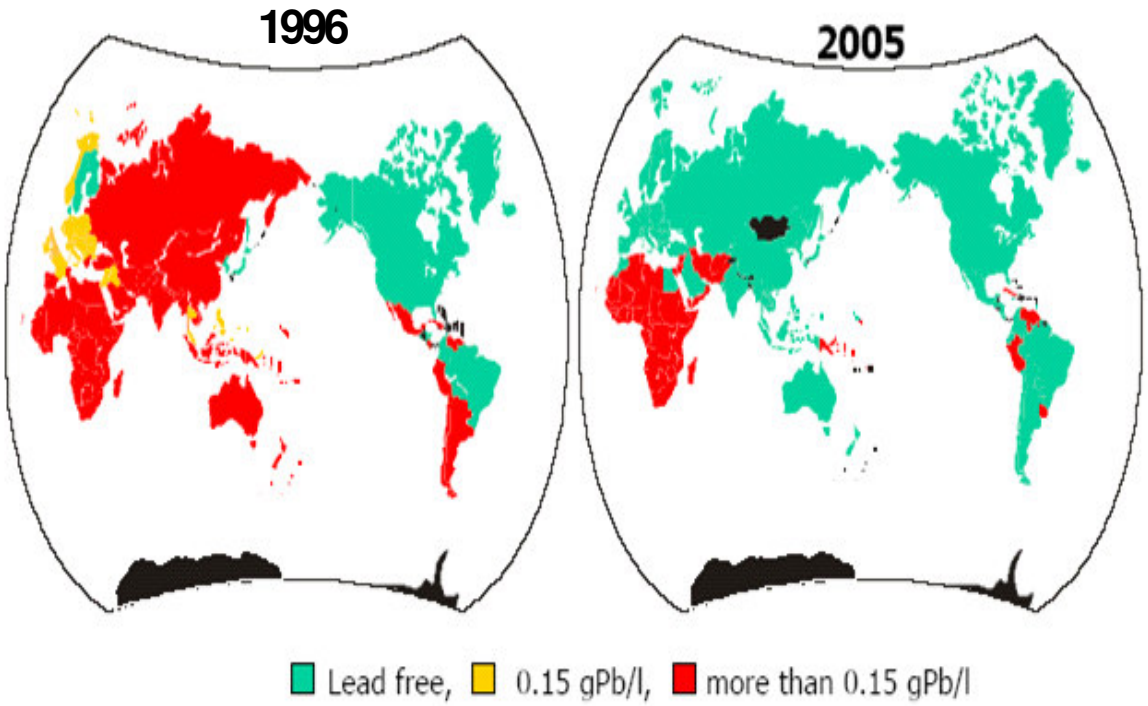


Figure 1.1: Leaded gasoline availability as of 1996 versus 2005 (prepared by the international fuel quality center, 2002, international fuel quality center, http://www.ifqc.org)

Although organolead compounds as a gasoline additive are banned in most countries, in some regions, lead is still added to gasoline in varying proportions of different tetraalkyllead compounds and contamination by organolead compounds occurs. Good examples include lead alkyl manufacturing, blending processes at oil

refineries, petrol stations and garage forecourts [Simon, 2002]. The map on figure 1.1 shows the distribution of leaded gasoline in the world.

The reported use of lead for petrol additives was 14,400 tonnes in 2003, and over 70 per cent of lead in gasoline is likely to enter the environment directly after combustion in motor engine [UNEP, 2003]. The speciation of organolead should be done, mainly in cities where there is high traffic. A systematic research should be done in countries where there is still leaded gasoline. There should be an inspection in garage and petrol station to confirm if there is no violation on lead use in gasoline. A study done in the USA just a few years after the ban of leaded gasoline in about 10000 service stations, between 1980 and 1987, shows a rate violation of 1.65 and 0.04 per cent respectively [USEPA, 2000, Great Lakes Binational Toxics Strategy U.S. Challenge on Alkyl-lead: Report on Use of Alkyl-lead in Automotive Gasoline].

1.2.3. Use and source of pollution of organomercury

The discovery of mercury in ancient Egyptian tombs dated from 1500 BC confirmed that mercury was known in the ancient time. In ancient China and India, the use of mercury was thought to maintain good health. It was also used as a cosmetic. It is only in 500 BC that mercury was used to make amalgams. [Wikipedia, 2006c, mercury]. This ignorance about mercury toxicity probably caused many deaths in the past. While the inorganic form of mercury was known long time ago, it is only recently that studies were focused on its organic

compounds [Monperrus, 2004]. The organometallic compounds of mercury occur naturally by methylation [Tessier, 2004].

Wang (2004) has reviewed the sources of mercury in environment. The major naturally occurring mercury emission processes include degassing from mercury mineral deposits, degassing from mercury contaminated aquatic and terrestrial systems (through reduction of Hg (II) to Hg (0), volcanic emissions, and forest fires.

Human activities may break the natural mercury cycle, and significantly increase the release of mercury into the environment. In agriculture area, mercury was used as pesticides and fungicides. It also has many applications in medicine, for instance in thermometers. In municipal waste, mercury comes from the disposal of mercury-containing products, such as batteries, fluorescent bulbs, and paints. Incineration, which is usually the method used to get rid of these solid wastes (municipal and medical wastes), will emit mercury into the environment. Coal and oil combustion, pyrometallurgical processes (iron, lead, and zinc), and gold production are the main sources resulting in mercury pollution from ores [Wang, 2004; ICMM, 2007, ICCM position statement on mercury]. Amalgamation was the method used in the beginning of gold mining, which resulted in a significant release of mercury vapours into the atmosphere. Furthermore, in this process, mercury remains in the processing of water [Nriagu, 1993; Lacerda, 1997; ICMM, 2007, ICCM position statement on mercury]. Among all these sources, coal

combustion and solid waste incineration account for more than half of the total global emissions [Pirrone et al., 1996].

About 80% of total mercury in the atmosphere is in Hg^0 gaseous form and could remain in the atmosphere for more than one year [Wang, 2004], which makes long-range atmospheric transportation of Hg^0 a major environmental concern. The question to ask is weather it reaches the surface in this form.

The global mercury emission is estimated between 4970 and 6050 tons per year [Themelis and Gregory, 2002], with a global natural source emission ranging from 2420 to 4000 tons per year [Seigneur et al., 2001, Bergan and Rodhe, 1999] while the anthropogenic emission sources contribute 2190 tons per year [Pacyna and Pacyna, 2005].

The contribution of CH_3HgX in these concentrations depends on the environment. Lakes, rivers, coastal waters and oceans contribute 0.01 to 10 percent, 20 to 80 percent in invertabra; 60 to 99 percent in fish and 10 to 30 percent in aquatic plant [Tessier, 2004].

The pollution of mercury is non specific due to its volatility. When Hg^0 reaches the atmosphere, some will be deposited on soil in its inorganic form, while the rest remain available for circulation. Pollution on the soil will affect air and water as well as other regions besides the source region. The control of mercury emission

becomes much more important, not only for the countries which are responsible for high emission but for also the countries which look unconcerned.

Many studies have been done and others are being carried out in order to understand the dynamics of mercury [Seigneur et al., 2001].

1.3. Organometallic compounds of tin, lead and mercury in South Africa

The speciation of organo-species of tin, lead and mercury compounds in South Africa is not well documented. To our knowledge, very few publications are available on the study of organometallic compounds in South African [Marshall, 2003; CSIR, Workshop: 7-8 March 2006, South African Mercury Assessment Programme].

Tin compounds in South Africa

A lot of studies have been done worldwide on organotin compounds especially in coastal areas and seawater. However, the South Africa environment has yet to be explored. There is very little literature on organotin studies on continental ecosystem (river, soil, sediment, plant) [Tessier, 2004].

The high amount of PVC used in daily life indicates the need to study Organotin especially on continental ecosystems that include soil, sediments, rivers and plants.

Lead compounds

Although leaded gasoline has been banned elsewhere, it is still used throughout the African continent (figure 1.1). The question to ask is whether the introduction of unleaded gasoline is driven by environmental concerns, rather than the needs of the automotive industry to keep abreast with current trends in engine technologies.

Nigeria and Libya are the biggest oil producers in Africa. Total oil production in South Africa currently accounts for approximately 10 percent of domestic needs. South Africa imports majority of its crude oil from the Middle East, with Saudi Arabia and Iran as its chief suppliers. Nigeria is now the third largest supplier of imported oil to South Africa due to the reduced imports from Iran [EIA, (2005) South Africa country analysis brief].

In early 1996, the unleaded gasoline was made available in South Africa but not all regions of South Africa have had immediate access [Mbendi, 2000, South Africa - Oil and Gas: Petroleum and Petroleum Products Wholesalers]. Leaded fuels are banned from 2006 [Alexander, 2005, Ethanol Africa: clean maize fuel]. In 2000, the penetration of unleaded gasoline was above 8% [Mbendi, 2000, South Africa - Oil and Gas: Petroleum and Petroleum Products Wholesalers]. A commitment was made by other African countries in 2002 to use only unleaded gasoline. The deadline was fixed as the 1 January 2006 [Allafrica, 2006, Slow progress on phase out of leaded fuel in SADC].

Figure 1.2 shows the regional fuel consumption in South Africa, combining total sales of liquid petroleum products and gaseous. Gauteng and Kwazulu Natal are the biggest consumers.

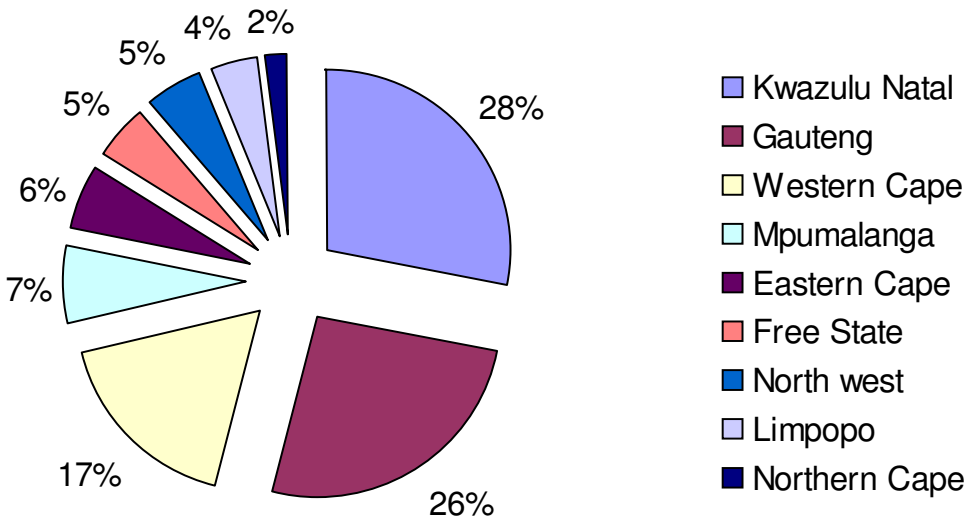


Figure 1.2: Regional fuel sales in South Africa (Mbendi, 2000 South Africa – Oil and Gas: Petroleum and Petroleum Products Wholesalers).

Though much has been done in an effort to reduce the release of organolead from gasoline into the environment, a lot still has to be done to eradicate their sources. The article in the Appendix 1 (from *"Sunday Times, 23rd October 2005")* reports high concentration of lead in children's toys in South Africa. This is total concentration of lead without any specification of the lead species.

Mercury and organomercury

South Africa is among the countries where there are significant man made mercury emission, generally from coal combustion as source of electricity (which provides 90% of SA electricity) and heat. Southeast Asia (China) is currently the biggest emitter, followed by South Africa, Central and Eastern Europe, and the Eastern USA [Pacyna et al., 2003, Atmospheric Environment, 1: S109-S117; Harvey et al, 2006, Mercury Emissions from Coal Combustion China], as shown in the figure 1.3.

Studies on coal used for heating and cooking purposes in Beijing have indicated large release of Hg to the atmosphere. From these studies, it was recognised that small-scale coal burning is a significant source of Hg, which is deposited in water bodies and sediments, where it is eventually methylated and bioaccumulates up the food chain as CH_3HgX or $(CH_3)_2Hg$. The mercury contained in Chinese coal is estimated to be about 0.150 mg kg^{-1} while south African coal contains 0.327 mg Kg^{-1} [CSIR, Workshop: 7-8 March 2006, South African Mercury Assessment Programme; Mercury Emissions from Coal Combustion, China].

While there appears to be substantial sources of Hg input in Southern Africa, the amount of Hg emissions in the region is unknown [Crouch, 2006, mercury facts]. Thus, the extent to which Hg and its derivatives contribute to ecosystem degradation is still unknown. A detailed study needs to be done on a larger scale.

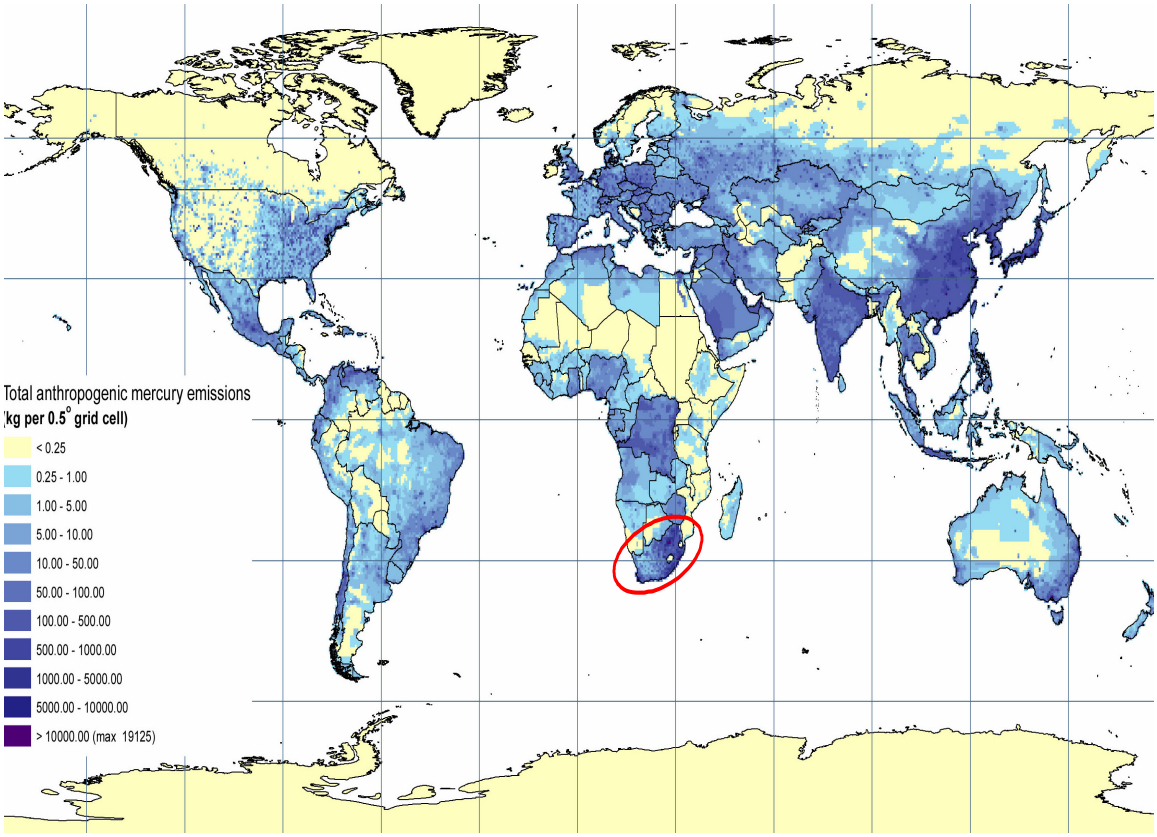


Figure 1.3: Spatially distributed inventory of global anthropologic emissions of mercury to the atmosphere, 2000 (Pacyna et al. 2005).

Coal combustion is not the only potential source which has to be monitored. The reprocessing of old gold tailings originally mined by amalgamation processes in South Africa poses significant health risk. [CSIR, Workshop: 7-8 March 2006, South African Mercury Assessment Programme]. Amalgamation extraction was used in the mining of gold in South Africa before 1886 [Tutu, 2006]. The reducing conditions in which non-reprocessed old tailing dams are kept might be a source of inorganic and organic mercury.

As mentioned before, there are few publications on total mercury concentrations in South African. The existing ones are also questionable, as the detections limits reported are very high (1 µg/L). This is substantially higher than the detection limits currently used in the USEPA, which is 0.1 ng L^{-1} total Hg. [CSIR, Workshop: 7-8 March 2006, South African Mercury Assessment Program]. There is therefore an urgent requirement for more sensitive methods to be developed.

Recently, the CSIR hosted a meeting in Pretoria, South Africa (7- 8th March 2006) to discuss the way forward in establishing an environmental Hg assessment program for the country. The workshop focussed on initiating a South African Mercury Assessment (SAMA) program, involving government departments, and national and international scientists. The aim of the meeting was to develop a framework for Hg research in the SAMA program, focusing initially on the sources, transport, fate and health consequences of Hg emitted from coal-fired power plants in South Africa. It was proposed to select a study area in South Africa where detailed research on the transport, fate and consequences of Hg will be undertaken initially. [CSIR, Workshop: 7-8 March 2006, South African Mercury Assessment Program]

1.4. Organotin, organolead and organomercury compounds in the environment

Air, water, and soil pollution are often thought of as distinct forms of pollution. However, each component of the environment -air, water, and soil- is in contact with the others and with plants and animals living within that environment. The relationships between all the living and nonliving species in an environment make up an ecological system, called an ecosystem. All the ecosystems are connected. Thus, pollution that seems to affect only one component of the environment may also affect the entire ecosystem. Furthermore, chemical species can be stable in one media and unstable in another. Thus a study of speciation should focus on the whole ecosystem, including the inter-conversion which may happen between chemical species. This section will discuss the stability of different species of organotin, organolead and organomercury in ecosystems.

1.4.1. Biogeochemical cycles

A biogeochemical cycle is defined as a pathway by which a chemical species (element or molecules) move through both biotic ("bio-") and abiotic ("geo-") compartments of an ecosystem. This pathway includes many physical chemical processes. The study of the degradation and other transformation processes of chemical species in the environment help to understand the fate of pollutants and their toxicity. The degradation can be biotic or abiotic.

There are different half-lives for each species. Depending on the compartment conditions, a species can remain the same for a long time or change to another. Air, sunlight, chemical radicals and oxygen are the main parameters which play a role in species transformation. The physical chemical properties play a big role in species degradation in water; pH and salinity being the more influential. The adsorption properties of soil or sediment are the main factors that control the transformation of species in soil. The extent of adsorption depends on the organic matter content and cation exchange capacity of soil or sediment [Blunden and Chapman, 1982].

Organotin compounds biogeochemical cycle

Figure 1.4 shows the known transformations of organotin compounds in sediment, water and air. Photodecomposition, biotic degradation and chemical decomposition are the principal reactions responsible for their transformation.

This study focuses on butyltin compounds. The degradation products of Bu_3SnX are the mono and disubsituted ($BuSnX_3$ and Bu_2SnX_2), to inorganic tin [Blunden and Chapman, 1982]. The process responsible for this degradation will be discussed later in section 1.4.2.

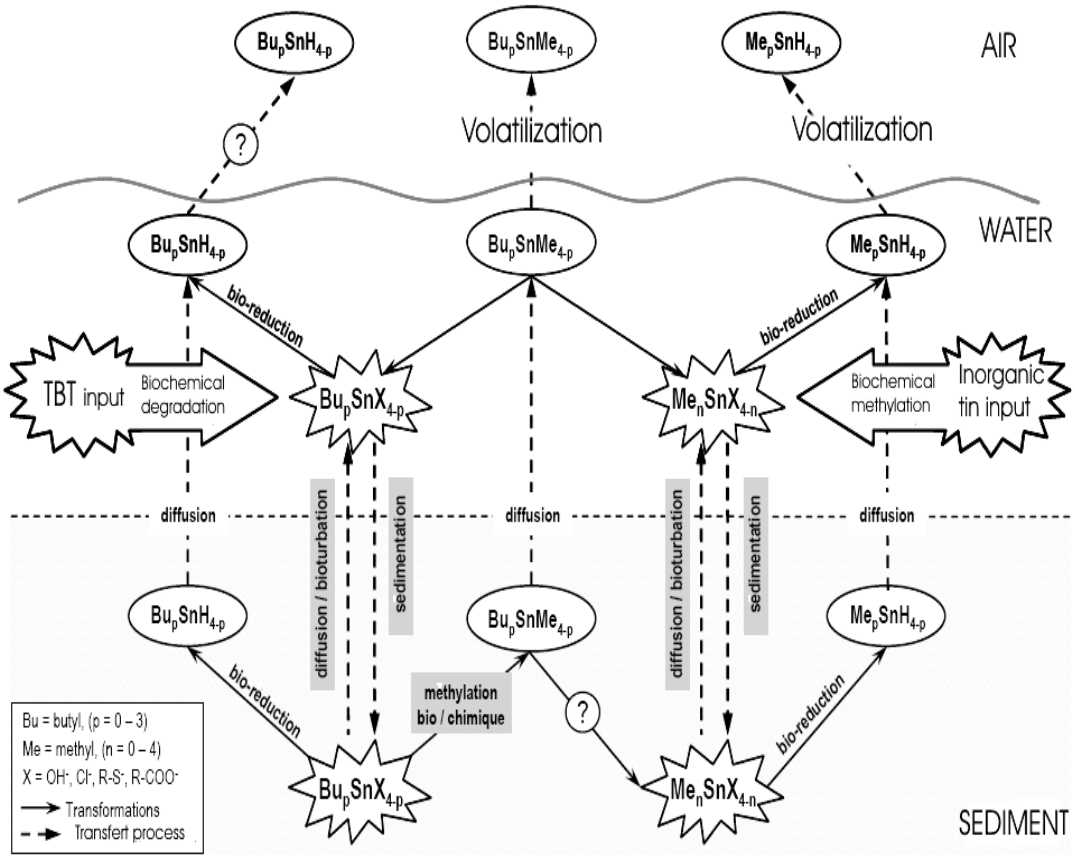


Figure 1.4: Transfers and transformations of OTC in ecosystem (Tessier, 2004)

Depending on the stability of a species in each compartment, its concentration will differ. Hoch 2001 undertook an overview of organotin compounds in the environment. Figure 1.5 gives the range of Bu_3SnX concentration found in different media. It also shows the possibility of bioaccumulation of Bu_3SnX in food chain. Seawater and freshwater show lower concentrations than the concentration found in algae mussels, fish and mammals.

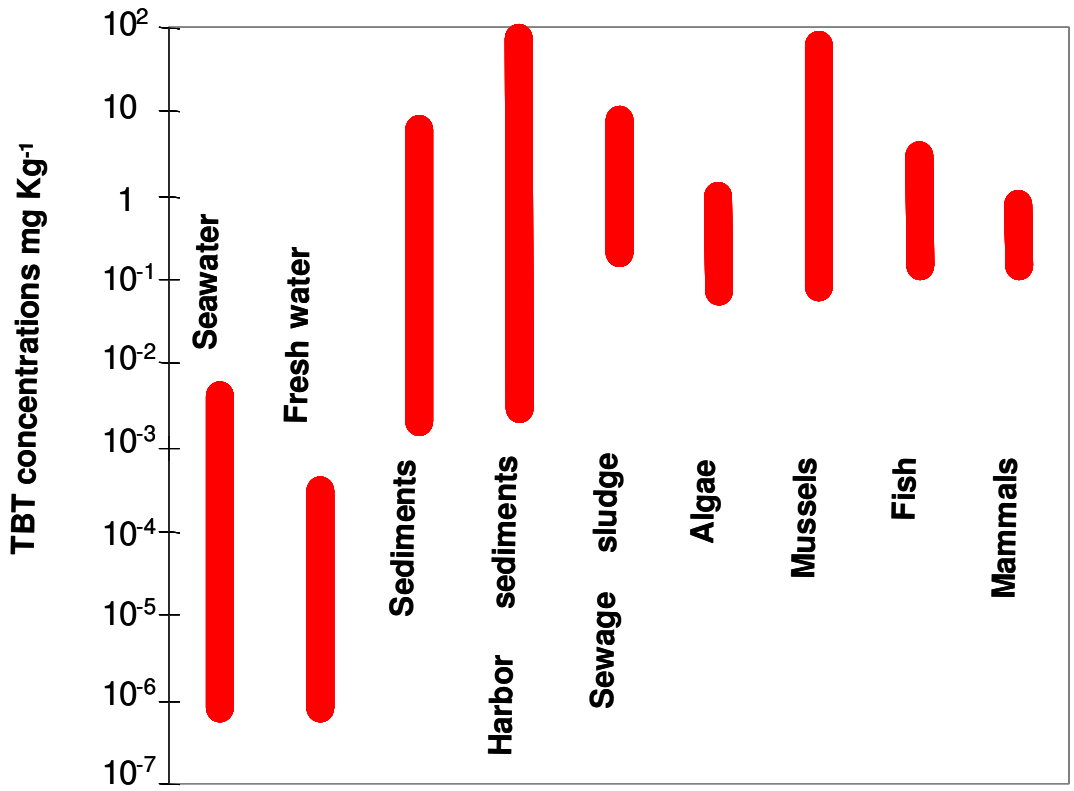


Figure 1.5: Range of Bu_3SnX concentration (mg kg^{-1}) in different environmental matrix (Hoch, 2001)

Organolead compounds biogeochemical cycle

Figure 1.6 shows the known transformation and transfer processes of lead compound in air, water and sediments. This cycle is from a compilation of many studies done on organolead transformation in environment [Jarvie et al., 1981; Harrison et al., 1986; Irwin et al., 1997]. This cycle shows two main organometallic compounds Et_4Pb and Me_4Pb. The ethylmethyllead compounds

(Et_nPbMe_{4-n}) found in this cycle, are known to be found in lower concentration than ethyllead or methyllead compounds [Gallert and Winter, 2004].

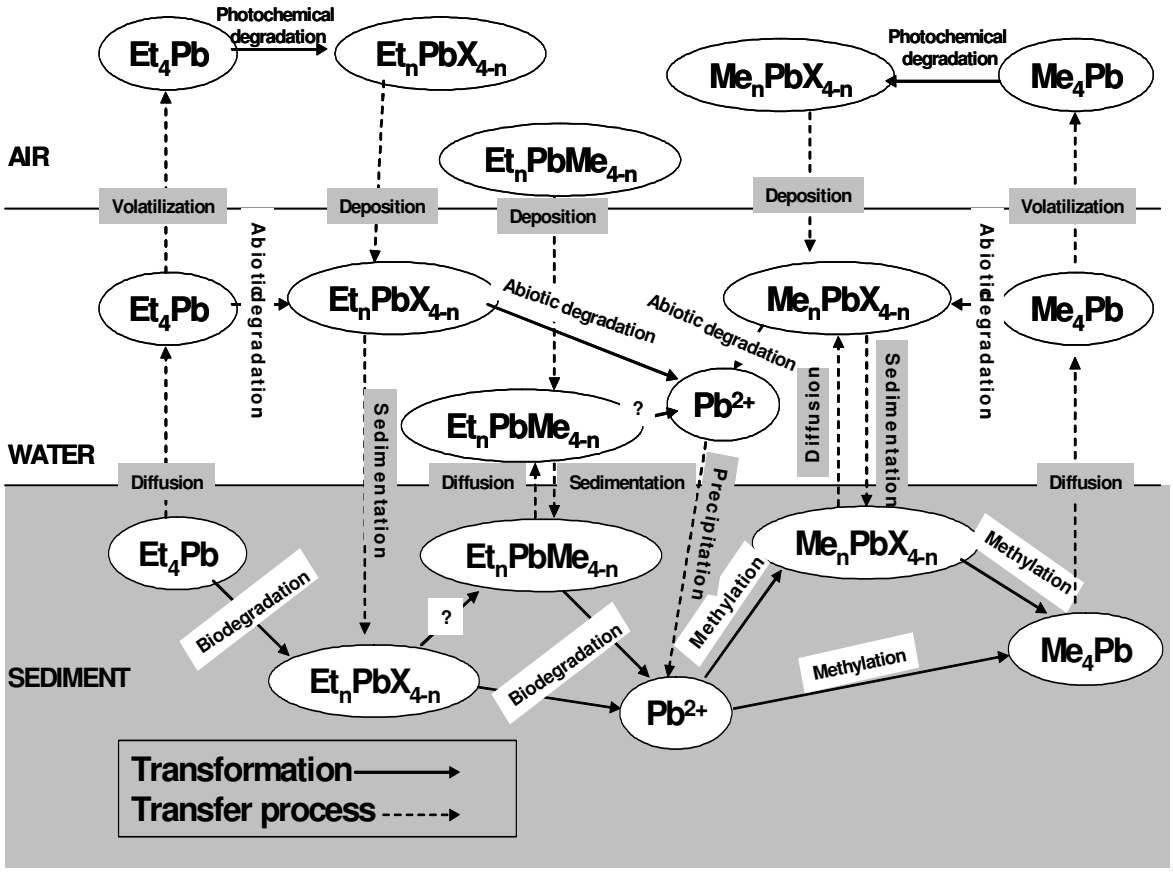


Figure 1.6: Organolead compound biogeochemical cycle

Air and rainwater are the main matrices that should be analysed in order to understand the pollution of organolead from gasoline. Figure 1.7 shows that rainwater and air contain higher concentrations of Pb than continental samples

such as fish or ocean water. The grass samples indicate that the concentration of lead from areas close to petrol stations would be higher than in areas far from the station. Therefore, an area far from high traffic network will contain low concentration in lead. This assumption would be correct if gasoline was the main source of organolead pollution.

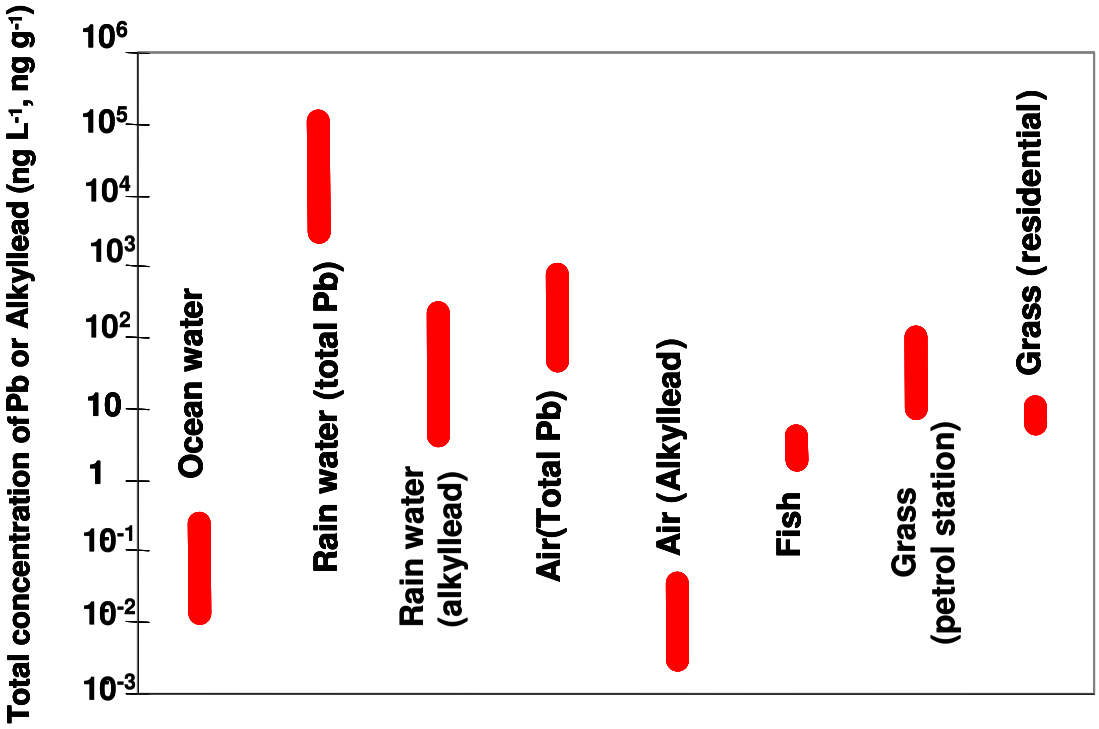


Figure 1.7: Range of total Pb concentration (ng L^{-1} for liquid and ng g^{-1} for solid sample) in different environmental matrix (IUPAC, 1995)

Mercury biogeochemical cycle

Figure 1.8 shows the known transformations of mercury in ecosystems, the principal reactions are the reduction and biomethylation processes; these processes modify mercury properties and make it much more available in environment by changing its volatility.

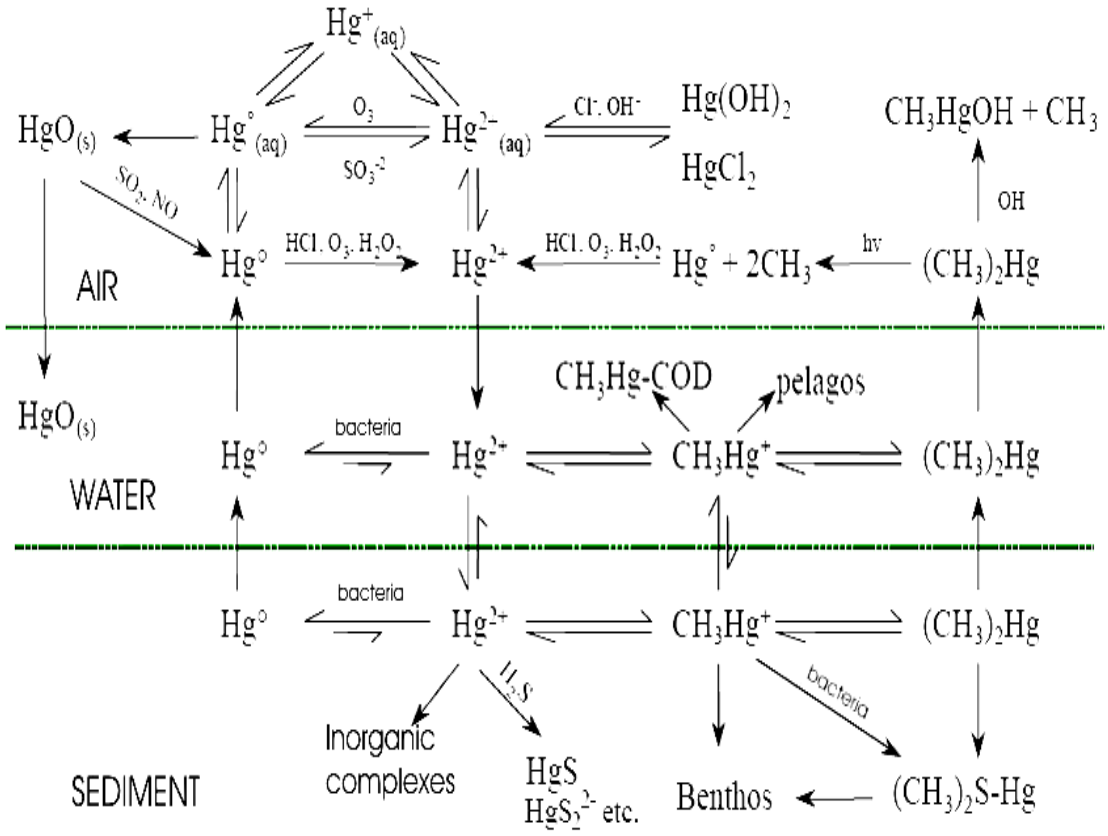


Figure 1.8: Transfers and transformations of Mercury in ecosystem (Monperrus, 2004)

The approach of mercury speciation will differ according to the matrix. Figure 1.9 represents the range of mercury concentration in different media. A high concentration in fish can be noticed. This high concentration is due to the accumulation of mercury in fish. In country and coastal regions where fish is the main food, there is a high risk of human intoxication.

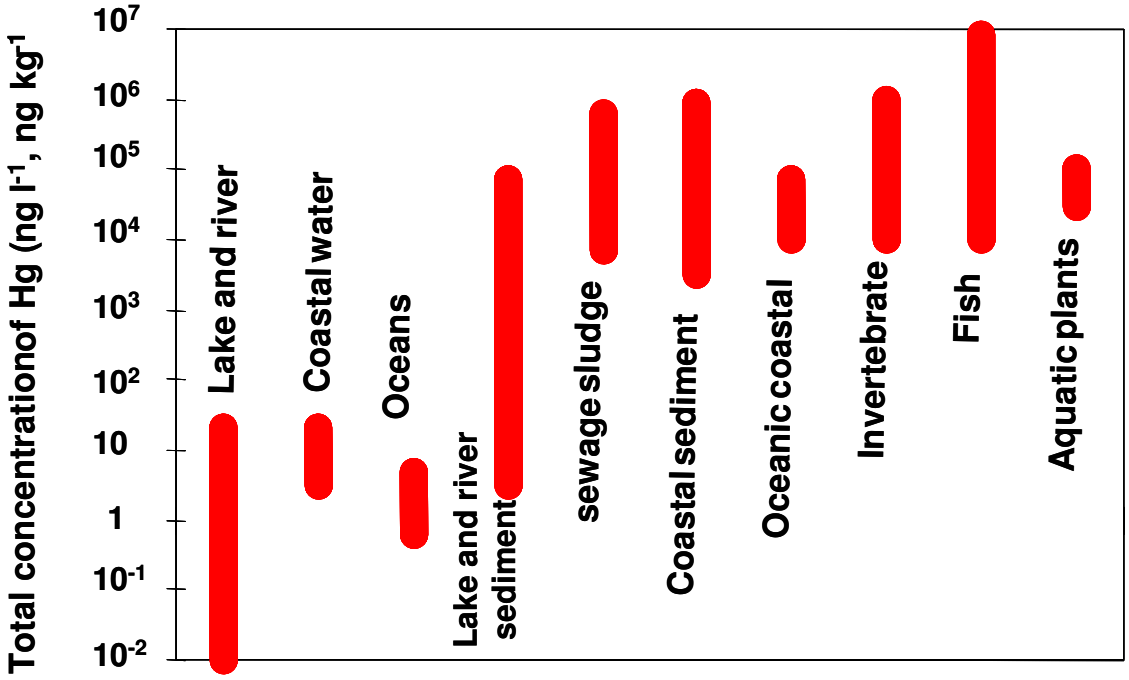


Figure 1.9: Mercury concentration (ng L^{-1} for liquid and ng kg^{-1} for solid sample) (Tessier, 2004)

1.4.2. Transformation process of tin, lead and mercury compounds

The biogeochemical cycle discussed previously in section 1.4.1 shows that organotin; organolead and organomercury are subject to transformations. The main species produced are the following:

- Tin compounds: $Bu_3SnX \rightarrow Bu_2SnX_2 \rightarrow BuSnX_3$
- Lead compounds: $R_4Pb \rightarrow R_3PbX \rightarrow R_2PbX_2 \rightarrow Pb^{2+}$ ($R = CH_3$-, C_2H_5-)
- Mercury compounds: $Hg^0 \rightarrow Hg^{2+} \rightarrow CH_3HgX \rightarrow (CH_3)_2Hg$

The main transformations which will be discussed in this section are methylation and demethylation for all three groups of metals and butylation and debutylation for organotin.

1.4.2.1. Methylation process

The term methylation refers to the delivery of the methyl (CH_3) group in a variety of ways. The methyl group can replace chemical groups such as halogen or hydroxyl. The methylation may involve use of electrophilic methyl (CH_3^+) for example, the use of iodomethane, or nucleophilic methyl (CH_3^-) such as methyllithium (CH_3Li) or Grignard reagents (CH_3MgX). [Wikipedia, 2006, methylation]

The methylation can also occur naturally by a biotic process, often in sediment or in water [Mason and Fitzgerald, 1990; Pongratz and Heumann, 1998; Leermakers et al., 2001, Guard et al., 1981]. Anaerobic bacteria are thought to be the main

agents of biomethylation in sediment and other anoxic environments. With the exception of a few examples (such as cobalt methylating like vitamin B_{12}) higher organisms do not seem to be capable of biomethylating metals. [Chemaly, 2002; Gilmour et al., 1998].

Methylation of organotin

The presence of methyltin in environment was investigated at the end of the 1970's [Tessier and Turner, 1996]. It is only recently that the methylation of Bu_3SnX in environment has been demonstrated [Amouroux et al., 1998; Weber, 1999, Amouroux et al., 2000; Tessier et al., 2002]. Investigations in the laboratory have shown that sulfato-reducing bacteria in anaerobic conditions, and algae in aerobic condition, can methylate inorganic tin [Yozenawa et al., 1994; Gilmour et al., 1985, Donard and Weber, 1988].

It is not only microorganisms that are responsible for tin methylation. The formation of $(CH_3)_4Sn$ from $(CH_3)_3SnOH$ was demonstrated in sterile sediment. The production of $(CH_3)_4Sn$ is attributed to the disproportionation of $(CH_3)_3SnOH$ to $(CH_3)_4Sn$ and $(CH_3)_2SnO$ [Tessier and Turner, 1996].

Interest in organotin has focused on Bu_3SnX as other forms of tin are considered less toxic (see section 1.5). In the toxicity context, the methyltin compound (from inorganic or butyltin) are more volatile and so more bioavailable, though there is little data currently available on the $(CH_3)_nSnX_{4-n}$.

Methylation of lead

Whether methylation of lead occurs in the environment or not is still under discussion [Wong, 1975; Craig, 1985; Rapsomanikis, 1984; Jarvie, 1988; Tessier and Turner, 1996]. The first studies of Pb methylation were published by Wong et al. (1975). Trimethyl acetate was transformed to tetramethyllead by bacteria in the presence of glucose. In the same year Jarvie and co-authors found that the presence of microorganism is not an absolute requirement for lead methylation.

In 1981, Reisinger and co-authors in their publication "Evidence for the absence of biological methylation of lead in the environment" concluded that there is no biotic lead methylation. They used ^{14}C-labeled substrates and ^{210}Pb as a radiotracer in an attempt to detect methylated compounds from mixed cultures incubated in both aerobic and anaerobic media. Only abiotic methylation was observed in the presence of sulfide.

Despite these studies giving evidence of non biomethylation, it is difficult to explain lead methylation only by abiotic process. In 1988, Walton and co-author published results done on sediment. The results show a highly seasonal dependence of Me_4Pb production, which suggests a biological control of overall reaction. The biomethylation of Pb is still an open subject and remain unclear. Walton suggested a two step mechanism, one biotic the second abiotic. He proposed that the first biotic step is a methylation of Pb (II) and the concentration

of the product is too low to be detected. This product can undergo an abiotic reductive disproportionation reaction leading to di-, tri-, and $(CH_3)_4Pb$.

The methylation studies were done on inorganic lead. The methylation of triethyllead and diethyllead should also be investigated as some studies indicate the presence of ethylmethyllead in environment [Gallert and Winter, 2004].

Methylation of mercury

The methylation reactions of mercury have received more attention than those of tin and lead, and are much more understood.

The kinetic of these reactions depend on the reactant mercury not on the total concentration of mercury. Mason and Fitzgerald (1990) and Fitzgerald et al. (1998) have studied these reactions and concluded that only the labile inorganic mercury participates in the reduction and methylation reactions in aqueous media.

The concentration of CH_3HgX and $(CH_3)_2Hg$ in the environment is controlled by the relative rate of methylation and demethylation [Monperrus, 2004]. Figure 1.10 shows the physico-chemical processes which are believed to be the origin of methylation and demethylation processes.

Abiotic methylation can occur in sediment or water. Two main mechanisms have been described; chemical reactions and photochemical processes [Weber, 1993]. The chemical reactions happen in the presence of natural methylating agents such

as methylcobalamine, iodomethane, methyllead and methyltin [Jewett et al.,1975; Rosenkranz et al. 1997]. Iodomethane is found as a metabolite generated by algae. Methylcobalamine is also produced in nature by bacteria or termites. [Wikipedia, 2006, Cyanocobalamin; Craig, 1982; Tessier, 2004]. Methylation of Hg by methylead or methyltin is called transalkylation [Craig, 1986; Rosenkranz et al., 1997]. Photochemical methylation occurs when there are methyl donors groups such as acetic acid, methanol or ethanol [Gardfeldt et al., 2003]. Photochemical methylation in the presence of humic and fluvic acid has also been demonstrated by Weber 1993 in the laboratory.

Many aquatic organisms are responsible for biotic methylation, some of them are bacteria [Compeau and Bartha, 1985, Gilmour et al., 1992, Pak and Bartha, 1998a,1998b; King et al., 2001], the macroalgae [Pongratz and Heumann, 1998] and the macrophytes [Mauro et al., 2002]. The sulfato-reducing bacteria are known to be the principle mediator of the methylation in non oxic marine sediment [King et al., 2000], estuarine [Compeau and Bartha, 1985], fresh water [Gilmour et al., 1992] and anoxic lake water [Matilainen, 1995]. Sulfato-reducing bacteria use sulfate in their respiratory chain which generates H_2S [Postgate, 1965]. The presence of sulfate increases the methylation process [Gilmour et al., 1992]. Nevertheless, high concentration of sulfide, the product of sulfato reduction, may lead to the limitation of methylation process [Gilmour et al., 1998, Compeau and Bartha 1985]. King et al. (2000), in their study on the mechanism of methylation,

found that the bacteria which use acetate as the carbon source are more effective at methylation. The authors suggest that these bacteria express a methyltransferase enzyme.

Methylation is usually in equilibrium with demethylation, the abiotic demethylation of CH_3HgX by photodegradation has been demonstrated [Sellers et al., 1996]. Biotic demethylation by microbial process involves a reductive degradation in aerobic condition [Omerland et al., 1991], or oxidative degradation in non aerobic conditions, using organomercurial lyase enzyme [Marvin-Dipasquale et al., 2000, Monperrus et al., 2004].

Though, high attention has been given to methylation, few studies have been done on other possible environmental alkylation. Hempel et al., (2000) published results on mercury transalkylation in soil samples from an industrial site contaminated with organolead compounds and inorganic mercury. $EtHg^+$ was clearly identified in high concentrations (up to 46 mg Hg kg^{-1}). Furthermore, methylmercury was found in concentrations up to 27 mg Hg kg^{-1}. It was concluded that this ethylation takes place at sites, where organolead compounds occur and Hg^{2+} is available.

Abiotic methylation

CH_3^+ transfer

CH_3^- transfer

Transmethylation

Photochemical

$Hg_2^{2+} + CH_3I \longrightarrow MeHg^+ + HgI^+$

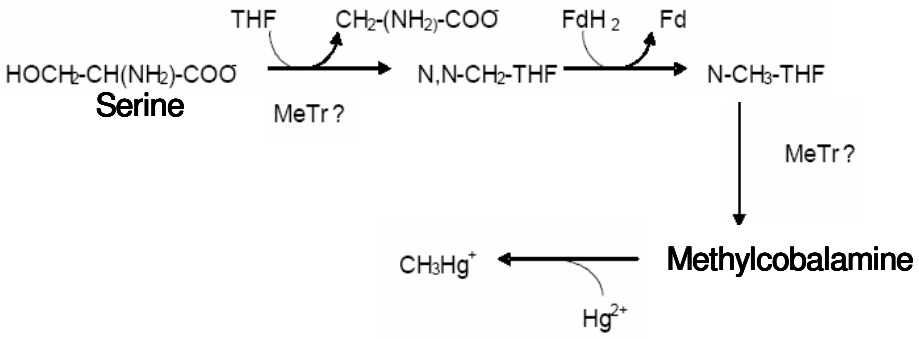
$Hg^{2+} + CH_3[Co] + H_2O \longrightarrow MeHg^+ + H_2O[Co]^+$

$Hg^{2+} + (CH3)_3Sn^+ \longrightarrow MeHg^+ + (CH3)_2Sn^{2+}$

$Hg^{2+} + CH_3CO_2^- \xrightarrow{h\upsilon} MeHg^+ + CO_2$

Biotic methylation

Methyltransferase



Abiotic demethylation

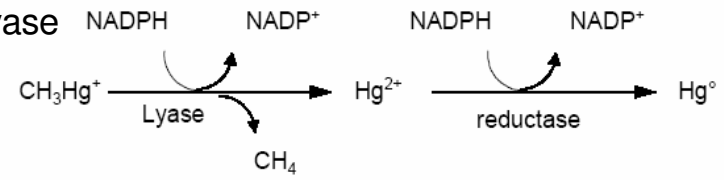
Photochemical

$MeHg^+ + CH_3CO_2^- \xrightarrow{h\upsilon} Me_2Hg + CO_2$

$Me_2Hg \xrightarrow{h\upsilon} C_2H_6 + Hg^\circ$

Biotic demethylation

Organomercurial lyase



$CH_3[Co]$: Methylcobalamine
THF: Tetrahydrofolate
MeTr: Methyltransferase
Fd: Formate dehydrogenase
NADP: Nicotinamide adenine dinucleotide phosphate

Figure 1.10: Mechanisms of Hg methylation / demethylation (Monperrus, 2004)

1.4.2.2. Stability to light, air and atmospheric reagents

Photochemical processes can in atmosphere, often decompose organometallics into radical species. However, other processes occur in the atmosphere, particularly where it is polluted. Relevant species identified include O_3, $^{\cdot}OH$, ClO_x, HO_x, and NO_x [Craig, 1986; Craig, 2003].

Photo properties are more dependent on the wavelength of excitation radiation for organometallic compounds than in the case for organic compounds. Light stability is more relevant for volatile organometallic (R_4Sn, R_4Pb and $(CH_3)_2Hg$) than their ionic forms (R_nSnX_{4-n}, R_nPbX_{4-n} and CH_3HgX) [Craig, 1986; Craig, 2003].

Organotin

Few studies detailing the release of Bu_nSnX_{4-n} into the atmosphere are available. However, the vapour pressure of these compounds is significant; it is possible to detect them in the atmosphere. For example, the vapour pressure of Bu_3SnCl is 0.0093 Torr at room temperature, higher than the vapour pressure of elemental mercury (0.0017 Torr at 25°C), which is considered to be a very volatile species [Mester et al., 2002].

Evidence suggests that organotin could be volatilised from the marine environment. Adelman et al. (1990) studied the fate of Bu_3SnX in laboratory experiments. They observed the degradation of radiolabeled Bu_3SnCl (^{14}C) in enclosed marine ecosystems during batch incubations. They were unable to

recover, at the end of experiment, all ^{14}C activity from Bu_3SnCl introduced in the system; this loss was related to the exchange of butyltin compounds at the air/water interface.

The volatile forms of organotin (methyltin, butylemethyltin and tin hydride) found in aquatic environment [Donard and Weber 1988, Amouroux et al. 2000] are considered as significant source of organotin in atmosphere. The transfer is estimated between 20 and 510 nmol m^{-2} per year [Amouroux et al. 2000, Tessier et al. 2002].

When organotin compounds reach the atmosphere, their high UV sensitivity may result in their photocatalytic decomposition, Bu_3SnX producing Bu_2SnX_2 and $BuSnX_3$ [Mester et al., 2002].

The other pathways of reaching atmosphere are the spraying of herbicides, antifouling paint sprays, incineration of materials treated or stabilized with organotin compounds, and glass coating operations. Incineration of organotin containing material is not believed to be a significant source of organotin compounds to air, since these compounds are readily decomposed to inorganic tin during combustion [Blunden et al., 1984].

Organolead

On the basis of the vapor pressure of tetraethyllead (0.26 mm Hg at 25 °C) and tetramethyllead (26.0 mm Hg at 20 °C), these two compounds are readily transferred to the vapor phase [Eisenreich et al., 1981].

As discussed in section 1.2, lead compounds are introduced into the atmosphere mainly by emission from vehicles using leaded gasoline.

When exposed to sunlight they are decomposed rapidly into trialkyl and dialkyl lead compounds and eventually into inorganic lead oxides by a combination of direct photolysis and reaction with hydroxyl radicals and ozone [Craig, 1986; Craig, 2003]. The half-life of Et_4Pb in reactions with hydroxyl radicals during summer is approximately 5.7 hours and about 65 hours for Me_4Pb [Nielsen et al., 1991]. In winter, both compounds have half-lives of up to several days since the concentration of atmospheric hydroxyl radicals is lower than in summer months [DeJonghe and Adams 1986]. Trialkyl compounds occur almost entirely in the vapor phase and have life-times in air that are three times longer than for the corresponding tetraalkyl compounds [Hewitt and Harrison 1986, 1987].

Because of the relatively high water solubility of trialkyl and dialkyl lead compounds, washout in wet deposition would be the major process for removing these compounds from air. Dialkyllead compounds would be removed from the air by dry deposition as they occur in particulate form. Adsorption of Et_4Pb and

Me_4Pb onto atmospheric particles does not appear to be an important process [DeJonghe and Adams, 1986, USEPA 1985].

Atmospheric pollution has no boundaries. Though, organolead would be found in high concentration in urban areas, it is also crucial to monitor their concentration in rural areas.

Organomercury

Mercury metal is considered to be highly volatile. The elemental Hg has a relatively high vapor pressure of 0.0017 Torr at 25ºC, whilst $(CH_3)_2Hg$ vapor pressure at 20ºC is 50 Torr [Sheu and Mason, 2001, U.S. Department of Health and Human Services and U.S. Department of Labor Occupational Health Guideline for Organo (Alkyl) Mercury, 10 September 2005]

Once in the atmosphere, Hg undergoes various physical and chemical transformations before being deposited in water or soil. Only a few chemical reactions of Hg have been studied in the laboratory under conditions relevant to the atmosphere [Fitzgerald et al., 1998; Schroeder and Munthe, 1998]. Sheu and Mason (2001) and references cited within reviewed these transformations. Elemental Hg can react with several oxidants in the aqueous phase, including O_3, $^{\cdot}OH$, and $HOCl/OCl^-$, to form divalent Hg^{2+}. Still in aqueous phase, the divalent mercury produced can be reduced by SO_3^{2-} and $HO_2^{\cdot}$. Hydrogen peroxide is not an important oxidant for Hg, but it can be a source of $HO_2^{\cdot}$. In the gaseous phase, O_3,

H_2O_2, NO_3 and other gaseous radicals are capable of oxidizing the elemental mercury Hg^0. There is no clear evidence of Hg^{2+} gaseous phase reduction occurring in the atmosphere. Photochemical reaction cannot be ignored, light and high temperature increase the production of elemental mercury by reduction of Hg^{2+} [Barkay et al., 2003] and $(CH_3)_2Hg$. Dimethylmercury can also be transformed to CH_3HgOH by reacting with OH [Monperrus, 2004].

Depositions and volatilization are the main phenomena that control water-atmosphere total Hg concentration [Mason et al., 1997, Lamborg et al., 1999]. Depending on the favoured reaction the Hg^{2+} or Hg^0 will dominate in atmosphere and consequently on land following deposition.

Given the life time of mercury with short (1-2days) or long (1-2 years) residence times in the atmosphere, mercury can be a local, regional and global pollutant. Consequently, it can deposit locally or travel long distances depending on its form [Dastoor and Larocque 2004].

1.4.2.3. Stability of organometallic compounds in water and sediments

Organometallic compounds in water and sediments are believed to be stabilized by coordination to sediments and suspended particulate matter containing sulphur. [Craig, 1986, Craig, 2003]

Organotin

Organotin compounds solubility range between 5.4-50 mg L^{-1} in seawater and 5–17 mg L^{-1} in distilled water for Bu_3SnX, and 4-50 mg L^{-1} in seawater and 92 mgL^{-1} in distilled water for Bu_2SnX_2. There is no value available for monobutyltin [Hoch, 2001]. The higher solubility in seawater might be due to its higher ionic strength. Bu_2SnX_2 which is more polar than Bu_3SnX shows high solubility in distilled water.

The speciation of organotin compounds is pH-dependent. The pKa of organotin ranged between 5 and 6.8. The cationic forms are favoured by lower pH and as the pH increases, the neutral hydroxide compounds will be the predominant species. In the environmentally relevant pH range (pH 5–9), the predominant organotin species will be the neutral hydroxide compounds (i.e., R_3SnOH, $R_2Sn(OH)_2$, and $RSn(OH)_3$). In seawater where high concentrations of chloride are found, the formation of chloro species is favoured. [Blunden et al., 1984; Fent, 1996].

Degradation of butyltin compounds in water or sediment is controlled by parameters such as temperature, sunlight, dissolved organic matter content and microorganism diversity and activity. It can be achieved by an abiotic or biotic mechanism [Maguire, 1996, Tessier, 2004]. In aqueous media, chemical and UV degradation are reported to be the most significant abiotic pathway [Maguire, 1996, Mailhot et al., 1999]. The bond strength of Sn-C bond is 190-220 KJ mol^{-1}

and UV radiation of wavelength of 290 nm correspond approximately to 300 KJ mol^{-1}, consequently, provided that absorption of light happens, the cleavage of Sn-C bond can occur under these conditions [Hoch., 2001, Maguire et al., 1983, Duhamel et al., 1987]. A study on phenyltin degradation demonstrated that some bacteria are able to degrade organotin [Barnes et al., 1973]. Tributyltin can also be degraded by microbial, microalgae, and fungal populations, as well as by some higher organisms [Anderson et al., 2002].

Recent research on organotin compounds transfers showed their diffusion at sediment-water interface [Tessier., 2004; Amouroux et al., 2000]. These results are a new challenge on the mobility of organotin in aquatic media. The main volatile organotin are the methylated compounds such as Me_4Sn, a product of transalkylation of Bu_3SnMe, or produced by inorganic tin methylation. The transalkylation reactions include Bu_2SnMe_2 and $BuSnMe_3$ as intermediates [Tessier, 2004].

The half-life of tributyltin in seawater varies, depending on pH, temperature, turbidity, and light [Alzieu, 1998, Tessier, 2004]. Biodegradation is the major process in seawaters rich in suspended solids, but photolysis, in surface waters, exceeds biodegradation in clean seawater. Calculated half-lives range from 6 days in summer time waters rich in suspended particles to 127 days in clean winter waters [Watanabe et al., 1992]. Degradation of organotin compounds in sediments is much slower than in water and half-lives have been estimated to be several

years [Alzieu, 1998]. Sediment appears therefore to act as an organotin sink and so represents a long term risk for water contamination, via desorption and remobilization processes.

Organolead

Solubility in water is 17.9 mg L^{-1} (Me_4Pb) and 0.29 mg L^{-1} (Et_4Pb) at 25°C [UNEP, 2003]. While Et_4Pb is almost insoluble in water, dialkylead and trialkyllead compounds are water soluble and quite stable [Craig, 1986, Hewitt, 1986].

In water, Et_4Pb and Me_4Pb are subject to photolysis and degradation precedes from a trialkyl species to dialkyl species, and eventually to inorganic lead oxides. Some of the degradation products include trialkyllead carbonates, hydroxides, and halides. These products are more persistent than the original tetraalkyllead compounds [DeJonghe and Adams 1986; USEPA, 1979c; UNEP, 2003].

In Gallert and Winter (2004), and references cited within, the degradation of tetraalkyllead to trialkyllead was found to be enhanced by light, oxygen, Fe^{2+} or Cu^{2+} ions, or enzymatically by bacteria.

Half-lives of tetraalkyllead compounds in seawater are estimated from a few hours in the light to a few days in the dark. The most stable product of tetraalkyllead degradation was found to be trialkyllead with a half life of a few days in the light

to up to one year in the dark. Consequently, in seawater and sediment more stable trialkylleads will prevail [Mikac et al., 2001].

Organomercury

The main compound of concern is methyl mercury which is classified as persistent toxic substance. Solubility in water of $HgCH_3Cl$ is 0.1 g L^{-1} at 21°C and 1.0 g L^{-1} at 25ºC for $Hg(CH_3)_2$ [UNEP, 2003].

The net amount of methylmercury produced in the aquatic environment depends on simultaneous processes (the kinetics of demethylation and methylation) which can give rise to elemental, inorganic, monomethyl and dimethylmercury [Rodrìguez Martìn-Doimeadios, 2004].

Inorganic mercury reduction is regarded as the main source for elemental mercury production in marine [Mason and Fitzgerald, 1993] and lake water [Fitzgerald et al., 1998; Amyot et al., 2000]. In abiotic reduction, the transformation of inorganic Hg to elemental Hg is caused by UV radiation [Amyot et al., 1994, Amyot et al., 1997; Costa and Liss, 1999]. Humic acid, fulvic acid and iron can also reduce inorganic Hg to elemental Hg [Allard and Arsenie, 1991; Matthiessen, 1996; Zhang and Lindberg (2001)]. Biotic reduction can occur in marine and freshwater [Mason and Fitzgerald, 1993, Fitzgerald et al., 1991; Mason et al., 1993; Siciliano et al., 2002].

Photochemical reactions are not the only responsible causes of the biotic reduction because elemental mercury and dimethylmercury have also been found in deep water [Mason et al., 1995a]. Heterotrophic bacteria and phytoplankton are suggested to be responsible for these biotic reduction and methylation [Mason et al., 1995b; Mason et al., 1998, Siciliano et al., 2002 ; Barkay et al., 2003; Poulain et al., 2004].

The oxidation of Hg° to inorganic Hg was neglected due to its supposed slow rate. Recent research indicates that, in the presence of chloride and by enzymatic catalysis, elemental mercury can be oxidized to inorganic mercury [Siciliano et al., 2002; Lalonde et al., 2001].

1.4.2.4. Absorption, distribution, metabolism and excretion (ADME)

To be able to understand the toxicity of organometallic compounds of tin, lead and mercury, we need to understand the "ADME".

This section will discuss these processes. Tessier and Turner (1995) reviewed the metal speciation and bioavailability in aquatic systems. In this book review, a chapter dedicated to lead, tin and mercury describes the current understanding of these processes.

Transport of organometals across biological membranes

The uptake of organometals by living organisms necessarily involves their movement across epithelial and internal cellular membranes. A detailed study of organotin and organolead describes a diffusion model for their transport. This model makes use of amphiphilic properties of organotin and organolead compounds. Being amphiphilic, they possess both hydrophobic and hydrophilic nature.

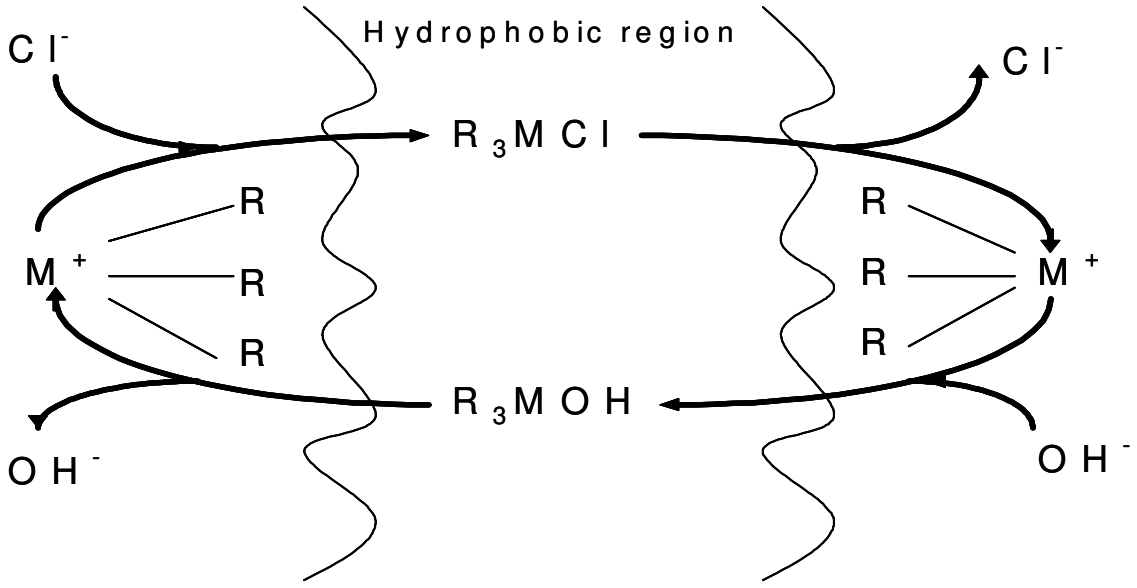


Figure 1.11: Transport of trialkyltin and trialkyllead across a cellular membrane (Tessier and Turner, 1996)

The model suggests the accumulation of trialkylmetal cations at the cellular membrane, with the hydrophilic metal cation oriented toward the aqueous phase and the alkyl groups projecting toward the hydrophobic region. As the cationic

metal combines with aqueous anion (usually Cl^- or OH^-) it becomes neutral and lipid soluble. It can then cross the membrane. The anion may be released on the other side of the membrane. This model is illustrated on figure 1.11.

This model ignores the interaction of organometals with enzyme and other proteins; it is just a simple passive diffusion process. It has been confirmed that these organometals interact with the thiols of protein. They have a high affinity for sulfur donors than for oxygen donors [Craig, 1996].

A study of organomercury transport demonstrated the attachment of mercury species to particular membrane site such as phospholipids and proteins. These proteins act as carrier through the membrane. This fixation is considered to be a key step in the bioaccumulation process. The other mechanism of organomercury transport is a rapid diffusion of neutral species such as $HgCl_2$ and CH_3HgCl. This diffusion is different from the mechanism of organotin and organolead transport [Tessier and Turner, 1996].

Bioaccumulation

Bioaccumulation is a general term for the accumulation of substances in an organism. These substances can reach the organism by different pathway. They can enter the organism through respiration, food intake, epidermal (skin) contact with the substance. The bioaccumulation results in the organism having a higher

concentration of the substance than the concentration in the organism's surrounding environment.

The level at which a given substance is bioaccumulated depends on the rate of uptake, the mode of uptake, how quickly the substance is eliminated from the organism, transformation of the substance by metabolic processes, the lipid (fat) content of the organism, the hydrophobicity of the substance and other environmental factors such as temperature, pH, alkalinity and turbidity [Toxic substance Hydrology Program, 13 September 2006, bioaccumulation]. As a general rule the more hydrophobic a substance is the more likely it is to bioaccumulate in organisms. The bioaccumulation of organometallic compounds of tin, lead and mercury will depend, among other factors, on the length of hydrocarbon chain.

Bioaccumulation can be divided into bioconcentration and biomagnification. Bioconcetration considers uptake from the non-living environment while biomagnification considers uptake through the food chain [Wikipedia, 2006, bioaccumulation].

An important factor is the bioconcentration factor (BCF), which is used to estimate the bioaccumulation of a species.

$BCF = [Species]_{organism} / [Species]_{water}$ Equation **(1.1)**

Theoretically, the bioconcentration factor can be calculated from the octanol/water distribution ratio of the species and the fat content of the studied organism [Looser et al., 1998]. This is just a theoretical value which should be treated critically, it does not take into account the other transformations which might happen when a species enter an organism. For example, degradation of organometal can happen and produce the species that are much hydrophilic, which might reduce the species bioconcentration factor.

Organotin compounds have a high affinity for lipid containing tissues. This property is the basis of their accumulation in biota; nevertheless, the simple partition in organic aqueous phase does not explain their bioaccumulation, the study done by Hunziker et al. (2001) shows an affinity of Bu_3SnX for the biological membrane. The liver and kidney of fish and mammal are the main organs affected by organotin toxicity [Fent and Looser, 1995; Looser et al., 1998; Looser et al., 2000].

Depending on the fish species, the BCF of Bu_3SnX ranges between 300 and 406 [Tessier and Turner, 1996]. The BCF of butyltin compound decreases from $Bu_3SnX > Bu_2SnX_2 > BuSnX_3$.

Organolead compounds occurrence in the environment has received much less attention than organomercury and organotin species and many aspects of their occurrence and fate in aquatic systems are still largely unknown. Data on

occurrence of alkylleads in living organisms and trophic transfer of organolead compounds through aquatic ecosystems are scarce. It was shown that fish found close to alkyllead manufacturers accumulate high concentrations of ethyllead compounds [Tessier and Turner, 1996].

A study done by Wang et al. (1996) on lead bioaccumulation in mussel and fish indicated that organoleads were more equally distributed between muscle and intestine. BCF between mussels and seawater indicates less efficient bioaccumulation of organolead. The concentrations in organisms and BCF between organisms and seawater were much higher in mussels than in fish, indicating the absence of biomagnification [Wang, 1996].

Et_4Pb has a BCF of 1000 while trialkyllead and dialkyllead have a BCF of 500 [CSF, 2002, Tetraethyl lead (TEL].

Organomercury compounds accumulate in the food chain, which lead to highly toxic effects on biota and humans [Quevauviller, 1996]. A BCF of 5000 for inorganic mercury and ranging from 4000-85000 for methylmercury have been found [USEPA, quality criteria for water, EPA 440/5-86-001, 1986].

The methylmercury can bioaccumulate then bioamplify in trophic chain starting from planktons. Planktons absorb methylmercury by passive surface absorption whereas heterotrophic organisms absorb it indirectly through their diet [Mason et al., 1996, Abreu et al., 2000].

90 % of mercury in fish is in the form of monomethylmercury [Burger, 2003]. A high concentration of inorganic mercury Hg^{2+} in fish has been found.

Many factors influence bioaccumulation such the size and the age of organism and diet. Depending on the organism and environment condition the results can differ for the same species.

Adsorption

The low solubility and hydrophobic properties of organo-species of tin, lead and mercury make them susceptible for adsorption on soluble and non soluble particles present in water. Among many other parameters, the concentration of the species, the presence of organic matter, the nature and the size of the particles and the salinity are the major factors which influence the adsorption [Unger et al., 1988 ; Randall and Weber ; 1986, Fent, 1996 ; Arnold et al., 1998a, Huang 2003].

Organotin compounds can adsorb on sediment and soil. The tributyltin present high tendency to bioaccumulate and bioamplify in aquatic organism, these are similar phenomena which lead to its adsorption in sediment and on suspended particles in water [Fent and Hunn, 1996; Stäb et al., 1996]. They form stable complexes in certain range of pH on surface of sediment rich in clay, silica or aluminium and Iron hydroxides [Unger et al., 1988; Weidenhaupt et al., 1997; Hoch and Schwesig, 2004].

The binding of tributyltin species to the sediments is most likely to be between the partial positive charge of the triorganotin species and the anionic centers found in the humic acid or clay that exist in freshwater sediments [Song et al. 2005].

Organolead compounds are much more adsorbed in wetland soil. This soil act as a sink for deposited alkyllead and inorganic lead [Huang, 2004]. Due to the high adsorption of alkyllead on soil with high organic matter content, their leaching to groundwater is very low.

Iron and manganese oxides can influence organolead and inorganic lead adsorption. They form stable complexes with lead compounds [USEPA, 2005a].

Organomercury compounds have high affinity toward organic matter. In sediment, they are complexed by organic compounds such as humic acid and inorganic compound such as iron and manganese oxide [Anderson et al., 1990, Gilmour et al., 1992, Bloom et al., 1988, Tseng et al., 2001]. Sediment acts as a sink of mercury compounds. It was found that 25 per cent of mercury compounds can be desorbed from sediments and diffuse in water [Covelli et al. 1999, Horvat et al., 2002].

Bioavailability

Bioavailability is a key parameter governing toxicity [Fent, 2004]. Figure 1.12 summarizes the relation between bioavailable concentration, bioaccumulation, sorption process and toxicity.

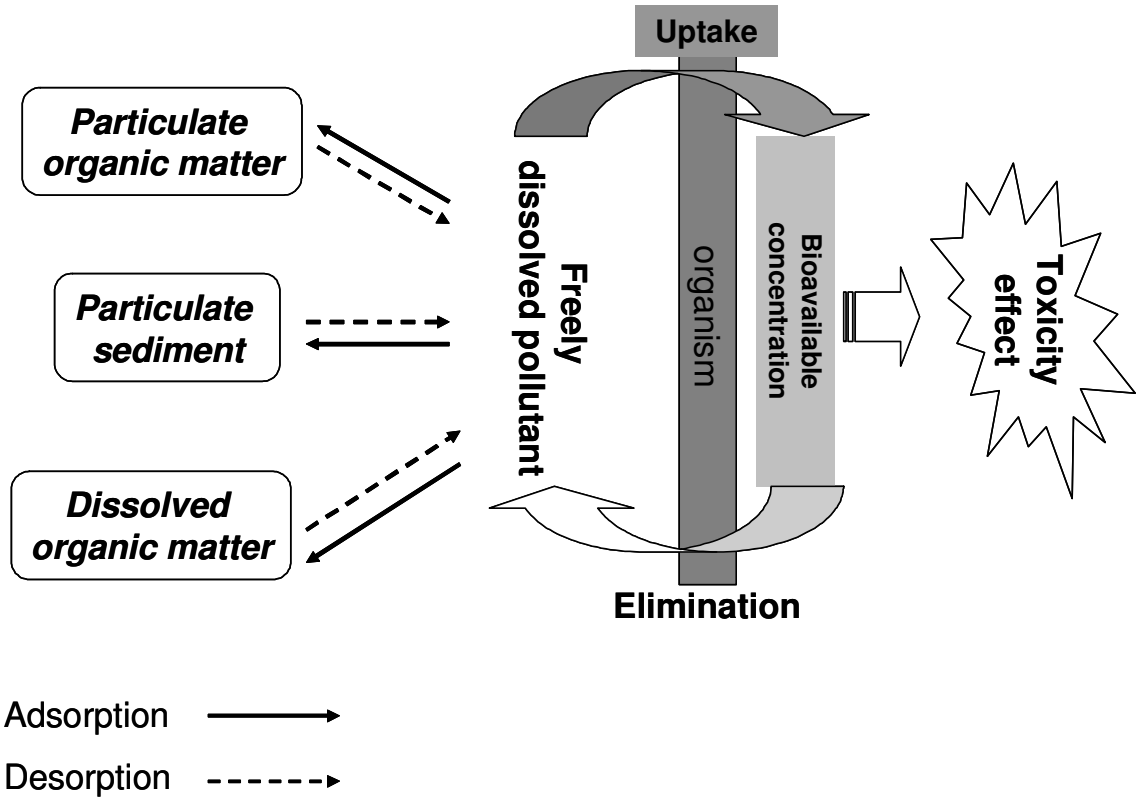


Figure 1.12: Bioavailability, bioaccumulation, sorption and toxicity interactions

Pollutants can be adsorbed on organic matter (particulate or dissolved) or on sediments. This mechanism is reversible, desorption of the pollutants will

remobilise them and they become available in the environment. Organisms may uptake the free pollutant by several pathways (oral, diffusion). Depending on the concentration of the pollutant it may cause acute toxicity or chronic toxicity following bioaccumulation.

Dissolved organic matter such as humic substances lead to a significant reduction in bioavailability [Fent and Looser, 1995; Looser et al., 1998]. Thus the free species of these compounds will be in very low concentration.

1.5. Toxicity of organometals of tin, lead and mercury

The inorganic form of tin, lead and mercury are less toxic than their organic species. The presence of the organic chain renders these compounds accessible to biological systems through membranes and allows them to bioaccumulate.

Generally, studies on toxicity focus on effects of a toxicant on one substance in organism. There is now a big interest in “mixture toxicity” [McCarty and Borgert, 2006].

When there are interactions of different substances, antagonistic and synergistic mechanisms can occur. The toxicity of organotin, organolead and organomercury needs to be studied simultaneously.

Antagonistic effect was found when $HgCl_2$ induced renal and intestinal necrosis in rats was completely abolished by simultaneous administration of Se (IV) in 1.5 molar ratios Se/Hg. [Gailer, 2006]

1.5.1. Organotin toxicity

Though the toxicity of organotin in aquatic life has been widely studied, little is known about human toxicity. The first reported case of organotin toxicity in humans was in 1954. The death of 100 people and 200 intoxications was reported in France, where a topical skin treatment containing Et_3SnI was administered orally [Hoch, 2001].

The toxic effects of organotin depend on the number and nature of constituent alkyl and aryl groups. The trialkyl and triaryl Sn compounds (R_3SnX) have been found to be more toxic than the di or mono substituted organotin compounds. The nature of X in these tri-substituted compounds have been found to have little or no effect on the toxicity, except in cases where X itself is a toxic component [Hoch, 2001, Bushong et al.,1988].

The prominent toxicological feature of organotin is its immunotoxicity, an effect produced by di- and trialkyltins as well as triphenyltins. On contact the organotin may cause eye and skin irritation [WHO, 1990; ATSDR, 1992; WHO, 1999a,b; KEMI, 2000; Bryan, 1986].

Tributyltin as an endocrine disrupter has imposex effect in animals and induces masculinization by increasing testosterone levels in different species of female gastropods. In some species of mussel (dog whelk), tributyltin causes the females to develop male sexual characteristics such as a penis, this causes them to become infertile or even die. In severe cases males can develop egg sacs [Wikipedia, 2006, Tributyltin, Fent, 1996, Fent, K. and Meier, W.1992, Horiguchi et al.,1997]. Tributyltin inhibits transport through cell membrane of major mineral element such as (Ca^{2+}, Na^{+}, K^{+}) [Selwyn, 1976] and alters the cell structure [Gray et al., 1987].

The highest toxicity of butyltin compounds is shown by trisubstituted species. In vitro work showed toxicity in rats [Bulten and Meinema, 1991]. Other investigations on dibutyltin toxicity have shown that it is accumulated in bodies and tissues of mammals [Takahashi et al.1999] and fish [Kannan et al., 1996; Stäb et al., 1996]. Monobutyltin has been demonstrated to have an effect on fish immune system [O'Halloran, 1998].

1.5.2. Organolead toxicity

Seyferth (2003), in his review on "the rise and fall of tetraethyllead", described the history of tetraethyllead. The toxicity of tetraethyllead was noted at the beginning of 1920. Its toxic effects caused major problems almost right from the start. As described (section 1.2) tetraethyllead use as antiknocking agent was discovered by Midgley. Midgley himself and three co-workers suffered digestive problems,

subnormal body temperature and reduced blood pressure, presumably the result of their work on synthesis routes to tetraethyllead during the latter part of 1922. Midgley was warned about the toxicity of tetraethyllead. However, despite of his own health problems he did not appear to be overly concerned about the health issues associated with the handling and use of tetraethyllead. In a report issued in October 1924, the Bureau of Mines in U.S concluded on the basis of its animal tests that the danger to the public of breathing lead in automobile exhaust was negligible.

During the same year (1924), in the Bayway plant, New Jersey, five workers died and 44 were hospitalised. This was mainly due to the equipment and procedures used and the lack of safety precautions. Four deaths and many illnesses were reported the following year. On the 1st of May 1925, the sale of leaded gasoline was suspended until its potential public health hazards could be assessed. But it was concluded that leaded gasoline was not a health hazard to the public.

On the 16th of May 1926, leaded gasoline was back on the market and tetraethyllead flourished for another 50 years. The renewed concerns about the automobile that used leaded gasoline as a source of toxic lead compounds in the environment led to many clinical and laboratory studies in the 1960's and 1970's and, ultimately, as many of these studies provided support for these concerns, in 1982 the Environmental Protection Agency (EPA) mandated a decrease of the

average lead content of gasoline sold in the USA from 0.52 to 0.28 g L^{-1} and, in 1986, to 0.026 g L^{-1}.

It was pointed out that not only was tetraethyllead highly toxic but also toxic inorganic lead compounds were formed in the engine and that a large percentage of these exited into the environment through the exhaust system. It is more dangerous than inorganic lead compounds since it is volatile and can be inhaled. It is also soluble in lipids and is rapidly absorbed through the skin [Seyferth, 2003b].

It can be observed that the delay in recognition of toxicity of tetraalkyllead was motivated by economical reasons, due to the success of the use of tetraalkyllead as antiknocking agent. Methylated compounds are in general less toxic than the corresponding ethylated lead compounds [Unob, 2003, Grandjean, 1984].

Lead and lead compounds have been found to cause cancer in the respiratory and digestive systems of workers in lead battery and smelter plants. However, tetra-alkyllead compounds have not been sufficiently tested for evidence of carcinogenicity [UNEP, 2003].

1.5.3. Organomercury toxicity

The awakening on mercury toxicity comes with what is called “Minamata disease”. It is a neurological syndrome caused by severe mercury poisoning. Symptoms include ataxia, numbness in the hands and feet, general muscle weakness, narrowing of the field of vision and damage to hearing and speech. In

extreme cases, insanity, paralysis, coma and death follow within weeks of the onset of symptoms.

Minamata disease was first discovered in Minamata (Japan) in 1956. It was caused by the release of methylmercury in the industrial waste from the Chisso Corporation's chemical factory. Over 1400 died. It is estimated that over 2 million people may have eaten contaminated fish from Minamata Bay.

Initially Chisso Corporation was producing fertilizers in 1908s, the factory followed the nationwide expansion of Japan's chemical industry in 1930s, branching out into production of other chemicals, among them acetaldehyde. Mercury sulfate was used as a catalyst in the chemical reaction used to produce the acetaldehyde. During the reaction methylmercury was also produced and released into Minamata Bay with other waste.

After an unknown disease which many people suffered in Minamata in 1956 and later found to be the consequence of mercury toxicity; in 1968 after nearly 40 years of production the method used to produce acetaldehyde was discontinued. [Wikipedia, 2006c, mercury]

Decontamination of Minamata Bay is now on course, dredging is the method used. It appears to be an effective remedy for systems heavily polluted by mercury. Minamata bay contained as high as 600 mg kg^{-1} of mercury in settled sediment. Dredging began in 1977 and ended in 1990. Monitoring data shows that dredging

did not cause a significant adverse impact on the environment from sediment resuspension. Mercury concentrations in water and fish were below the safety requirement during and after dredging [Wang, 2004].

Long-term exposure to either inorganic or organic mercury can permanently damage the brain, kidneys, and developing fetus. The most sensitive target of low level exposure to metallic and organic mercury following short or long term exposures appears to be the nervous system [UNEP, 2003]. Though both types of mercury are toxic, the methylmercury is more toxic than inorganic mercury [Cai et al., 1997], the reason being the high bioaccumulation property of methylmercury.

1.6. Environmental legislations

Three organizations are concerned with the regulation of toxic substances, the World Health Organization (WHO), then the United State Environmental Protection Agency (USEPA) and European Economical Community (EEC).

In South Africa, the Department of Water Affairs is responsible for environmental legislations.

Regulations on organotin compounds

The restrictions on its use have been implemented in many countries from the mid to end 1980's. France was the first to ban the use of antifouling paint on boats of less than 25 m length in 1982. This was followed by the United Kingdom in 1987,

then the USA, Canada, Australia and New Zealand in 1988-89. The European Union generalized the restriction on the whole continent in 1991. In 1997 the OMI (International Maritime Organization) adopted a worldwide principle on the total elimination of tributyltin based antifouling paint [Tessier, 2004].

As a consequence, tributyltin concentrations in harbor waters decreased significantly at many locations in industrialized countries [Chau et al., 1997; Fent and Hunn, 1995]. Due to a moderate permanent persistency of these compounds, they are still found in marine and continental environment.

Table 1.1 gives the concentration of organotin in environment. In many cases, only the concentration of the more toxic tributyltin is given.

Table 1.1: Limit concentration of OTC in environment

Description	Information	Reference
TBT (microorganism)	1 ng L^{-1}	WHO-IPCS, 1989 Alzieu, 2000 INERIS, 2003
TBT (aquatic plant)	100 ng L^{-1}	
TBT (fish)	100 µg L^{-1}	
Triphenyl acetate (oral)	81 mg kg^{-1} (LD_{50})	FAO/WHO, 1971
TBTCl	5.26 µg L^{-1}	Meador, 1986
TBTO	66.3 µg L^{-1}	Foster, 1981

Due to different sources of results and different conditions some results are given in ranges. Many studies are underway in order to harmonize the concentration limit of different organotin in environment; limit of concentration in water has been established. The low concentration limit established for a chronic effect is 0.4 ng L^{-1}, and 50 ng L^{-1} for acute toxicity [Tessier, 2004].

Regulations on organolead compounds

Under the Clean Air Act (CAA), the EPA regulated lead and has designated lead as a hazardous air pollutant [USEPA, 2004]. In the early 1970s, after determining that lead additives would impair the performance of emission control systems installed on motor vehicles and that lead particle emissions from motor vehicles presented a significant health risk to urban populations, the EPA began regulating the lead content in gasoline [USEPA, 1996b]. The EPA instituted a phase-down program in 1973 designed to minimize the lead content of leaded gasoline over time. The Clean Air Act Amendments (CAAA) of 1990 banned the sale of leaded gasoline as of December 31, 1995

The international and national regulations and guidelines regarding lead compounds in air, water, and other media are summarized in Table 1.2.

Table 1.2: Regulations and Guidelines Applicable to Lead and OLC

Description	Information	Reference
Air quality	0.5µg/m^3	WHO 2000
Drinking water	0.01 mg/l	WHO 2004
Air quality	1.5 µg/m^3	EPA 2005, 40CFR 50.12
Action level	0.015mg/l	EPA 2002
Hazardous waste	5.0 mg/l	EPA 1990c
Lead Residential lead hazards standards – TSCA Section 403	Floors 40 µg/ft^2; Interior window sills 250 µg/ft^2; Bare soil in children's play areas 400 ppm; Bare soil in rest of yard 1200 ppm average	EPA 2005i
PEL (permissible exposure limit) , 8-hour TWA(time weighted average) for toxic and hazardous substances for lead	50 µg/m^3	OSHA 2005d, 29CFR 1910.1025
Action level	40 µg/100 g of whole blood	
Removal of employee from exposure	50 µg/100 g of whole blood	
PEL (8-hour TWA) for general industry for tetraethyl lead	0.075 µg/m^3	OSHA 2005c, 29CFR 1910.1000
PEL (8-hour TWA) for construction industry for tetraethyl lead	0.01 µg/m^3	OSHA 2005b, 29CFR 1926.55
PEL (8-hour TWA) for shipyard industry for tetraethyl lead	0.01 µg/m^3	OSHA 2005a, 29CFR 1915.1000

Regulations of organomercury compounds

The European Union in 1976 put mercury on the list of dangerous substances for aquatic media in the decision 76/464/EEC. The Hague conference of the European Union of the 8th of March 1990 [CEE, législation communautaire en vaguer,

document, *391L015*, 1999], fixed the limit concentration of Hg in manganese alkaline battery at 0.025%. The European Union in its decision 91/157/CEE planned a ban on the market of manganese battery with a concentration of Hg higher than 0.025% in other batteries as of 1993. The decision 98/101/CEE fixed the Hg concentration at 0.0005% in battery from January 2000 except the button battery containing Hg inferior to 2%. Table 1.3 gives a summary of the regulations other than that mentioned.

The EU banned the use of pesticide containing mercury compounds in agriculture in 1979 (79/117/CEE); limitation of discharging mercury product in underground water (80/068/CEE) and all mercury use in paints, textile, wood preservative and industrial water in 1989 (89/677/CEE) and banned in seed treatment in 1991 (91/118/CEE).

A lot is still to be done in harmonization of recommended exposure limits. The question to ask is why what is toxic in one part of the world at this given concentration, is not toxic at the same concentration in other parts of the world or completely non toxic? Is it economically related, as shown previously in the case of early age of tetraethyllead use? Is it related to politics? Or it is just the lack of adequate methods, with a very low detection limit?

Table 1.3: Regulations and guidelines applicable to Mercury and OMC

Description	Information	Reference
Mercury chloride (oral)	RfD: 3 10^{-4} mg kg^{-1}	EPA 1995
Methylmercury (oral)	RfD : 10^{-4} mg kg^{-1}	EPA 2001
Drinking water (total mercury)	2.0 µg L-1	EPA 440/5-86 001,1986
Seafood (methylmercury)	1.0 µg L-1	EPA 440/5-86-001,1986
Methylmercury(oral)	TDD = 1,6 10^{-3} mg kg^{-1}	WHO 2003
Total Mercury(oral)	TDD = 6 10^{-4} mg kg^{-1}	WHO 1996
Drinking water	1 µg L-1	WHO 1996
Mercury chloride (oral)	MRL= 7 10^{-3} mg kg^{-1} (acute) MRL = 2 10^{-3} mg kg^{-1} (sub chronic)	ATSDR 2001
Methylmercury (oral)	MRL = 3 10^{-4} mg kg^{-1}	ATSDR 2001
Surface water, for water supply for total mercury	1 µg L^{-1}	75/440/CEE
Hg in water intend to human consumption	1 µg L^{-1}	80/778/CEE
Hg discharged from chloro alkaline industry	75 µg L^{-1}in 1983 50 µg L-1 in 1986	82/176/EEC
Industry discharge other than chloro alkaline in continental water	1 µg L^{-1}	84/156/CEE
Hg in soil	0.1 kg ha^{-1} per year	86/278/CEE
Atmospherique Hg discharge from incineration	0.2 mg m^{-3}	89/869/CEE
Hg in Sea food	0,5 mg kg^{-1}	93/351/CEE

RfD:Reference Dose per day, MRL:Minimun Risk Level, cTDD: Tolerable Dose per Day

CHAPTER 2

CRITICAL LITERATURE REVIEW OF CURRENT ANALYTICAL METHODOLOGIES

The analysis of organometallic compounds as other trace metal species in the environment requires many steps. This section gives a comprehensive overview of the sampling method, sample preparation and analytical techniques (instrumental analysis) that can be used for organometallic compounds of tin, lead and mercury.

2.1. Sampling and sample preparation

Sampling should be planned meticulously. The plan starts with the cleaning of containers which will be used to collect samples. A good understanding of the analyte is required. This is important for selection of the right type of containers to be used and to determine what parameters to measure during sampling.

2.1.1. Sampling and storage

Batley (1991) reviewed the analytical methods and problems in trace element speciation. He described the methods that should be used in water sampling and solid sampling. The species and the state of the sample dictate the method that should be used.

2.1.1.1. Water samples

Water sampling, pretreatment and storage

A major problem in all sampling procedures is to obtain a representative sample. In natural water, the concentration of pollutant depends on several physico-chemical parameters. Its concentration will vary with depth, salinity, proximity to discharge point, pH, dissolved oxygen and temperature. The sampler must decide initially upon the sample information required, and design a collection strategy accordingly [Batley, 1991].

Treatment depends on analyses required. In general, for trace metal analyses, particulate matter is first removed from the sample by filtration and centrifugation. Then preservation reagents may be added to the sample and the sample stored in an appropriate container under conditions which minimize contamination or losses of the analyte [Batley, 1991]. It is advisable that filtration be performed as soon as possible after collection.

Storage starts by choosing a container. A range of containers have been used, including polyethylene, polypropylene, teflon, polycarbonate and borosilicate glass. The choice of container is determined both by its adsorptive properties and surface impurities.

It is believed that polyethylene and teflon bottles are the best for trace metal [Batley, 1991]. However, studies on mercury showed surface reaction of

polyethylene with the metals, therefore for mercury samples a teflon container should be used [Parker and Bloom, 2005, Stoichev et al., 2006]. Glass containers have a problem of adsorption. The adsorption of metal ions on glasses and oxides surfaces depends on the ability of metal to be hydrolysed. Little adsorption is observed in acidic sample hence the sample should be directly acidified after filtration [Batley, 1991]. Acidification prior to filtration may extract metals from particulate surfaces and increase the dissolved metal concentration. The effective pH for acidification was found to be less than pH 2.

The containers used have to be thoroughly cleaned. The method used is usually acid cleaning with HCl and/or HNO_3, to remove possible metal impurity from the container wall and prevent further metal adsorption.

After pre-treatment, a sample should be stored at 4ºC but kept frozen for mercury analysis. The studies done on mercury reported transformation of mercury species when samples are not frozen [Batley, 1991].

From sampling to analysis, there are many steps subject to contamination. To be able to determine the contamination from container, filtration, storage and other sample preparation steps, blank samples should be collected and submitted to the same procedure as the real samples. It is advisable to collect one to three blank samples [Dadfarnia et al., 1994].

2.1.1.2. Solid sample

Soil and sediment sample

Depending on the purpose of the analysis, surface sampling of soil/sediment or profile sampling can be done.

For soil profiles, the initial operation is digging out soil profile and determining its genetic classification. Sampling starts from the lowest part of the profile [Pawliszyn, 2002] to avoid contamination.

For sediment profile, an "auger" is usually used. An auger has a cutting end (bit) which advances the device through the subsurface as it is turned to the desired level [SDSU, department of geological sciences, 2007, Sediment sampling].

Pretreatment is completely dependent on the type (coarse, fine grained, debris) and the environment (wetland, forest, river, lake). Sediment or soil interstitial water can lead to sediment - water interface transformation process (section 1.4.2). This water should be removed. The centrifugation of small portion of solid has been found to be effective [Batley, 1991].

When a sample contains fine grained sediments or soil (clay and silt) without debris dry sieving is not required but wet sieving is necessary. Sieving should be done after sampling using a nylon sieve (2 mm or 300 µm in size) or a clean stainless steel sieve (not roasted).

Storage conditions are very important. Soil or sediment sample can be stored at 4°C in the dark for a few hours and then rapidly deep frozen. The temperature used is generally -20°C. Afterwards it is necessary to dry freeze the frozen samples (lyophilized) under vacuum at -50°C. This is should be followed by grinding and the dry powder can be stored in clean plastic ware at room temperature in a dark room for several months [Tessier, 2004]. Lyophilization prevents the loss of volatile organometals.

Biological plant material

Sampling of plant is the same for all parts of the plant. Depending on the study, a part of the plant to be sampled is chosen. Obtaining a representative composite sample of the plant is especially difficult. Plants of different characteristics should be gathered so that the overall data are representative of the area studied [Pawliszyn, 2002].

Pretreatment involves cleaning and drying then grinding. The plants have to be washed to avoid contamination by dust [Pawliszyn, 2002].

Storage of plant samples is the same as for soils and sediments (section 2.1.1.2).

2.1.2. Extraction methods

The speciation of organometals of tin, lead and mercury requires extraction step prior to the detection. To our knowledge, there are no methods for speciation of

these compounds directly from solid samples. HPLC is used for liquid [Chao, 1998] with limitation for volatile species.

Many extraction methods have been used for organometallic analysis in solid or liquid samples.

2.1.2.1. Liquid – liquid extraction

In liquid-liquid extraction the analytes are partitioned between two immiscible solvents in which they have different solubility. One phase is usually water and the other phase organic solvent. The selection of the optimum solvent is of particular importance, where general principle "like dissolves like" applies [Pawliszyn, 2002]. For organotin, organolead and organomercury compounds, hexane and isooctane have found much more application.

The speciation of organometals includes all species, polar (less volatile) and non polar (more volatile). There is a need of transformation of polar species to non polar species. This is achieved by derivatisation.

The formation of emulsion often renders phase separation difficult. The addition of acid (HCl) and centrifugation breaks up the emulsion for organometals extraction [Monperrus, 2004].

The use of low volumes allows simultaneous extraction and preconcentration.

2.1.2.2. Liquid membrane extraction

Liquid membranes can be used to extract trace amounts of heavy metal ions from process streams. The liquid membrane is a thin layer of organic phase, separating two aqueous phases. To achieve metal ion extraction, an extractant is dissolved in the organic membrane phase. It acts as a shuttle, extracting the metal ion from the feed phase and releasing it again at the other side of the membrane. In this way, extraction and stripping are performed in one process step [Runner, 1993, Zhou et al., 2003].

The use of liquid membranes for extraction is a common way of avoiding problems associated with liquid-liquid extraction, such as emulsion formation.

2.1.2.3. Solid Phase Extraction

In solid phase extraction, a liquid is passed over a sorbent packed in a cartridge or embedded in a disk. As a result of the strong attraction, analytes are retained on the sorbent. In the second step, analytes are eluted by passing a small volume of organic solvent through a cartridge or a disk which disrupts the bonds between analytes and sorbents [Pawliszyn, 2002].

This extraction method can be used for organometallic preconcentration. Organotin, organolead and organomercury give cationic ions that can be preconcentrated on reversed-phase columns or cartridges. The adsorbent used generally is silica-gel C_{18}. This method can preconcentrate organometallics to a

concentration factor of 1000. The preconcentration of Hg species requires the use of reagents to retain them in the solid surface. Sulfydryl, cotton microcolumn, crown-ethers and chelating agents such as dithizone or 2-mercaptoethanol are used [Gomez-Ariza et al, 2001].

Gomez-Ariza et al (2001) reviewed sample preparation for organometallic speciation and reported the advantages and disadvantages of solid phase extraction methods. One of the undoubted advantages is that this method allows online flow analysis. In addition, SPE has other advantages such as the use of less volume compared to liquid-liquid and membrane extraction, the cartridges can be used for storage of species, and it provides high enhancement in preconcentration. The main disadvantage of this method is the poor reproducibility in comparison with liquid-liquid extraction and less selectivity with common sorbets [Pawliszyn, 2002].

Solid phase extraction was used in online modified system. There is no use of cartridge; the adsorbent is packed into the column. The sample is adsorbed at very low temperature and desorbed by heat. This method is called "*Cryogenic Purge and Trap Solid Phase Extraction"* [Dietz, 2000].

This technique is based upon in situ derivatisation, trapping, desorption and gas chromatographic separation and detection. The purge and trap system allows for identification and quantitative determination of low concentrations of organotin,

organomercury and organolead forms in water, sediment and soil samples. It may also be able to simultaneously analyze various species for more than one element in complex matrices [Runner, 1993].

Figure 2.1 shows a modified on line cryogenic and traps solid phase extraction developed by Tseng et al. (1997), followed by gas chromatographic separation and detection. This system is illustrated the way it is used in our laboratory.

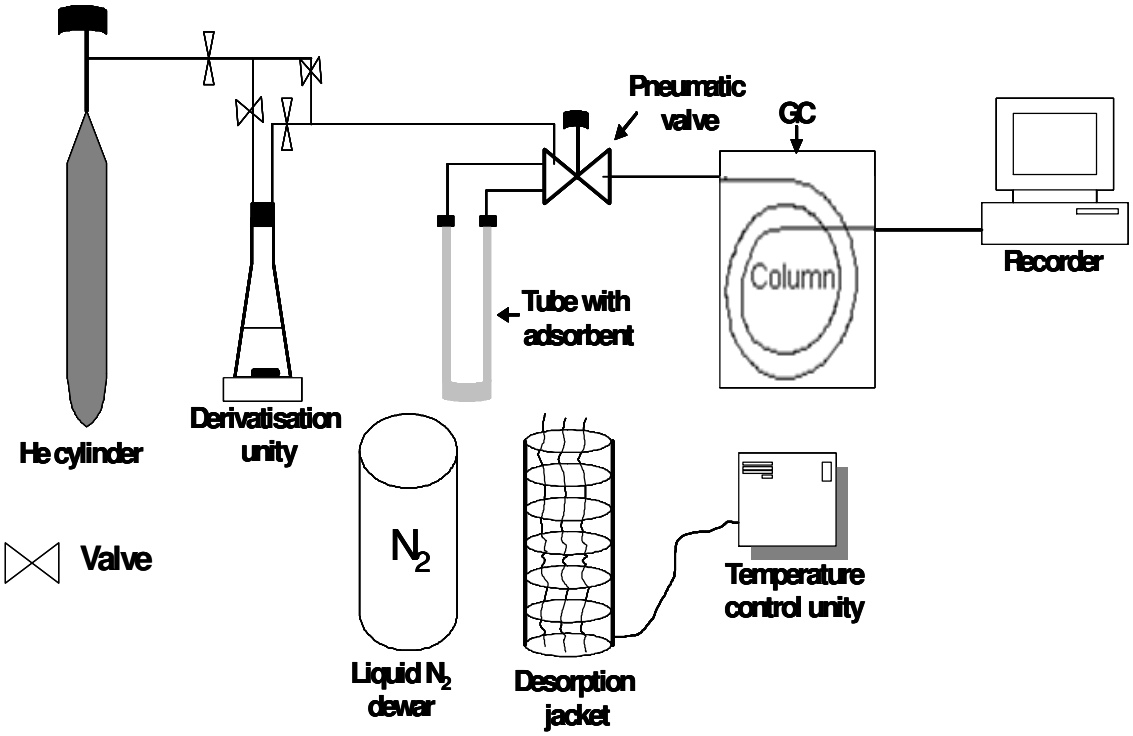


Figure 2.1: Cryogenic and trap solid phase extraction

2.1.2.4. Microwave-assisted extraction

Microwaves are defined as the electromagnetic radiation within a frequency range of 300 MHz to 300 GHz.

Depending on the dielectric constant, the material exposed to microwaves can reflect the radiation (metal), allow the permeation of radiation without being heated (glass and plastic), or absorb microwave energy allowing the heating-up of the substance (polar substances) due to the oscillation of polar molecules. The microwave radiation heats up the medium directly, which results in significant time savings [Anton Paar GmbH, 2002].

Microwave assisted extraction has been used for several solid matrices (soils, sediments, and biological tissues), and has been found to be an appropriate tool for rapid treatment of solid samples in organometallic speciation. It is faster than most other sample preparation and extraction procedures, with typical sample treatment taking only 3 minutes for organometallic extraction [Tessier, 2004; Monperrus, 2004]. Its disadvantage is that organometallic compounds can be greatly degraded during the extraction procedure [Runner, 1993].

Anton Paar 2006 improved the technique in microwave assisted extraction; currently the development in microwave assisted extraction gives the possibility of using non polar solvent in extraction. It renders sample preparation easier, due to the possibility of conducting both extraction in polar solvent and non polar solvent simultaneously. The non polar solvent can then be collected and directly injected into the gas chromatography.

2.1.3. Overview of the derivatisation methods

In the environment, organometallic compounds can be in a polar form with low volatility to enable gas chromatography separation.

Derivatisation procedures may be used to separate trace elements from their matrices, to concentrate the analyte species and to generate species which are more easily separated from each other by chromatography. Reactions employed are mostly centered on the addition of simple groups. These reactions can be divided in two groups, one includes the hydride generation reactions and the other one the alkylation reactions.

2.1.3.1. Hydride generation derivatisation

In organometallic speciation, hydride generation method as a tool of derivatisation was used in 1970s. The polar organometallic compound was converted to non polar hydride form by sodium tetrahydroborate (Na BH_4). (Equation 2.1)

Hydride generation [Morabito et al., 2000]:

$$R_nM^{(4-n)+}(aq) + Na\,BH_4 + H^+ \rightarrow R_nSnH_{(4-n)} + H_2 \qquad (2.1)$$

(R = organic group, n=1, 2 or 3)

This method requires acidic conditions, thus HCl is usually added prior to the derivatisation step.

It has been applied in the speciation of tin, lead and mercury. The critical steps include sample pre-treatment and interferences control. This method was used for the analysis of butyltin compounds [Takeuchi et al, 2000], analysis of organolead [Quevauviller et al, 2000] and mercury [Gerberwnann et al 1997].

Hydride generation has an advantage over alkylation for dilute aqueous samples [Ko et al., 1995] due to the high reactivity of borohydride. For direct in situ derivatisation, hydride formation was usually the preferable method, [Lu et al., 2001]. Hydride generation produces a large volume of hydrogen as by-product, which facilitates the purging of organometallic hydrides from large volumes of sample [Champ, 1996].

However, the studies done on harbor sediment by Quevauviller et al. 1994 and Martin et al. 1994 on certified material, revealed the great analytical difficulties in the determination of tributyltin using hydride generation due to the presence of high amounts of inorganic and organic interferences. The resulting organometallic compounds hydrides are not stable; they are prone to dismutation reaction [Liu, 1999]. This has lead to the decline of hydride generation use in speciation analysis.

2.1.3.2. Alkylation derivatisation

The alkylations derivatisations may be produced by two different reactions. The first one is the derivatisation by Grignard reagent which has been used for

speciation since 1970s. The second one is alkylation by boron reagent, which started in the 1990s.

Grignard reagent alkylation

The Grignard reaction is an organomeatallic reaction involving alkyl- or aryl-magnesium halides (RMgX) also called Grignard reagents [Wikipedia, 2006, Grignard reaction]. The reaction which is involved in derivatisation is represented in equation 2.2 for organotin. Prior to the derivatisation step the analyte has to be extracted in organic solvent, due to the instability of Grignard reagent in water [Liu, 1999].

$$R_nSn^{(4-n)+} + R'MgX \rightarrow R_nSnR'_{(4-n)} \qquad (2.2)$$

(R, R' = organic group, n = 1, 2 or 3)

A variety of Grignard alkylations exist depending on alkylating reagents with different alkyl-groups. Methyl-, ethyl-, butyl-, propyl-, butyl-, pentyl-, hexyl- and phenyl are available. Long chain alkyl group such as pentyl- and hexyl have the advantage of providing derivatisation compounds with relatively low volatility which allows easy preconcentration steps without losses [Liu, 1999, Takeuchi et al., 2000].

This method has been applied in several studies on organometallic compounds speciation. In many cases the derivatisation is combined with complexation. The

application to organolead compounds by propylation with propylmagnesium chloride, complexation with diethyldithiocarbamate was successful. Other Grignard reagents were also applied for organolead speciation, such as butylmagnesium chloride and pentylmagnesium bromide [Quevauviller et al, 2000]. They were used in butyltin and phenyltin speciation [Takeuchi et al 2000, Dirkx, 1992] and in mercury speciation [Emeteborg et al, 1999].

The main advantage of the Grignard reagent is the introduction of long alkyl chains. However the longer the alkyl chain is of the Grignard reagent, the greater is the risk of formation of degradation product and peak broadening effects in the chromatographic analysis [Quevauviller, 1998.a, Quevauviller, 1998 b].

Boron reagents alkylation

This derivatisation method includes ethylation by $NaBEt_4$, propylation by $NaBPr_4$ and $MgBPr_4Br$, then phenylation by $NaBPh_4$.

The equation 2.3 gives an example of alkylation by sodium tetraalkyl borate [Cai et al., 1993].

$$R_nSn^{(4-n)+} + NaBR'_4 \rightarrow R_nSnR'_{(4-n)} \qquad (2.3)$$

(R= Me, Et, Phe and R'= Et, Pr and Phe)

This method was used for the first time for ethylation of inorganic lead by Honeycutt and Riddle (1961) for its identification after sodium tetraethylborate

synthesis. The first method to be used for environmental purpose was ethylation in 1990's [Liu, 1999]. Since then it has found several applications in speciation of organometallic compounds.

This procedure allows for in situ analysis. Extraction and derivatisation can be performed simultaneously [Arnold et al. 1998b; Hoch, 2001; Cassi et al., 2002; Bowles et al., 2003].

This method is now widely used for organotin and organomercury derivatisation. It allows the simultaneous determination of these organometals. The derivatisation is done by ethylation or propylation with good limits of recoveries and reproducibility [Tessier, 2004, Monperrus, 2004]. It has also been used for organolead compounds in aqueous solutions for ionic lead compounds such as Me_3PbX and Me_2PbX_2 [Rapsomanikis et al., 1986].

This method of derivatisation by $NaBEt_4$ is inappropriate for mixed ionic methylethyllead species, which may exist in the environment due to anthropogenic input. It is also inappropriate to use this method in the presence of Et_4Pb and inorganic lead. It will not be able to differentiate between tetraethyllead and inorganic lead [Rapsomanikis et al., 1986].

During derivatisation from complex matrices (such as sediments) a large number of unwanted components can co-extracted, which can lead to incomplete derivatisation and interfere with the analysis [Takeuchi et al, 2000].

The alkylation with boron reagent compared to hydride generation shows a significant improvement in detection limit, more reproducible results and no inorganic interferences. In comparison with the Grignard reagents, the boron reagents are stable in water hence derivatisation can take place in aqueous media [Liu, 1999].

2.2. Separation and detection methods for organometallic compounds speciation used in this study

A variety of organometallic compounds of the same element may be in a matrix (butyl / methyltin) and must be separated before individual detection [Craig, 1986, Craig, 2003]. Very sensitive methods are required for this.

The need for improved control of environmental contamination has led to the development of hyphenated techniques. These methods are based on successive steps which may vary from one procedure to another. The hyphenated techniques combine separation and detection. An example is gas chromatography combined with inductively coupled plasma mass spectrometry (GC-ICP-MS).

In this study, organometallic compounds species were analysed using gas chromatography equipped with different detectors such as Flame Ionisation detector (FID), Mass Spectrometry (MS) and GC coupled with Inductively Coupled Plasma Mass Spectrometry (GC-ICP-MS).

This section summarizes some results published recently, using GC-MS, GC-FID and GC-ICP-MS (table 2.1).

Table 2.1: Analytical methods using GC-MS and GC-FID

Species/matrix	***Sample preparation***	***Derivatisation***	***LOD (ng L^{-1})***	***Reference***
TBT, DBT, MBT, TPT (water)	SLMPE and SPE / GC-FID	$NaBEt_4$		Cukrowska et al, 2004
Organolead/ urine	SPME GC-MS	$NaBEt_4$	7	Dunemann et al.1999
Organolead	LLE hexane/GC-MS	$NaBEt_4$	2.5	Zufiaurre et al.1997
Organolead	SPME GC-MS	$NaBEt_4$	83–130	Yu and Pawliszyn, (2000)
Organolead	SPE GC-MS	PrMgCl	1–4	Baena et aL2000
MeHg, EtHg; standard aqueous solutions (2)	GC-MS	Phenylation (NaBPh4)	0.03 pg EtHg as Hg	Cai et al. 2000
TML.Del,DML,DEL / water	NaDDA/SPE GC-MS	$NaBPr_4$	6-12	Baena et al, 2001
MBT, DBT, TBT	Methanol, Acetic acid, Ultrasound (30W) GC-MS	$NaBEt_4$		Centieno et al,2004
MBT, DBT, TBT/water	LLE (Hexane) GC-MS	$NaBEt_4$	0.18 – 0.25	Centieno et al, 2005

Table 2.2: Analytical methods using GC-ICP-MS

Species / matrix	*Sample preparation*	*Derivatisation*	*LOD (ng L^{-1})*	*Reference*
organolead	SPME	NaBEt4	0.1	Moens et al. 1997
organolead	LLE (hexane)	PrMgCl	0.05–0.08	Heisterkamp et al.1997
organotin			0.052–0.17	De Smaele et al.1996
organotin			0.050	Prange A andJantzen E (1995)
organotin			0.05–0.08	Aguerre et Al.2001
IHg,MMHg	MAE (TMAH)	$NaEt_4B$	0,15 pg (Hg)	Wasik et al. 1998
MBT,DBT,TBT, MPhT,DPhT,TPh / biologic tissue	MAE (TMAH)	$NaEt_4B$	0,05 pg	Pereiro et al. 1999
MMHg, IHg, MBT, DBT, TBT, Sediment	acide acétique (Ultrasons)	$NaPr_4B$	52-170 fg (Sn) 210 fg (Hg	De Smaele et al. 1998
MMHg, MBT, DBT, TBT/water	SPME	$NaEt_4B$	0,6 ng/l (Hg) 0,3-2 ng/l (Sn	Moens et al. 1997
MBT, DBT, TBT /Sediment	MAE (Acetic acid-Methanol) LLE (Hexane)	$NaEt_4B$	0.03 – 0.09 ng g-1	Rodriguez-Gonzalez, 2005(a)
MBT, DBT, TBT /Rat	MAE (Acetic acid-Methanol) LLE (Hexane)	$NaEt_4B$		Rodriguez-Gonzalez, 2005(b)
MBT, DBT, TBT /Sea water	MAE (Acetic acid-Methanol) LLE (Hexane)	$NaEt_4B$		Garcia Alonso, 2002
MBT, DBT, TBT /Sea water	MAE (Acetic acid-Methanol) LLE (Hexane)	$NaEt_4B$	0.04 – 0.09 ng l^{-1}	Rodriguez-Gonzalez, 2002
MBT, DBT, TBT /PACS-2 CRM	MAE (Acetic acid-Methanol) ASE (Accelerated solvent extraction)	$NaEt_4B$		Ruiz Encinar, 2002 (a)
MBT, DBT, TBT/ Sediment	MAE (Acetic acid-Methanol) Ultrasonic LLE (Hexane)	$NaEt_4B$		Ruiz Encinar, 2002 (b)
MBT, DBT, TBT /PACS-2 CRM	MAE (Acetic acid-Methanol) Ultrasonic LLE (Hexane)	$NaEt_4B$		Rodriguez-Gonzalez, 2003
MBT, DBT, TBT/ In vitro gastrointestinal	MAE (Acetic acid-Methanol) LLE (Hexane)	$NaEt_4B$		Rodriguez-Gonzalez, 2003 (c)

Table 2.2 continued

Species / matrix	*Sample preparation*	*Derivatisation*	*LOD (ng L^{-1})*	*Reference*
MBT, DBT, TBT, MMHg/ Oysters	MAE (TMAH) LLE (Isooctane)	$NaEt_4B$	1.3 -2.8 % (RSD)	Monperrus, 2003
MBT, DBT, TBT, IHg, MMHg/ Water, snow	LLE (Isooctane)	$NaEt_4B$, $NaPr_4B$	10 – 60 pg L^{-1}	Monperrus, 2005 (a)
MMHg/ CRM 580, IAEA 356, IAEA 405, sediment	MAE (HNO_3) LLE (Isooctane,CH_2Cl_2)	$NaEt_4B$, $NaPr_4B$, BuMgCl		Martin-Doimeadios, 2003
TBT, MMHg/ Microbenthic	MAE (TMAH) LLE (Isooctane)	$NaEt_4B$	12 – 250 pg g^{-1}	Monperrus, 2005 (b)
TBT/ Sediment, biological sample	MAE (Acetic acid-Methanol) LLE (Isooctane)	$NaEt_4B$	0.3 – 5.1 % (RSD)	Monperrus, 2003
Volatile organotin/ sediment, water, air	Cryogenic trap			Amouroux, 2000
Volatile organotin/ sediment, water, air	Cryogenic trap			Tessier, 2002
IHg, MMHg/ Sediment	MAE (HNO_3)	$NaEt_4B$		Rodriguez, 2004
Elemental Hg/ Air, water	Cryogenic trap			Amouroux, 1999

Gas chromatography is a major analytical tool for speciation of organometals. The ability to combine gas chromatography with advanced detectors such as ICP-MS and others has resulted in wide range of applications. But still, it has some drawbacks such as the tedious sample preparation by derivatisation. This has shifted the attention of scientists from gas chromatography to other analytical techniques such as HPLC and capillary electrophoresis (CE).

2.3. Other separation and detection methods which can be used

Different methods have been applied in order to minimise the number of step during organometallic analysis by gas chromatography. High performance liquid chromatography and electrophoresis methods are mostly used now.

2.3.1. High Performance Liquid Chromatography (HPLC)

One of the advantages of the HPLC as a tool of organometallic speciation is the low cost of analysis, while GC uses high amount of gas as carrier, HPLC uses aqueous buffer as the mobile phase. Another advantage is that HPLC does not need the derivatisation step as it can perform separation of nonvolatile species [Ali and Aboul-Enein, 2006, Fairman and Wahlen, 2001].

HPLC method has few drawbacks in metal ion speciation. The separation power of organometals species is not high hence some metal ions species cannot be speciated satisfactorily using this method. The other disadvantage is the coupling with element-specific detectors due to the introduction of a liquid sample to the detector [Ali and Aboul-Enein, 2006].

2.3.2. Capillary Electrophoresis (CE)

Though GC and HPLC method are accurate for organometallic compounds determination, there is still a great need for speciation techniques that are fast and inexpensive. This need is fulfilled to some extend by capillary electrophoresis (CE).

CE is an automated analytical technique that separates species by applying voltage across buffer filled capillaries. It is generally used for separating ions, which move at different speeds when the voltage is applied depending on their size and charge. The solutes are seen as peaks as they pass through the detector and the area of

each peak is proportional to their concentration, which allows quantitative determinations. Analysis includes purity determination, assays, and trace level determinations [Harris, 1998].

With the introduction of capillary zone electrophoresis (CZE), also called free-solution CE), as instrumental technique for speciation of organometallic compounds, a number of steps in gas chromatographic methods will be avoided. In CZE separation, the difference in ionic mobilities has a decisive effect on the resolution. The principle mechanism of separation depends on whether the analytes are charged or not. There are aqueous electrophoretic and micellar phases. For charged analytes, both electrophoretic migration in the aqueous electrolyte and solubilisation into micelles phase play a role. The separation of electrically neutral compounds, on the other hand, is dominated by distribution between the aqueous electrophoretic and micellar phases only. Accordingly, the analyte's hydrophobicity governs the distribution ratio and determines its migration behaviour [Dabek-Zlotorzynska, 1998]. Thus the derivatisation step in gas chromatography could be avoided.

A capillary electrophoresis (CE) method was used by Liu et al (1997) for simultaneous speciation of lead and mercury species. They reported an effective CE with a limit of detection equal 40, 80, 90, 100 ng ml^{-1} successively for triethyllead chloride, trimethyllead chloride and phenylmercury acetate, diphenyllead dichloride respectively [Liu. et al., 1997]. Keiichi et al. (2000) had

used that method for determination of tributyltin and triphenyltin cations, the limit of detection consecutively was 0.46 and 0.57 µg/ml respectively.

Ali and Aboul-Enein (2006), reported certain advantage and disadvantages of CE compared to chromatographic method in their review on analytical methods. The advantages include simplicity, low cost of running, high speed of analysis, unique selectivity and high degree of matrix independence. Among the disadvantage of CE is the reproducibility which is still not good and the coupling of CE to the element-specific detectors is very difficult to achieve due to the very low flow rate of the buffer in CE. CE is also not useful for neutral ion species.

CE methods still need much improvement to be able to compete with the chromatographic methods in organometallic compounds speciation. A number of modifications are still under study and include the development of fluorescence and radioactive complexing agents as their detectors are more sensitive and reproducible with low detection limit in these conditions [Crouch, personal communication].

2.4. Sources of errors

The discussion of sources of errors will mainly focus on hyphenated techniques in speciation analysis. These methods involve several analytical steps such as extraction derivatisation procedures, separation steps (GC or HPLC) followed by detection of the compound or the element by a wide variety of methods (AAS,

ICP-MS). Quevauviller (1996) in his review on improvements of quality control of speciation analysis reported sources of errors when using hyphenated techniques.

Each method has its own source of error. For instance errors may occur during derivatisation step from incomplete derivatisation in gas chromatography which is not the case in high-performance liquid chromatography (HPLC). The latter technique, however, may have errors such as incomplete separation which is not encountered, or is encountered to a lesser extent, in the former technique [Ali and Aboul-Enein, 2006; Maier et al, 1994, Quevauviller 1996].

2.4.1. Errors from extraction

The extraction method depends on the method which will be used for separation and finally the detection of the analyte. Extraction is also dictated by the sample analysed; liquid, sediments or soil. A wide variety of acid extraction procedures have been used for sediments, soil and biota analyses particularly when AAS, ICP-MS and MIP-AED are used as the final determination step. These involved the use of acids such as hydrochloric acid, acetic acid, hydrochloric-acetic acid mixture, nitric acid for organotin and organomercury compounds [Astruc,1992; Ritsema, 1992; Stoichev, 2004; Tseng',1999]. Organic solvent such as dichloromethane, chloroform, toluene, hexane and isooctane are used in GC and HPLC [Rubio, 1992; Larsen et al.1992; Tolosa et al., 1992**;** Monperrus, 2003a; Rodríguez Martín-Doimeadios, 2002b].

Reliable results from the extraction are given by calculating percentage recoveries. This can be done by spiking a sample of similar composition as the sample analysed with a known content of the analyte being analysed, leaving it to equilibrate and determining the concentration of the analyte after extraction. The spike is not always bound in the same way as the naturally occurring compounds. An alternative method for recovery would be to perform the experiment on a previously extracted real sample by spiking, equilibration and extraction and this is only if the extraction procedure does not change the matrix composition and appearance [Quevauviller, 1996]. However, recovery assessments can often be overestimated. The analysis of certified reference material may be a good tool to ascertain accuracy; with a limitation of being only useful in cases where they contain integral, and not spiked, species. All experiments for extraction efficiency might be conducted in duplicate extraction over the same sample. Extraction recoveries may vary from one chemical species of the same metal to another and they should, consequently, be assessed independently for each compound [Donard, 1992, Quevauviller, 1996].

For a thorough evaluation of extraction methods, materials with an integral analyte (bound to the matrix in the same way as the unknown) which is preferably labeled (radioactive labeling would allow for verification of recovery) would be necessary [Rodríguez Martín-Doimeadios, 2003, 2004; Centineo, 2004, 2006].

The ideal should be a direct analysis of sample without extraction but this is not possible in many cases such as in solid samples. The very low concentration of organotin, organolead and organomercury compounds in environment enforce extraction and preconcentration prior to the analysis.

2.4.2. Errors during derivatisation

The main reactions employed in derivatisation of organometals of tin, lead and mercury currently are mostly alkylation (ethylation, propylation, butylation or pentylation).

At present, derivatisation reactions are far from being well controlled. Despite of the many publications, the reaction mechanisms of derivatisation are not well understood. In general, the risk of producing a wrong result increases with the number of steps and with their complexity in a determination. Therefore, if derivatisation can be avoided it is worthwhile to consider such possibility [Quevauviller, 1996, Emteborg et al., 1999].

The major problems which can be countered during the derivatisation step are often matrix dependent. The increased number of analytical steps prior to and after derivatisation (such as extraction, preconcentration and clean-up) increases the overall uncertainty. The stability of some derivatives is poor and may be affected by uncontrollable factors, such as initial sample composition [Quevauviller, 1996].

2.4.3. Errors during separation procedure

Gas chromatography, using capillary or packed columns and liquid chromatography are the main separation methods used so far. The cold trapping (U-tube filled with chromatographic material) can be used in the case of gas chromatography. Note that the separation method has to be non destructive of the chemical form of the analyte. GC has become a powerful tool in the determination of traces of organic compounds. Whereas for most environmental applications packed columns are abandoned for the determination of traces of organic compounds because of poor separation and time-consuming procedures. They are still widely used for speciation, for example, for methyl-mercury determinations as well as for butyltins (packed GC in a U-tube).

In gas chromatography the separation is done by changing temperature, injection, controlling oven and detector temperatures and precautions should be taken to preserve the compound integrity in the column. For example, a heat-induced decay may lead to deposition of Sn oxides in the capillary column which in turn may cause peak tailing during Sn speciation. Cold trapping has been used successfully for the determination of for example alkyltin [Cukrowska et al., 2004], Pb compounds [Harrison, 1988] and mercury and alkylmercury [Tseng et al 1999, Stoichev et al., 2004]. The technique presents the advantage of concentrating the species and sequentially separating them according to their specific volatility.

One drawback of this method is that only volatile forms of elements (hydrides, alkylated forms) may be separated; other molecules of low volatility, such as triphenyltin, cannot be separated. In addition, cold trapping requires derivatisation steps that are difficult to validate and it is still unclear which physical and chemical parameters may hamper, for a given matrix, the formation and separation of volatile forms. Although the technique is not always applicable, its simplicity and the fact that it can operate on-line with derivatisation steps makes it a commendable method for a variety of compounds [Quevauviller, 1996].

In liquid chromatography systems such as HPLC, stationary phases; ion-exchangers and ion-pairing; are less commonly used than in GC. The stability of silica based ion-exchange columns is still a problem. Consequently, separation problems may still exist for some species. However, this method is better suited for element specific detections such as ICP-AES or ICP-MS, AAS, XRF and electrochemical detectors [Quevauviller, 1996].

The quantification of the analytes are generally based on peak area or the peak height, therefore the peaks have to be well resolved. In environmental samples many unwanted compounds can be co-extracted and may interfere with the analyte, thus well resolved peaks are needed. Today, there are new developments in GC, using hyphenated GC-GC. These new techniques resolve the concern of interferences; it uses successive separations that ensures the identity of the peaks being integrated.

2.4.4. Errors of detection

The choice of detector in speciation analysis depends strongly on the chemical forms to be determined and on the mode of separation used.

ICP-AES or ICP-MS can be used on-line after HPLC or GC separation, using a proper interface. MS can be specific in certain cases and even would allow an on-line quality assurance in the isotope dilution mode. Classical detectors such as FPD or FID can be applied after GC separation [Quevauviller, 1996]. The elemental specific detectors (ICP-MS) have fewer errors than the universal detectors (FID). The drift of the signal during analysis is the main error in the elemental specific detector, whereas in the universal detectors, the elution time of the analyte is the main source of error.

Apart from extraction, derivatisation, separation and detection errors, other sources of errors should also be taken into consideration. Quevauviller et al (2000) in their study of speciation of lead in urban dust found that there were losses related to filtration step, taking back the trimethyllead in water, leading to possible instability and / or adsorption on the filter.

An independent method should be used to verify results of routine analysis. Inter-laboratory studies are therefore valuable tools to evaluate analytical techniques in particular. A good procedure to evaluate the performance of hyphenated techniques is to separately examine the different steps and evaluate the sources of

error which may arise [Ali and Aboul-Enein, 2006; Maier et al., 1994, Quevauviller, 1996]. The inter-laboratory studies exclude the systematic analytical errors which may occur in one laboratory.

CHAPTER 3

RESEARCH OBJECTIVES

The fundamental aim of this research was to develop the methods for speciation of organometallic compounds of tin, lead and mercury in different environmental samples such as water, sediment, soil and plants.

By looking at literature review, little has been done on speciation of organometallic compounds of tin, lead and mercury in South Africa. To add to this poor literature and to the current analytical methods used worldwide the following specific objectives were addressed:

- to make quantitative assessment of organometallic compounds of tin, lead and mercury in different environmental matrix in South African environment,
- to develop and optimise an affordable derivatisation method using a new derivatisation agent (bromomagnesium tetraethylborate),
- to develop and optimize analytical methods for extraction and determination of organometallic compounds,
- to assess the stability of organotin and organolead compounds in environment by studying the degradation and adsorption / desorption processes.

- ❖ to compare different analytical techniques for organometallic compounds speciation.

CHAPTER 4

SYNTHESIS AND DERIVATISATION APPLICATION OF Et_4BMgBr

It was mentioned in the chapter 2, that gas chromatography is the best separation method used prior to detection of organometallic speciation. Therefore, to make the non volatile ionic organometallics of tin, lead and mercury amenable to GC, they have to be converted into volatile, thermally stable compounds.

Derivatisation by alkylation with Grignard reagents or by hydride generation methods have a number of drawbacks when compared to alkylation using a boron reagent, as discussed in chapter 2. Alkylation by boron reagents has the advantage of allowing *in situ* analysis.

These boron derivatisation agents are all organoboron that is they have a boron-carbon bond. The pioneer of organoboron chemistry was H.C. Brown, who initiated their study in the 1930's. He discovered the hydroboration reaction in 1959, and was awarded the Nobel Prize in 1979 for his work on boron [Omae, 1999]. Hydroboration is a process of producing organoboranes by the addition of a compound with a B-H bond to an unsaturated hydrocarbon, for example, the reaction of BH_3 ion with a carbonyl compound such as CH_2=CHR.

During the 1960's much effort, almost entirely on the part of H.C. Brown research group, went into the exploration of the scope of the hydroboration reaction for the

synthesis of organoborons. In the 1970's as a number of research groups joined Brown's group in exploring the reactions on organoborons, many synthetically useful applications were discovered.

Three organoboron compounds have found application for tin, lead and mercury derivatisation. These are sodium tetraethylborate ($NaEt_4B$), sodium tetrapropylborate ($NaPr_4B$) and bromomagnesium tetrapropylborate. Rapsomanikis et al. (1986) were the first to introduce the derivatisation using organoboron for organolead speciation and Ashby and Craig, (1989) were the first to use it for butyltin speciation in environmental samples. They used $NaBEt_4$ for ethylation in sediment. De Smaele et al. (1998) used sodium tetrapropylborate ($NaPr_4B$) for simultaneous speciation of organotin, organolead and organomercury compounds in water. Recently Bergmann and Neidhart, (2001) reported an *in situ* propylation method for organometallic compounds in water using bromomagnesium tetrapropylborate ($[MgBr][B(C_3H_7)_4].4THF$).

The high cost of commercial available $NaEt_4B$ (290 $ per 5g, Sigma-Aldrich, 2006) inspired the synthesis of bromomagnesium tetraethylborate (Et_4BMgBr) used in this thesis. 100 g Et_4BMgBr obtained after synthesis would be estimated at 150 $.

4.1. Synthesis of Et_4BMgBr

Direct preparation of triorganoboranes are usually made by a reaction between organic halide, boron trifluoride etherate and a metal (Li, Na, Mg…) in diethyl ether. An example is shown in equation 4.1.

$$3BuBr + 3Mg + BF_3.OEt_2 \xrightarrow{Et_2O} Bu_3B + 3MgFBr \quad (4.1)$$

Brown and Racherla (1986)b, while preparing the triorganylboranes observed unexpected results. By checking the effect of different solvent, the reaction in tetrahydrofuran (THF) did not conduct to expected product. Instead of the predicted formation of tributyl borane (Bu_3B) (equation 4.1), the addition of bromo butane (BuBr) to a mixture of Mg turnings and boron trifluoride etherate ($BF_3.OEt_2$) led to the formation of the bromomagnesium tetrabutylborate $Bu_4BMgBr.nTHF$ complex (equation 4.2). This complex was observed at -17.7 δ in ^{11}B NMR and at 16.5 δ [Brown and Rachelia, 1986a]. Only this complex and unreacted $BF_3.OEt_2$ were observed by NMR.

$$4BuBr + 4Mg + BF_3.OEt_2 \xrightarrow{THF} Bu_4BMgBr.nTHF + 3MgFBr.nTHF \quad (4.2)$$

By using bromo ethane in place of bromo butane, they encountered the same results.

$$4EtBr + 4Mg + BF_3.OEt_2 \xrightarrow{THF} Et_4BMgBr.nTHF + 3MgFBr.nTHF \quad (4.3)$$

The work described in this thesis, bromomagnesium tetraethylborate (Et_4BMgBr) was synthesised by a modified method of that developed by Brown and Racherla.

4.1.1. Chemicals

The chemicals used were all obtained commercially, magnesium turnings (Mg), bromoethane (EtBr), boron trifluoride etherate ($BF_3.OEt_2$) and tetrahydrofuran (THF) which was freshly distilled from sodium wire, under argon.

4.1.2. Experimental

The apparatus include 2 or 3 necked flask round-bottomed, reflux condenser (double walled), ice bath, fume hood. All apparatus were dried by heating slowly with a flame, whilst dry passing the inert gases N_2 or Ar through the apparatus.

Magnesium turnings (9.73 g, 400mmol) were placed in a three necked flask equipped with a reflux condenser in the fume hood. Prior to the addition of other reactants, the flask containing Mg was heated over a flame and cooled under argon. 100 ml of THF was added followed by 12.3 ml of $BF_3.OEt_2$ and crystal of iodine. The reaction was initiated by the addition of 5 ml of bromoethane. A vigorous exothermic reaction started. The ice bath was used to cool down the reaction. Further 15ml of bromoethane were added to 55 ml of THF then added dropwise for about 30 to 45 minutes to maintain a gentle reflux. The reaction was allowed to continue for 1 to 1.5 hours with continuous stirring.

A white crystalline solid was formed at the end of reaction. Solid product was recrystallised from THF to give after filtration 147.5 g. It was then heated gently for about 20 minutes under argon to realise exces of THF.

4.1.3. Identification

The product obtained was found to be deliquescent; and was stored under inert gas (Ar). It was stable in water, when dissolved in water no ethane evolved. These observations were consistent with other metal tetraethylborate compound studies [Honeycutt and Riddle, 1960].

Possible contaminents were Et_3B and MgFBr. For the purification of our product, we performed solubility test. Knowing that triethylboron dissolve in ether and $Et_4BMgBr.nTHF$ does not dissolve in ether [Brown and Racherla, 1986a]. The product was treated by ether; we found that there is a little loss of mass (3 g), which suggests there was little Et_3B contaminating the product or dissociation of our product (equation 4.5). The loss might also due to the dissolution of residual THF in ether (equation 4.6).

$$Et_4BMgBr.4THF \xrightarrow{Et_2O} Et_3B + EtMgBr \qquad (4.4)$$

$$Et_4BMgBr.4THF \xrightarrow{Et_2O} Et_4BMgBr \qquad (4.5)$$

To make sure that we reduced the quantity of magnesium dihalide we increased the volume of THF used during synthesis. We intended that the $Et_4BMgBr.nTHF$

remain in the viscous layer of THF which was filtered from white solid believed to be MgFBr (1.2 g) (Table 4.2). [Brown and Racherla, 1986a]. Any of EtMgBr produced was tested by addition of water. There was not ethane evolution.

During derivatisation study by Sunil et al (2004) on phenyltin using sodium tetraethylborate in THF they found no interferences by residual THF and the same conclusion were reached by D.R. Parkinson et al. (2004). Any of THF that may remain in the product would not interfere in derivatisation reaction.

After obtaining a product believed to be pure, we did other confirmation test. The boron NMR results gives us a shift of 16.973 ppm. The experimental determined shift of tetraethylboron are between 16.6 and 17.7 [Brown and Rahcerla, 1986]. The Boron NMR results are in Appendix 4, including the ^{1}H NMR and ^{13}C NMR results.

Microanalysis for the product were also performed. B and Mg were analysed by ICP-OES, Fluoride and Bromide by IC and C and H by CHNS. Table 4.1 shows the concentrations obtained.

The preparation of the analysis solution was done by dissolving 0.5g in 100ml of water.

Table 4.1: Elemental analysis of Et_4BMgBr

Composition (%)	Mg	B	Br	C	H	F
Theoretical	10.38	4.76	34.67	41.56	8.66	0
Determined	10.09	4.41	34.17	41.33	8.49	0

Assuming that the product does not contain residual THF, Et_4BMgBr can be written as $C_8H_{20}BMgBr$. By using the value of the composition determined to find the chemical formula of the product ($C_xH_yB_zMg_wBr_v$.), $C_{8.4}H_{20.7}BMgBr$ was found. This formula is in good agreement with the theoretical. Slight difference found might be due to the residual THF.

Microanalysis of the solid mentioned early, believed to be $Mg_xF_yBr_z$ was in good agreement with the theoretical formula. Using the value of the composition determined in table 4.2, MgFBr was found to be the formula.

Table 4.2 : Elemental analysis of MgFBr

Composition (%)	*Mg*	*F*	*Br*
Theoretical	*19.71*	*15.41*	*64.88*
Determined	*19.21*	*15.09*	*64.37*

4.2. Derivatisation application

This section presents the organotin and organolead results obtained using GC-MS and the organotin and organomercury results using GC-ICP-MS.

After conducting tests on standards, the speciation of organotin in water environmental samples by comparing the ethylation using sodium tetraethylborate and the novel ethylation by bromomagnesium tetraethylborate was undertaken.

4.2.1. Derivatisation test

The derivatisation tests were undertaken on organotin, organolead and organomercury. Environmental samples were used to expose the derivatisation agent to realistic environmental matrix interferences. These are screening tests; a detailed application on organotin compounds was done (section 4.2.2).

Organotin standards (Ph_3SnCl, Bu_3SnCl, Bu_2SnCl_2 and $BuSnCl_3$) were obtained from LGC Limited (Teddington, U.K.) or from Sigma–Aldrich (Steinheim, Germany). Organolead compounds standards ($PbCl_2$ and $(CH_3)_3PbCl$) were purchased from Sigma–Aldrich (Steinheim, Germany). Mercury compounds ($HgCl_2$ and CH_3HgCl) were from Strem Chemicals. Sodium acetate–acetic acid buffer, hydrochloride acid, ammonia and isooctane were from Sigma–Aldrich (Steinheim, Germany).

4.2.1.1. GC-ICP-MS analysis

Sample preparation

An environmental sediment sample from Klipriver (South Africa) was used. The sampling area is described in section 6.5. The sediment sample was extracted by microwave extraction. The extraction procedure will be discussed in chapter 6.

This sample was analysed stepwise for butyltin and mercury compounds screening using Et_4BMgBr as a derivatisation agent. It was found to contain detectible amount of butyltin and very low concentration of mercury compound thus we spiked the sample with 0.1 ng L^{-1} of CH_3HgCl and 10 ng L^{-1} of $HgCl_2$. These concentrations were chosen due to their usual environmental concentrations. In environment monomethylmercury as a results of inorganic mercury methylation, is found at lower concentrations than inorganic mercury.

Extraction

Liquid-liquid extraction was done by adding 500 µL of isooctane to 25 mL of water sample solution buffered with sodium acetate / acetic acid at different pH (4, 5 or 6); followed by the addition of an appropriate amount of the derivatisation agent solution depending on the experiment. The mixture was manually shaken for 5 minutes then allowed to equilibrate for about 5 minutes. The organic phase was then collected in vials for GC-ICP-MS analysis

pH optimisation

The ethylation process was investigated by several authors and found to be ranging between pH 4 and 6 [Mathiasson and Ndungu, 2000, Grinberg, 2003]. This range was chosen for pH optimisation. The figure (4.1) shows the chromatogram for pH screening test for mercury compounds.

For mercury compounds, at pH 4 and pH 6 they were no peaks observed. At pH 5, the first peak at around 100 seconds is the peak for the CH_3HgX and the second peak at around 135 seconds is the peak for HgX_2.

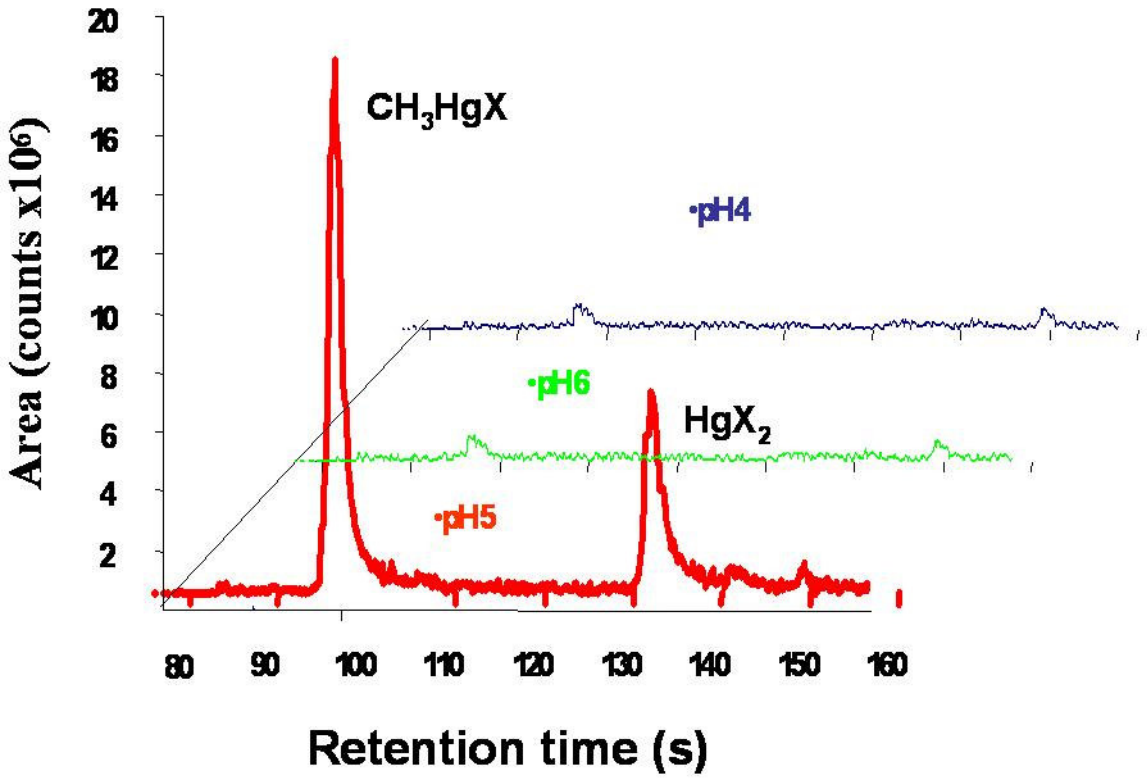


Figure 4.1: Optimisation of pH for mercury compounds

The peak corresponding to the CH_3HgX is a response for EtMeHg obtained by derivatisation with Et_4BMgBr (equation 4.8), while the peak corresponding to HgX_2 is a response for Et_2Hg (equation 4.9). The eluting peak were identified by standard analysis of individual analyte (CH_3HgCl and $HgCl_2$), the standard solution were prepared in deionised water. For environmental sediment sample, co-injections were done for retention time confirmation. Blanks samples were also analysed prior to analysis.

$$MeHgX + Et_4BMgBr \rightarrow EtMeHg + Et_3B + MgBrX \quad (4.6)$$

$$HgX_2 + Et_4BMgBr \rightarrow Et_2Hg + Et_3B + MgBrX \quad (4.7)$$

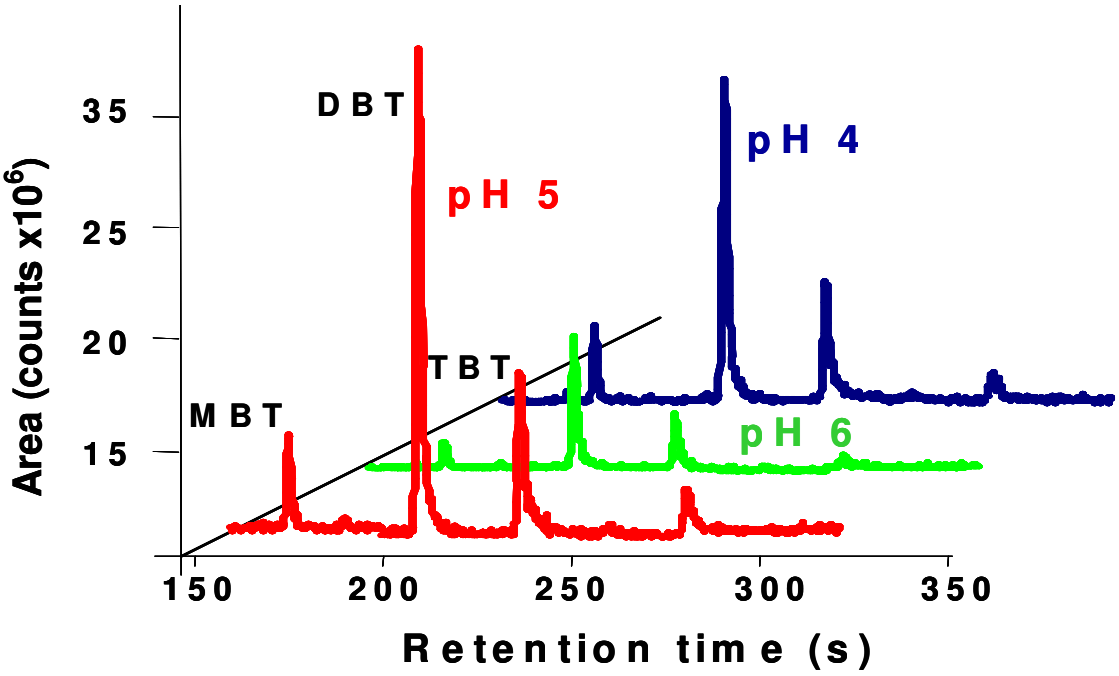


Figure 4.2 : Optimisation of pH for butyltin compounds (^{120}Sn)

For butyltin compounds, signals were adequate at all pH's, but much more considerable at pH 5 (figure 4.2). Thus, the pH 5 was chosen as the optimum pH for both organomercury and organotin compounds. The fourth peak found at a retention time of around 280 seconds corresponds to a non identified organotin compounds.

Volume optimisation

The volume to be used for derivatisation agent solution should be optimised. It depends strongly on the concentration of the analytes into the sample. The derivatisation agent should be added at excess quantity to make sure that all analytes were derivatised.

The minimum volume was determined by adding various volumes of the derivatisation solution to the sample. Following extraction and analysis as described before, the chromatogram was integrated. The volume at which, constant values were observed, was taken as the minimum volume for optimal recovery.

After screening test in the range of 200 µL and 800 µL, the optimum derivatisation agent volume was found to be 500 µL. This volume can't be generalised to all samples. Depending on the matrix the analyst should always optimised the derivatisation agent volume to be able to achieve a complete derivatisation.

While doing this experiment, an observation on derivatisation kinetic was made on a previously analysed sediment sample containing both CH_3HgX and HgX_2. This sample was from Arcachon basin in France. The derivatisation occurred first with organometallic (CH_3HgX) then inorganic mercury (HgX_2).

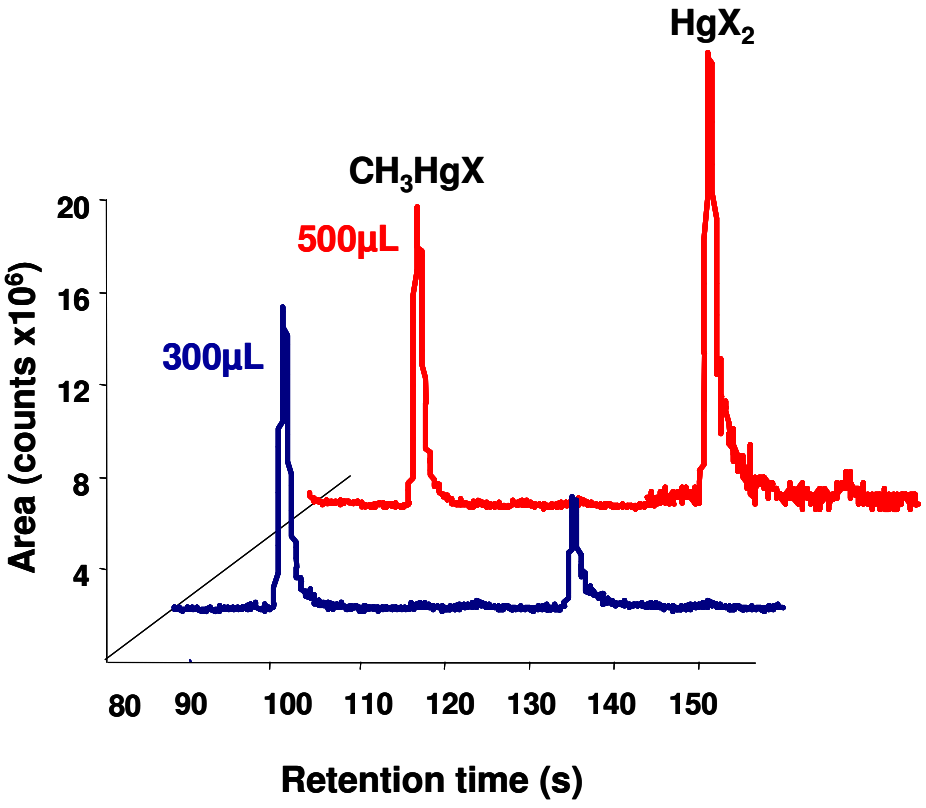


Figure 4.3: Derivatisation and species properties (for ^{202}Hg isotope)

The figure 4.3 shows the tendency of derivatisation in organic and inorganic mercury form.

When sub-optimal quantities of derivatisation agent were added, it appeared that the CH_3HgX was preferentially ethylated, as shown by the two chromatograms in figure 4.3. After the addition of 300 µL of Et_4BMgBr, the whole CH_3HgX was derivatised, this is shown by no increase in CH_3HgX peak area while the total inorganic mercury was only derivatised after 500 µL. The reason being is unclear. It is possible that this effect is due to faster reaction rate of the ethylation in case of CH_3HgX. It is also possible that the solubility and polarity of these mercury compounds play a role. This will suppose that there was not enough amount of derivatisation agent, after 300 µL some of inorganic mercury compounds were totally substituted, while another amount was still at non volatile form. Those non volatile forms could be HgX_2 or EtHgX.

4.2.1.2. GC-MS analysis

Sample preparation

For GC-MS analysis, an environmental water sample (rain water) was spiked with a concentration of 25 µg L^{-1} for Ph_3SnCl, Bu_3SnCl, Bu_2SnCl_2, $BuSnCl_3$, $PbCl_2$ and $(CH_3)_3PbCl$. A solution of derivatisation agent (Et_4BMgBr) was prepared as discussed previously.

Extraction

Liquid-liquid extraction was done by adding 500 µL of isooctane to 25 mL of water sample solution buffered with sodium acetate / acetic acid to pH 5 followed by the addition of 400 µL of the derivatisation agent solution. The mixture was

manually shaken for 5 minutes then allowed to equilibrate for about 5 minutes. The organic phase was then collected in vials for GC-MS analysis.

For this screening experiment all working conditions were fixed. In detailed application of Et_4BMgBr as a derivatisation agent for organotin speciation, pH and other parameters will be optimised. Ethylation derivatisation method is not usually chosen for organolead speciation due to the reasons discussed previously in chapter 2. As $(CH_3)_3PbCl$ was the only organolead spiked in the sample, no other interference was encountered.

Results

Organotin analysis gives consistent peaks as what was found in literature for ethylation derivatisation using $NaEt_4B$ [Banoub et al, 2004; Gallina et al, 2000; Ndungu and Mathiasson, 2000, Takeuchi et al. 2000]. The characteristic peaks were 120, 197 and 351 for triphenyltin, 121, 179, 235, 291 for tributyltin, 149, 207, 263, for dibutyltin and 149, 177, 235 for monobutyltin. The characteristic peaks for methyllead and inorganic lead compounds were also consistent to those found in literature [Zufiaure, 1997; Baena, 2001], for trimethyllead the characteristic peaks were at 207, 236, 266 m/z, for dimethyllead at 208, 223, 267 and at 208, 237 and 295 for tetraethyllead.

Figure 4.4 shows the chromatogram obtained for the speciation of organotin by GC-MS analyses. The example of a typical mass spectrogram of tributyltin is given on the figure.

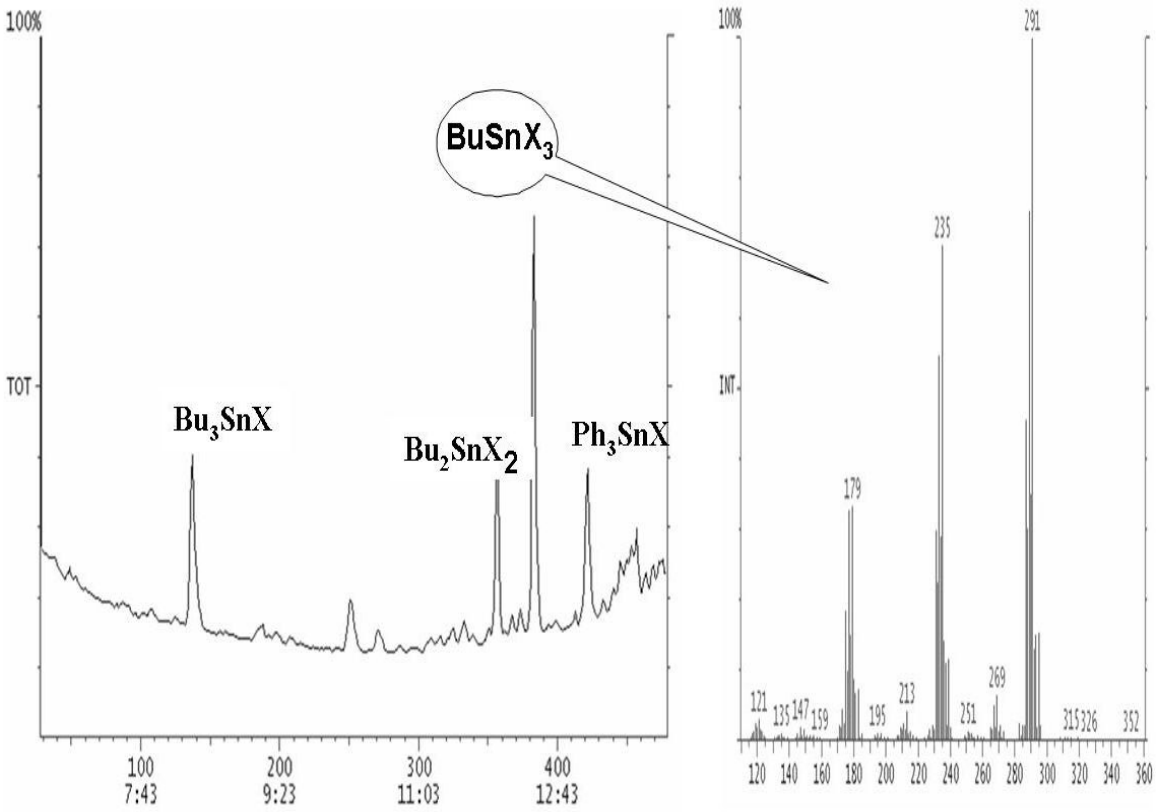


Figure 4.4: Organotin chromatogram by GC-MS analyses and Bu_3SnX mass spectrogram

Figure 4.5 gives the chromatogram obtained for ethylation derivatisation test of organolead with the example of the trimethyllead mass spectrogram.

The peak symbolised by Pb^{2+} stands for inorganic lead, but it is given by Et_4Pb obtained after derivatisation.

GC-MS analysis confirmed the possibility of using Et_4BMgBr for organotin and organolead derivatisation. A detailed study undertaken on organotin speciation in environmental samples using Et_4BMgBr for derivatisation step is discussed in the following section.

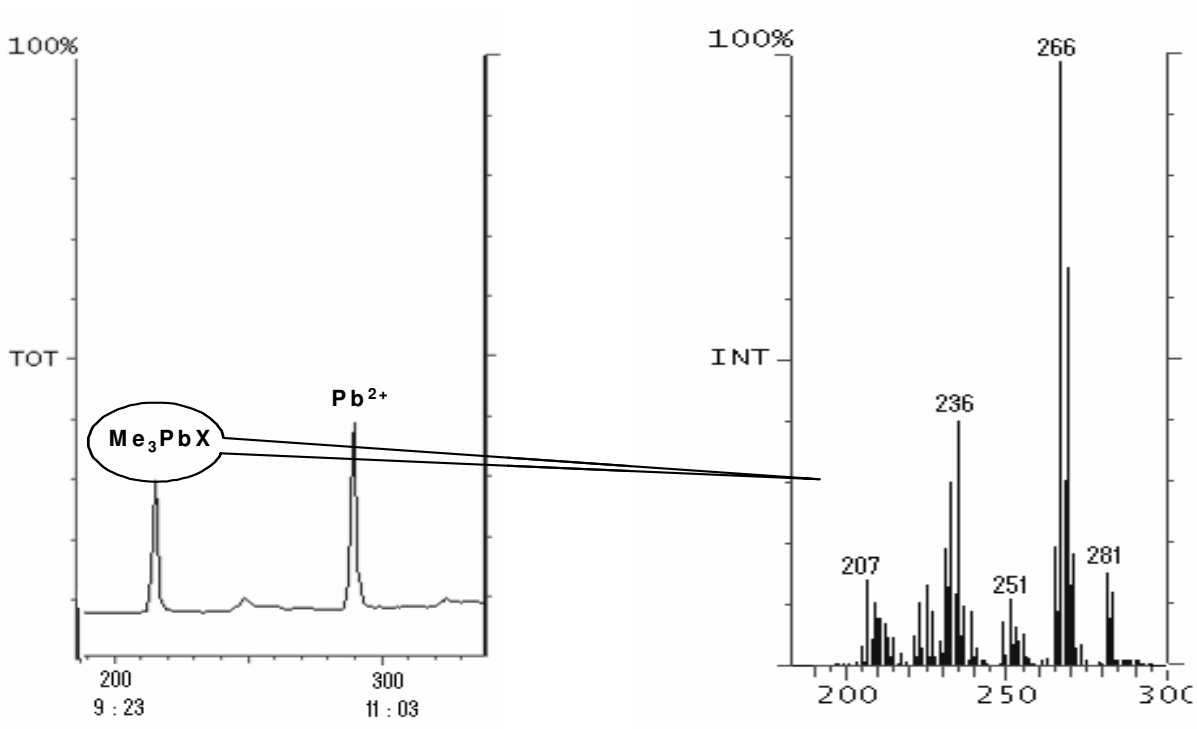


Figure 4.5: Lead species chromatogram and Me_3PbX mass spectrogram by GC- MS

4.2.2. Derivatisation comparison of $NaEt_4B$ and Et_4BMgBr: application to speciation of organotin in environmental water sample

The environmental water samples were collected from a polluted mining area in Johannesburg (South Africa). Field measurements including pH and redox potential were made. The samples consist of wetland water, stream water and

ground water in the vicinity of Fleurhof dam situated in the Central Rand of Johannesburg.

4.2.2.1. Optimisation of the method

Standard samples for the optimisation experiment of all organotin compounds (Bu_3SnCl, Bu_2SnCl_2, $BuSnCl_3$ and Ph_3SnCl) were prepared in deionised water.

Chemicals

Monobutyltin trichloride, dibutyltin dichloride, tributyltin chloride, and triphenyltin chloride (TPhT) were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium tetraethylborate (NaBEt4) was purchased in 1 g sealed ampoule from Merck (Darmstadt, Germany). Sodium acetate–acetic acid buffer and isooctane were from Sigma–Aldrich (Steinheim, Germany).

Experimental design

Extraction time, pH and stirring rate were found to be the main parameters which influence organotin extraction by supported liquid membrane probe. It was found that a probe depth of 2mm into the sample is the optimum [Cukrowska et al, 2004]. Extraction time, pH and stirring rate parameters were optimized in order to find the optimum working parameter. An experimental design was performed, using Umetric Modd 5.0. 27 experiments were each replicated 3 times. Three factors were investigated, time ranging from 15 to 60 minutes, pH ranging from 2 to 9 and stirring rate ranging from 3 to 10. (Table 4.3)

Table 4.3 : Experimental design for SLMPE of organotin

Experiment	PH	Time	Stirring speed
1	2	15	3
2	5.5	15	3
3	9	15	3
4	2	37.5	3
5	5.5	37.5	3
6	9	37.5	3
7	2	60	3
8	5.5	60	3
9	9	60	3
10	2	15	6.5
11	5.5	15	6.5
12	9	15	6.5
13	2	37.5	6.5
14	5.5	37.5	6.5
15	9	37.5	6.5
16	2	60	6.5
17	5.5	60	6.5
18	9	60	6.5
19	2	15	10
20	5.5	15	10
21	9	15	10
22	2	37.5	10
23	5.5	37.5	10
24	9	37.5	10
25	2	60	10
26	5.5	60	10
27	9	60	10

Figure 4.6 gives the experimental conditions and optimum parameters. Each corner of the cube diagram represents an experiment at various conditions.

The effect of pH on the derivatisation of organotin compounds has been investigated by several groups; it was found that the ethylation occurs between pH 4 and pH 6. A substantial reduction in recovery especially with monosubstituted species has been reported [Ndungu and Mathiasson, 2000]. It is evident on the figure 4.6 that pH 5.5 is the optimum pH. This is shown by the high (red color) and middle (yellow color) in the range of pH 5.5

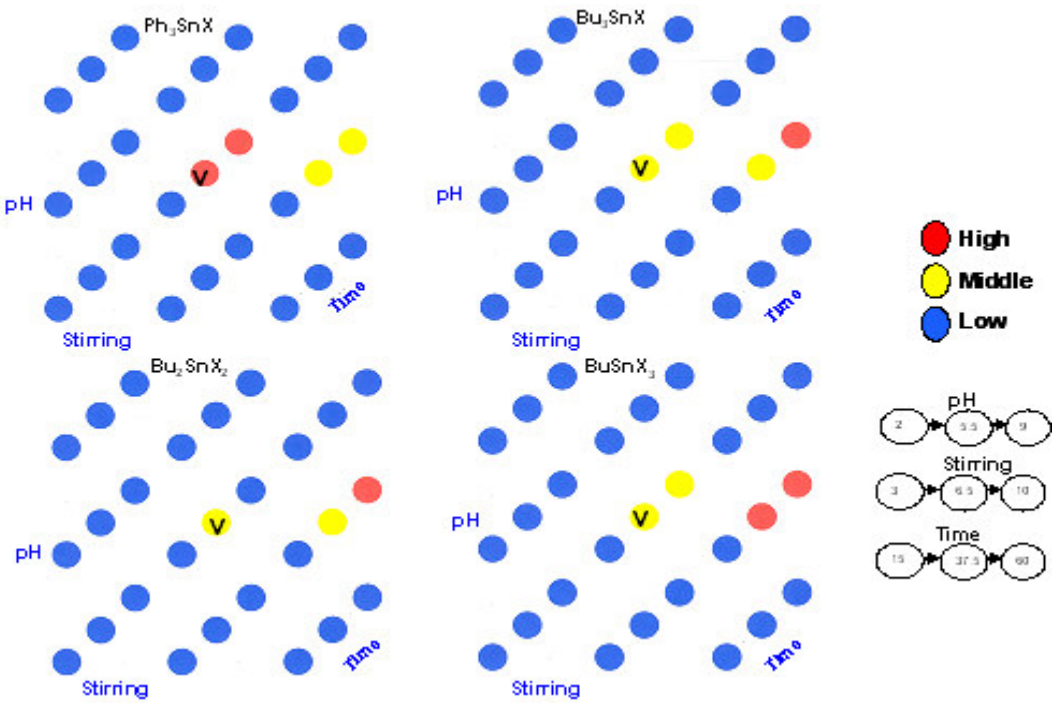


Figure 4.6: Optimisation of pH, time and stirring level versus area response

The effect of extraction time was also evaluated. The extraction time play a big role in determining the diffusion on the interface donor solution - membrane-acceptor solution. A longer extraction time enables greater degree of diffusion of the non-polar analyte from the donor to the acceptor phase through the hydrophobic membrane [Cukrowska et al, 2004]. Looking at the time (at pH 5.5), the optimum values are found in the range of 37.5 – 60 and 37.5 minutes were chosen. The reason being is that after 60 minutes some of the solvent were volatilized which may cause the loss of analyte. The other reason is saving time. As the supported liquid membrane is simple device this would allow the simultaneous extraction of many samples.

The importance of controlling the stirring rate may be explained in terms of the contact time between the analyte, the hydrophobic membrane and the pressure exerted on the membrane. An increase in the stirring rate enables more effective mixing of the donor solution. The result of the stirring is that fresh portions of the donor solution are allowed to come in contact with the organic acceptor. This enhances the extent to which diffusion can occur, hence increase the concentration of organotin compounds on the interface donor phase and membrane. The stirring level was set at 6.5 in this analysis even if there is good area response between 6.5 and 10 levels. The 6.5 level has been chosen due to bubbles observed when using the 10 level.

4.2.2.2. Method validation

The performance of an analytical method is tested by many parameters. A measurement should be linear in a certain range of concentration; this is confirmed by undertaking a calibration curve. The method should allow the determination of very low concentrations, (to allow good estimation of environmental relevant levels); this is given by the limit of detection (LOD) and limit of quantitation (LOQ). All these parameters constitute what called *figures of merit*.

Calibration curves

Reverse calibration method was used by preparing standard solutions containing varying concentrations of organotin compounds.

Solutions were prepared in the range of 10 to 40 µg L^{-1}, by combining varying concentrations for example one combination can be 10 µg L^{-1} of tributyltin, 20 µg L^{-1} of dibutyltin, 25 µg L^{-1} of monobutyltin and 10 µg L^{-1} of triphenyltin. Calibration curves for organotin compounds are shown in figure 4.7. It shows linearity in the range of 10 to 40 µg L^{-1}. The correlation coefficients are given in the following paragraph together with other figures of merit.

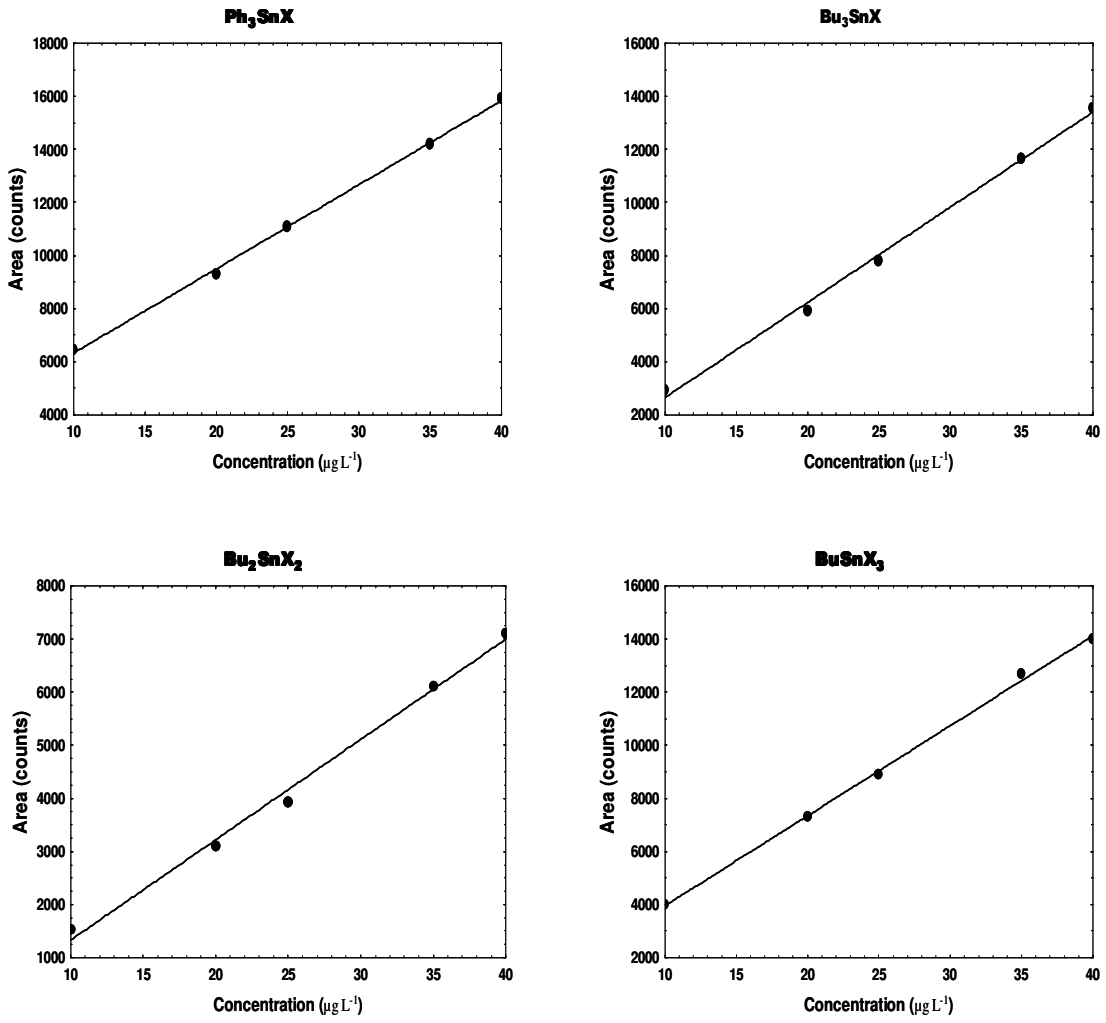


Figure 4.7: Organotin compounds calibration curves

Figures of merit

As it was mentioned in chapter 2, sodium tetraethylborate was proven to be efficient for the derivatisation of organometallic compounds. Validation of the derivatisation using Et_4BMgBr bromide was accomplished by comparison with commercial $NaEt_4B$. Table 4.4 gives the comparison of both derivatisation agents. The results are based on seven analyses.

Table 4.4: Figures of merit for Et_4BMgBr(1) and $NaEt_4B$ (2)

Parameters	Deriv.	Ph_3SnX	Bu_3SnX	Bu_2SnX_2	$BuSnX_3$
R^2	[1]	0,9747	0,9897	0,9845	0,9873
	[2]	0,9982	0,9974	0,9954	0,9977
Repeatability	[1]	3,79	6,76	19,96	9,08
	[2]	2,99	5,47	17,82	9,01
reproducibility	[1]	5,40	7,30	21,60	11,20
	[2]	4,90	6,10	19,70	10,80
LOD	[1]	4,30	3,90	2,80	3,10
	[2]	4,50	3,70	3,30	2,90
LOQ	[1]	14,30	13,00	9,30	10,30
	[2]	13,90	12,70	9,60	9,90

LOD: $\frac{3S}{mean}$, *LOQ:* $\frac{10S}{mean}$, *Reproducibility and Repeatability:* $\frac{SX100}{mean}$ *(for n = 7)*

Good correlation coefficient were obtained, they are close to one that shows good linearity of the method in the working range. The repeatability and reproducibility are also good, below 10 percent, only in the case of dibutyltin where both methods give high percentage. The dibutyltin is used in many materials, even if the blank samples didn't show any dibutyltin, this higher value might be from the unknown source of dibutyltin in the GC-MS system. As long as the working temperature is high, there is a possibility of volatilising dibutyltin if any is present in the system. If dibutyltin is the analyte of interest, the way to overcome this problem is the use of internal standard.

By looking at the concentration of organotin compounds found in environment (chapter one), this method with the lower values of LOD and LOQ offers the

possibility of analysis of organotin compounds using Et_4BMgBr as derivatisation agent.

Extraction efficiency

The extraction using supported liquid membrane probe extraction was also evaluated, previously designed. The extraction after derivatisation using $MgEt_4BBr$ and after derivatisation using $NaEt_4B$ was compared (Table 4.5).

Table 4.5: Extraction efficiency Et_4BMgBr(1) and $NaEt_4B$ (2)

Parameters	**Deriv.**	**Ph_3SnX**	**Bu_3SnX**	**Bu_2SnX_2**	**$BuSnX_3$**
Conc. Spiked (µg L^{-1})	[1]&[2]	30	30	30	30
Determined (µg L^{-1})	[1]	28,3 (9,8)	25,8 (8,8)	22,6 (7,2)	19,4 (6,7)
	[2]	28,3 (9,8)	26,1 (9,5)	27,3 (8,1)	17,1 (9,8)
Recovery %	[1]	94,3	86,0	75,3	64,7
	[2]	96,0	87,0	91,0	57,0
Coef. Efficiency %	[1]	7,5	6,9	6,0	5,1
	[2]	7,5	7,0	7,3	4,6

Coefficient efficiency % $= \frac{CaVa}{CdVd} x\ 100$

The accuracy of the method was evaluated by analyzing known concentrations of OT standards and determining individual percentage recoveries for each compound. The results are shown in the table below. The coefficient was calculated using the formula:

$$\text{Coefficient efficiency } \% = \frac{CaVa}{CdVd} \qquad (4.8)$$

4.2.2.3. Application to environmental samples

The development of any method should include the analysis of realistic environmental samples. In this study, the environmental water samples were analysed. The samples were collected in the Johannesburg town in South Africa. The samples include wetland water, stream water and groundwater. The field measurements were taken and after sample preparation the speciation of organotin compounds were done by GC-MS. Table 4.6 compiles the speciation results.

Table 4.6: Speciation of organotin compounds

Samples	pH	Conductivity (µs cm^{-1})	ORP vs SHE(mV)	Sn (µg L^{-1})	Ph_3SnX (µg L^{-1})	Bu_3SnX (µg L^{-1})	Bu_2SnX_2 (µg L^{-1})	$BuSnX_3$ (µg L^{-1})
W	3.2	290	724	0,389 (3,11)	6,7 (5,9)	22,3 (7,1)	38,7 (9,5)	25,1 (4,9)
S1	3.03	386	741	0,506 (3,15)	7,2 (6,1)	14,6 (4,6)	42,4 (4,6)	39,2 (5,5)
S2	3.21	267	741	0,322 (2,37)	5,9 (7,7)	10,5 (7,7)	33,7 (5,5)	19,6 (6,2)
G	3.86	212	573	0,108 (7,5)	ND	ND	ND	10,7 (6,3)

The pathway of these organotin can be proposed. Table 4.6 shows the high concentration of dibutyltin and monobutyl on expenses of tributyltin. This is due either on the degradation of tributyltin or the presence of these organotin compounds in the area studied, from the other use of material containing organotin other than antifouling paint. The second opinion a high supply of water for mining reasons thus the use of PVC material that may contain organotin as explained.

The underground water contains only a small amount of monobutyltin. This region is a wetland thus it contain high amount of organic matter. The organotin are bound to these organic matter which renders difficult the leaching to the ground water.

The acidic conditions and high redox potential has been found to be favorable for organotin compounds by Cukrowska et al, 2004. That why in all samples other done ground water, the organotin are found with significant concentration.

4.3 Conclusion

It can be concluded that bromomagnesium tetraethylborate can be used as a derivatisation agent in organotin, organolead and organomercury compounds. As in any other study the condition of the analysis should be always optimised taking in account the matrix properties. Thus the cost of the analysis can be cut down by using these boron magnesium compounds which are:

- Pr_4BMgBr used by Bergmann and Neidhart, (2001)
- and Et_4BMgBr used in this thesis.

CHAPTER 5

ANALYTICAL METHOD DEVELOPMENT FOR SPECIATION OF TIN, LEAD AND MERCURY COMPOUNDS

The speciation of organometallic compounds of tin, lead and mercury require several steps from sampling to detection. Depending on the study, several methods have been developed and validated.

5.1. Sampling

Development of sampling and analysis plans should be done meticulously. The contaminations that might occur during sampling process are difficult to monitor. The plan should include sample amounts, preservation procedures, field measurements, observations and historical data if available [Pawliszyn, 2002].

Historical data is essential in sampling. This gives information on the site and an insight into the behaviour of pollutants.

In this study, three basic approaches to sampling were used as described by Kegley and Andrews (1996) (figure 5.1).

These methods are defined by Barnard (1995). Judgemental sampling is a result of a bias of the sampler and usually occurs when samples are taken where the concentration of the pollutants is thought to be high (or low). This type of

sampling is not usually representative of the entire site but is useful in that it can point to worst or best case scenario of a pollutant source.

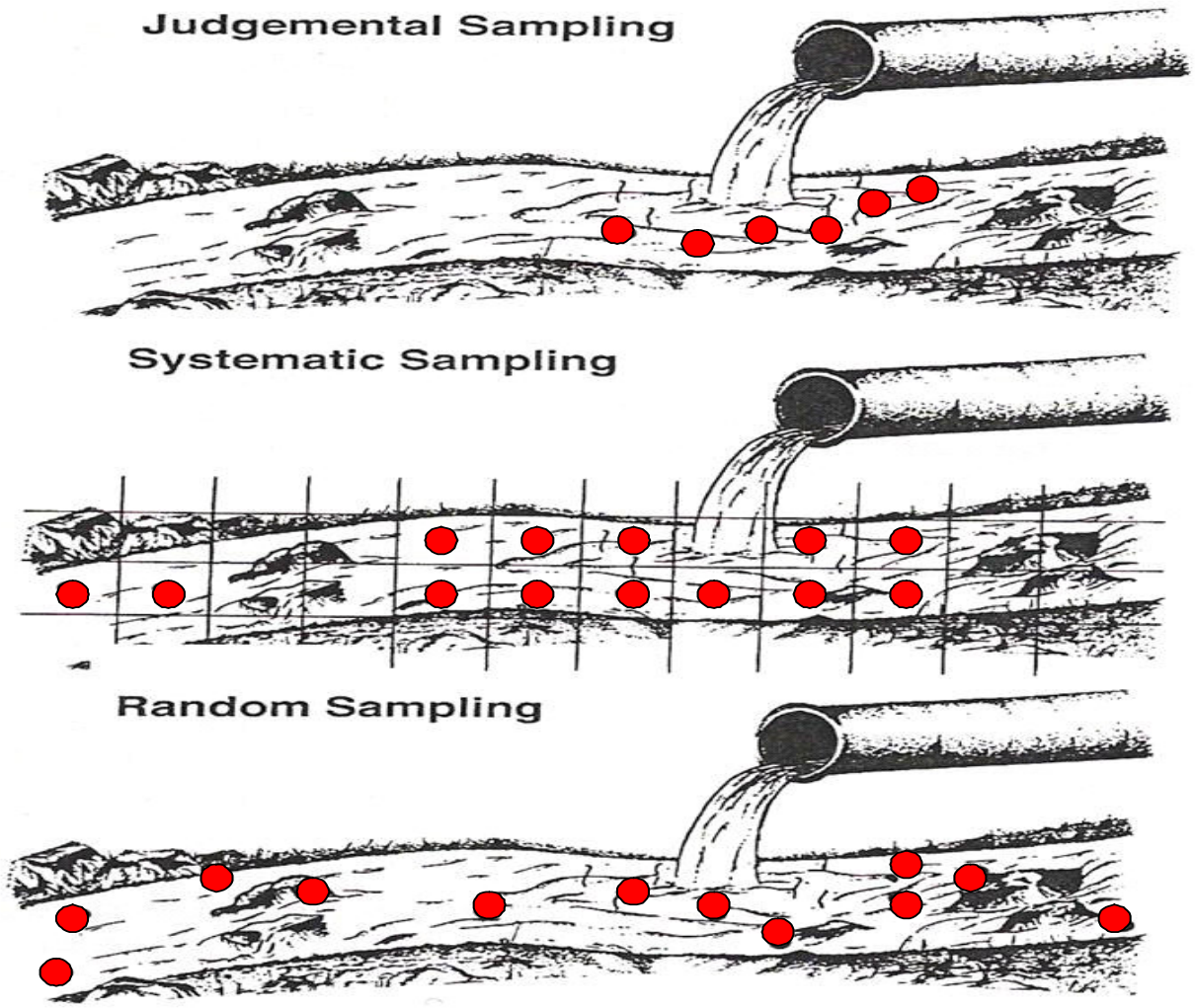


Figure 5.1: Basic sampling approaches (Keith, 1991)

The systematic sampling involves dividing the site into equal sized areas and sampling each area. A regular sized grid is normally used to set up a systematic sampling scheme.

Random sampling involves selecting sampling sites with no particular pattern. Combinations of random sampling with judgemental or systematic sampling can also be done.

Composite sampling is used where the number of samples that can realistically be taken is limited by time or cost. In this case, equal quantities of sample taken from different sites are combined and analysed as a single sample.

In this study, judgemental sampling and a combination of random and judgemental were used. Judgemental sampling was used to obtain organolead degradation water samples for speciation studies. Random and judgemental sampling was used obtain samples for adsorption/desorption studies and for simultaneous speciation of organotin and organomercury.

The method described in section 2.1 was used for sample collection and storage. The details of sampling are further elaborated on in chapter 6.

5.2. Experimental design

Experimental design has become indispensable as a good tool for one to be able to achieve reliable results and avoid tedious work by running unnecessarily large

numbers of experiments without optimum parameters values. In many cases, several kinetic and dynamic parameters have to be controlled during the experiment; these include pH, redox potential, temperature, time, stirring rate and others. Thus optimum values for these parameters are required.

Umetric Modd 5.0 and Statgraphics 5.0 softwares were used for experimental design, mainly for extraction step. The liquid-liquid extraction (LLE), supported liquid membrane probe extraction (SLMPE), cold trap solid phase extraction (CT-SPE) and microwave assisted extraction (MAE) were used.

5.3. Sample preparation

5.3.1. Derivatisation methods

The choice of a derivatisation method depends upon the analyte, the cost and the selectivity of the derivatisation agent. Different methods of derivatisation are described in chapter 2.

In this study, boron based chemicals were used. This method as discussed in chapter 2 allows in situ analysis and is not subject to much interference. Sodium tetraethylborate ($NaEt_4B$) was used for organotin speciation, sodium tetraphenyl borate for organolead speciation ($NaPhe_4B$) and sodium tetrapropylborate for simultaneous speciation of organotin and organomercury compounds. The use of magnesium tetraethylborate bromide ($MgEt_4BBr$) is discussed in chapter 4.

Organolead compounds were ethyllead and methyllead. The only simple way of simultaneously analysing them is by the use of a derivatisation agent which does not contain either ethyl or methyl group. By using ethylation, inorganic lead and ethyllead compounds combine to the same tetraethyllead derivative. Tetraphenylborate was used to simplify the analysis without removal of inorganic lead. Tetrapropylborate could also be used, but it is not readily available commercially.

Tetraphenyltin was used because it is cheaper than tetraethyborate, but screening analysis done for simultaneous speciation of organolead and organotin was not successful due to lack of separation of the analytes (appendix 5). Similar results were obtained and reported by Won et al. (2004). Won et al., 2004 studied the comparison of ethylation and phenylation of organotin and found that phenylation requires much longer time, provides very low yield and produces considerable amounts of side reaction products. The phenylation of butyltin produces phenybutyltin compounds which are big molecules. This renders the separation in GC very difficult. Phenybutyltin compounds are less volatile than ethylbutyltin produced by ethylation. In simultaneous speciation of organolead and organotin in this study, it could be said that both liquid-liquid extraction and supported liquid membrane probe extraction were successful but separation using GC did not give good results.

Tetrapropylborate has proven to be the best derivatisation agent for simultaneous organotin and organomercury speciation in almost all environmental matrix. It is also much more sensitive for mercury compounds than tetraethylborate and tetraphenylborate [Tessier, 2004; Grinberg et al. 2003]. This motivated us to use it for simultaneous analysis in water, sediments and plant samples.

5.3.2. Extraction

Three extraction methods were used. Depending on the matrix and the purpose of the analysis, either, liquid-liquid extraction (LLE), supported liquid membrane probe extraction (SLMPE) or microwave assisted extraction (MAE) was used. This section outlines the parameters used and their optimisation.

Solvent choice

Before performing extraction, a choice of solvent was made. Hexane and isooctane have been used in a lot of studies reported in literature and were used in this study too for choice of solvent. Screening experiments were done at fixed pH 5, extraction time (20 minutes) and stirring rate (6 levels). The parameters for supported liquid membrane probe extraction (SLMPE) system will be discussed later in this section.

The samples used were deionised water spiked with different concentrations of organotin or organolead compounds.

LLE can be used for organolead, organotin and organomercury speciation but was used for organolead speciation. Simultaneous speciation of organotin and organomercury was done by a method developed by the Laboratoire de chimie analytique et bioinorganique (LCABIE-France) [Tessier, 2004; Monperrus, 2004]. Figure 5.2 (a) shows that both hexane and isooctane can be used for LLE. There is no significant difference.

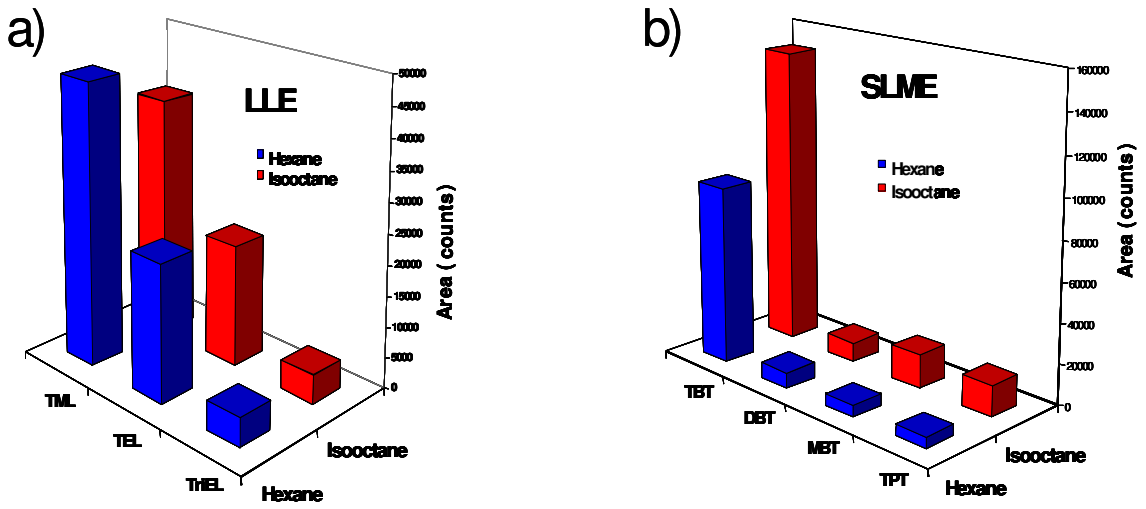


Figure 5.2: LLE and SLMPE obtained using both isooctane and hexane

Isooctane clearly looks the best for this extraction and should be used for SLMPE as shown in figure 5.2 (b). The ethylbutyltin compounds produced by ethylation are not very volatile and are retained well in isooctane. Similar observations were also made in speciation of organolead using phenylation as a derivatisation

method. The phenylalkyllead derivatives that are not very volatile were found to be stable and preconcentrate better in isooctane than in hexane.

SLMPE allows for simultaneous extraction and preconcentration. To be able to achieve a well preconcentrated extract, a small volume of solvent should be used. In this system (SLMPE) 0.5 to 1 mL of solvent is used. Using a highly volatile solvent may results in the loss of analyte with solvent and so the diffusion system donor-membrane-acceptor may be disturbed. Further detailed studies on this system needs to be done.

Liquid-liquid extraction

In organolead degradation study, the LLE had to be optimised. The pH and time of extraction were monitored. Using statgraphics 5, a 12 experiment experimental design was obtained (table 5.1) and results plotted in figure 5.3 .Each experiment was done in triplicates.

Deionised water samples were spiked with Me_3PbCl, Et_3PbCl and Et_4Pb and buffered to experimental pH (2, 4.5, 7 or 9.5) using acetic / acetate, ammonia / ammonium buffer or HCl. Hexane was then added prior to the addition of derivatisation agent ($NaPh_4B$). This prevents any loss of volatile derivatives that may occur when the acceptor phase (hexane) is added after the derivatisation agent. The mixture was shaken manually for 5, 10, 15 minutes (depending on the experiment) then the organic phase collected for GC-MS analysis. Et_4Pb, in its

volatile form, was used as internal standard and not as a derivatisation agent. Et_4Pb is present in environment, for environmental sample, blank samples were analysed before Et_4Pb was added.

Table 5.1: Experimental design for LLE

Experiment	PH	Time (minute)	Stirring level
1	2	15	4
2	4.5	15	4
3	7	15	4
4	2	37.5	4
5	4.5	37.5	4
6	7	37.5	4
7	2	60	4
8	4.5	60	4
9	7	60	4
10	2	15	6.5
11	4.5	15	6.5
12	7	15	6.5
13	2	37.5	6.5
14	4.5	37.5	6.5
15	7	37.5	6.5
16	2	60	6.5
17	4.5	60	6.5
18	7	60	6.5
19	2	15	9
20	4.5	15	9
21	7	15	9
22	2	37.5	9
23	4.5	37.5	9
24	7	37.5	9
25	2	60	9
26	4.5	60	9
27	7	60	9

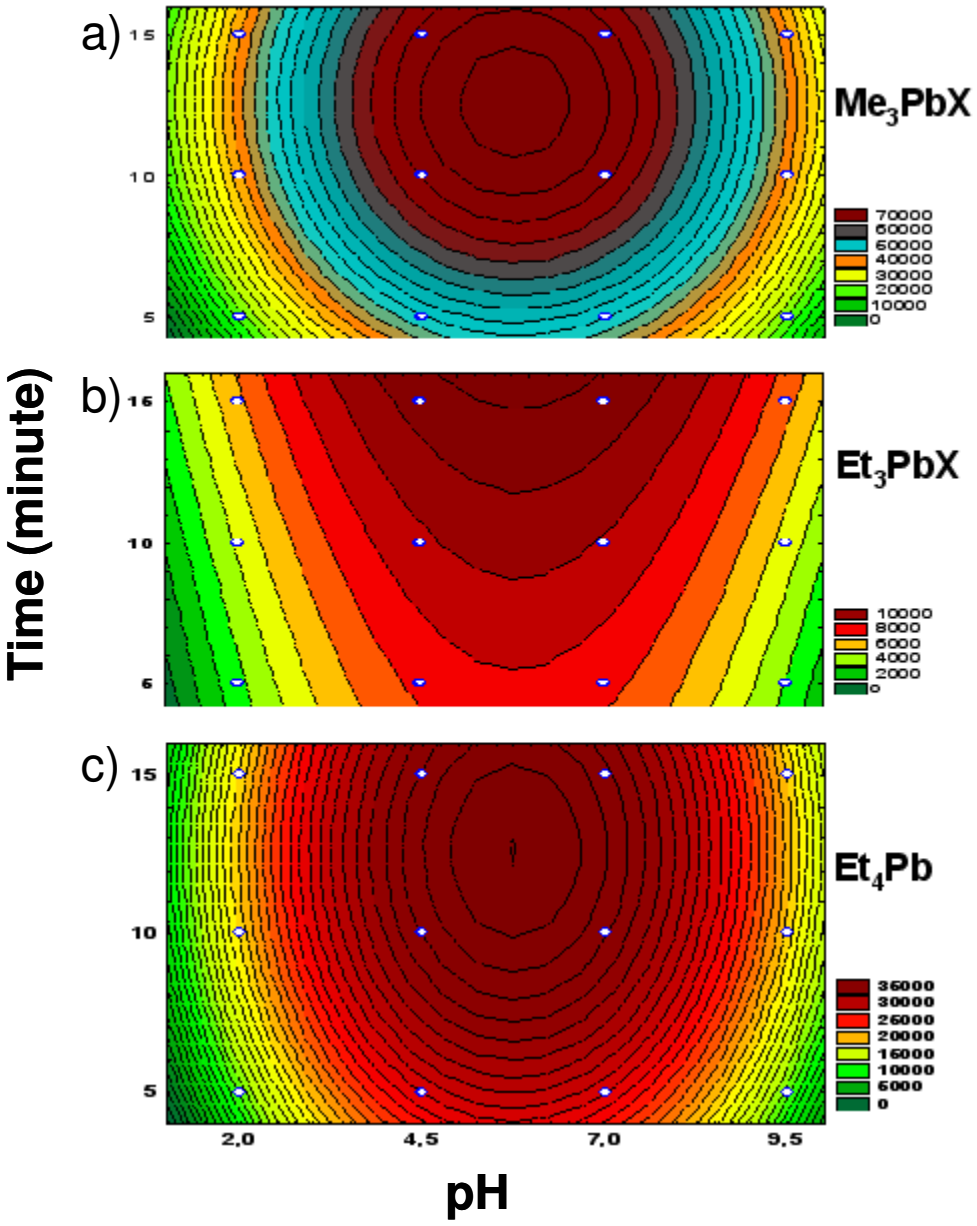


Figure 5.3: Time and pH optimisation for LLE

The 12 experiments from the method development were done at different times and pH values to find the optimum conditions. The graphs, figure 5.3, were plotted after GC analysis.

Figure 5.3 gives the optimum results for organolead extraction. Triethyllead and trimethyllead which undergo derivatisation prior to the extraction shows that phenylation occur in the range of pH 4-7, this is a broader range when we compare with the ethylation or propylation which happen between pH 4-6 (as found in many publication) and become inhibited at high pH [Mathiasson and Ndungu, 2000]. This high range is probably due to the high stability of phenyl carbanion. The proposed mechanism should be the dissociation of organometallic (equation 4.1), followed by a nucleophilic attack (equation 5.2)

$$Et_3PbX \rightarrow Et_3Pb^+ \quad (5.1)$$

$$Et_3Pb^+ + Ph^- \rightarrow Et_3PbPh \quad (5.2)$$

The overall reaction will be (equation 5.3):

$$Et_3PbX + NaPh_4B \rightarrow Et_3PbPh + Ph_3B + NaX \quad (5.3)$$

The high range of the pH of 3 to 8 for Et_4Pb found, figure 5.3 (c), is independent of derivatisation.

The optimum time was found to be 10 minutes, reaching the plateau after this time. 10 minutes is relatively high in comparison to the time usually used in LLE when ethylation and propylation derivatisation was applied. A similar observation was also made by Won (2004). It is probably due to the low rate of phenylation step during derivatisation reaction.

These extraction parameters were used for tetraalkyllead and dibutyltin compounds sorption.

Supported liquid membrane extraction

SLM probe can be made from plastic or glass material, the choice depends on the analyte and the solvent used for extraction. A glass probe was selected because of the organic solvent involved. It was made from glass tube of dimensions; 13 mm i.d.; 16 mm o.d. and 92 mm length. One end of the glass tube was closed with porous Millipore filter sealed with PTFE tape and soaked with organic solvent (isooctane or hexane) which serves as an acceptor phase (figure 5.4).

Deionised water samples were spiked with Me_3PbCl, Et_3PbCl, Me_4Pb, Et_4Pb, Bu_3SnCl, Bu_2SnCl_2, $BuSnCl_3$ and Ph_3SnCl then buffered to experimental pH (2, 4.5, 7) using acetic / acetate, ammonia / ammonium buffer or HCl followed by the addition of derivatisation agent ($NaPh_4B$). Isooctane (0.4 mL) was then placed inside the probe. Depending on the experiment, the mixture was stirred at 4, 6.5 or 9 levels using a magnetic stirrer and extraction was allowed for 15, 37.5 or 60 minutes. The organic phase was collected at the end for GC-MS analysis. The SLM probe was prepared by soaking into the organic solvent (isooctane) for 30 minutes. It was then flushed with deionised water both inside and outside to remove excess of organic solvent from the surface. The inside of the probe served as the acceptor phase and was filled with 400 µl of isooctane. The outside of the

membrane was immersed at 2 mm depth into 5 ml of the stirred sample solution (figure 5.4).

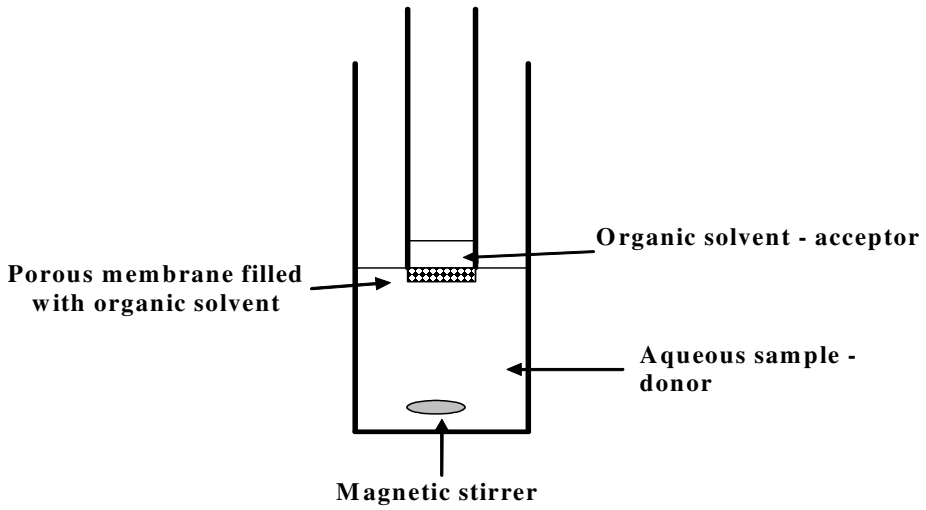


Figure 5.4: Supported liquid membrane probe unit

The optimum values used for speciation of organotin in environmental aqueous samples are discussed in the study published in A*nalytica Chimica Acta 523 (2004)* in the appendix 6. Figure 5.5 gives the optimum parameters for organolead degradation.

To determine the working parameters for organolead degradation, 27 experiments were designed using speciation Umetric Modd 5.0 (table 5.8).

Table 5.2: Experimental design for SLMPE

Experiment	PH	Time (minute)	Stirring level
1	2	15	4
2	4.5	15	4
3	7	15	4
4	2	37.5	4
5	4.5	37.5	4
6	7	37.5	4
7	2	60	4
8	4.5	60	4
9	7	60	4
10	2	15	6.5
11	4.5	15	6.5
12	7	15	6.5
13	2	37.5	6.5
14	4.5	37.5	6.5
15	7	37.5	6.5
16	2	60	6.5
17	4.5	60	6.5
18	7	60	6.5
19	2	15	9
20	4.5	15	9
21	7	15	9
22	2	37.5	9
23	4.5	37.5	9
24	7	37.5	9
25	2	60	9
26	4.5	60	9
27	7	60	9

After integration of chromatograms, the area responses were plotted, figure 5.5.

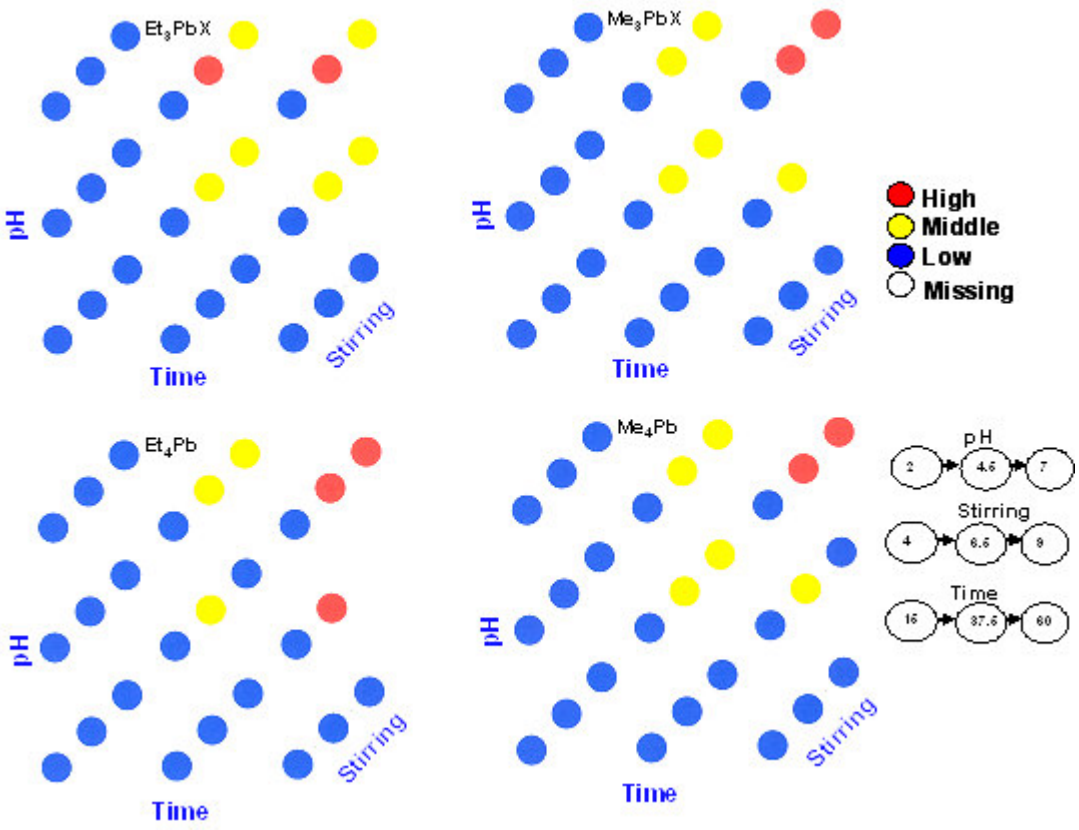


Figure 5.5: SLMPE and phenylation optimisation for organolead

From figure 5.5, between pH 4.5 and 7 the response area is high (red) or in the middle (yellow). An increase in response area for pH 7 is noted. A higher pH of 9 was used to check if there was still an increase in area response with an increase in pH. Figure 5.6 shows that after pH 7 the response area value dropped. Hence the optimum pH ranged was between Ph 4.5 and 7.

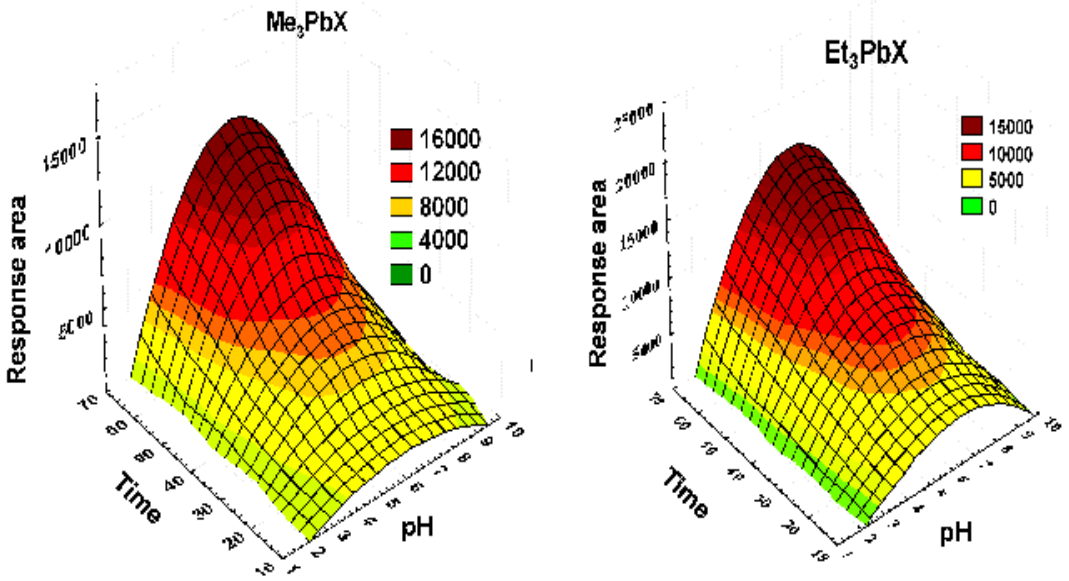


Figure 5.6: Optimisation of pH at high range (pH 2 – pH 9)

The optimum values were found to be pH 6 for 37- 45 minutes at a stirring rate of 6.5 levels. The influence of these parameters can be seen in appendix 6.

This method was used in the study of organolead compounds degradation and in the speciation of organotin in environment.

5.4. Calibration methods

The organometallic compounds of tin, lead and mercury analysed by GC are quantified by measuring the signal given by the detectors. The signal that is produced in the form of a chromatogram is integrated to give peak area or height.

In this study, peak area responses were used. This was evaluated by using standards solution.

External calibration, internal standard and isotope dilution calibration procedures were used in this study.

5.4.1. External calibration

This method is based on the calibration curve [Harris, 2002] and linearity is shown by the calculation of correlation coefficient (R^2).

External calibration was used for organotin and organolead compounds. Known concentrations of different analytes were prepared and analysed. Calibration standards matrices were matched to those of real samples, that is, every chemical treatment of the standard and every step was matched with those of sample preparation. Calibration points also included blank solution values to determine background concentrations.

The main drawback of this method is that all analyses rely on the purity and preparation of the standard and operating conditions free of contamination. There is no way of correcting errors made during analysis.

5.4.2. Internal standard

An internal standard is a known amount of a compound different from the analyte, which is added to the unknown. The signal from the analyte is compared with

signal from the internal standard to find out how much analyte is present [Harris, 2002]. It should have similar properties as that of the analyte.

Tetraethyllead was used as internal standard in degradation of organolead. It has similar properties as its homologue methyllead and ethyllead compounds.

In this case the concentration of the analyte was calculated using the equation:

$$\frac{A_x}{[x]} = F(\frac{A_s}{[S]}) \qquad (5.4)$$

Where A_x is the area of the analyte signal, X the concentration of the analyte, A_s the area of the standard signal and S the concentration of the standard. In this case A_x is the peak area of tetramethyllead, trimethyllead or triethyllead and X their concentration. A_s is the area of tetraethyllead and S is its concentration. At the end of experiment the only unknown is X in the formula.

This method is not affected by errors made in sample preparation or other errors during detection such as signal drift during detection. It can be used in conjunction with external calibration for improved results.

The main drawback of this method is the assumption that the internal standard would behave the same way as the analyte.

5.4.3. Isotope dilution

Isotope dilution is considered today as the ideal internal standard method. Instead of spiking a sample with a totally different compound, the sample is spiked with the same analyte of different isotope.

This method is based on the measurement of isotopic ratios in a sample. This method was reviewed by Rodríguez-González et al. (2005). It relies on the intentional alteration of the isotope abundance of an element in a sample by an addition of a known amount of the enriched isotope (spike) of the same element. The element to be analysed must have therefore at least two stable isotopes or long-lived radioactive isotopes able to be measured by mass spectrometry.

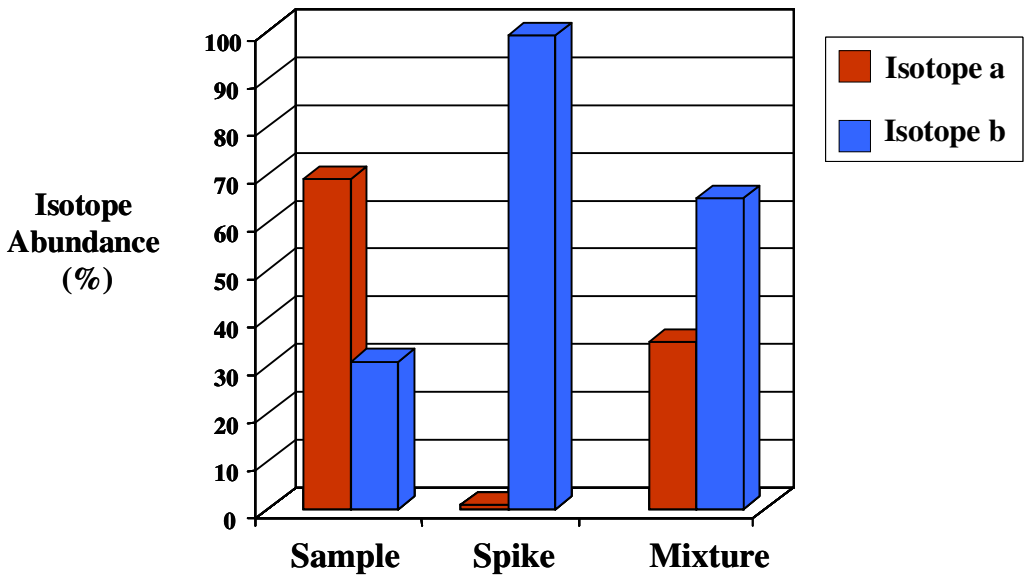


Figure 5.7: Illustration of the isotope principle for an element containing two isotopes

Figure 5.7 shows that isotope "a" is abundant in the sample and isotope "b" in the spike. The abundance of both isotope and the isotope ratio in the mixture would be intermediate. Their values will depend on the amount of the spike added to the sample and the initial amount of the analyte in the sample.

The calculation of the concentration of the analyte is done using equation 5.5. The development of this equation is discussed in details by Rodríguez-González et al. (2005).

$$C_s = C_{sp} \frac{m_{sp}}{m_s} \frac{M_s}{M_{sp}} \frac{R_m A_{sp}^b - A_{sp}^a}{A_s^a - R_m A_s^b} \tag{5.5}$$

where C_s is concentration of the element in the sample, C_{sp} is concentration of the element in the Spike, m_{sp} is mass taken from the spike in the mixture, m_s is mass taken from the sample in the mixture, M_s is atomic weight of the element in the sample, M_{sp} is atomic weight of the element in the spike, A_{sp}^b is isotope abundance of isotope a in the spike, A_{sp}^a is isotope abundance of isotope b in the spike, A_s^a is isotope abundance of isotope a in the sample, A_s^b is isotope abundance of isotope b in the spike and R_m *is Isotope ratio of isotopes a and b in the mixture.*

Determination of the concentration of the analyte becomes simple because in equation 5.2, R_m (isotope ratio of isotopes a and b in the mixture) is the only unknown parameter.

This method combined with GC-ICP-MS was used in this study for simultaneous speciation of organotin and organomercury in different environmental matrix. The results are discussed in chapter 6. Figure 5.8 shows the chromatograms obtained for spike analysis.

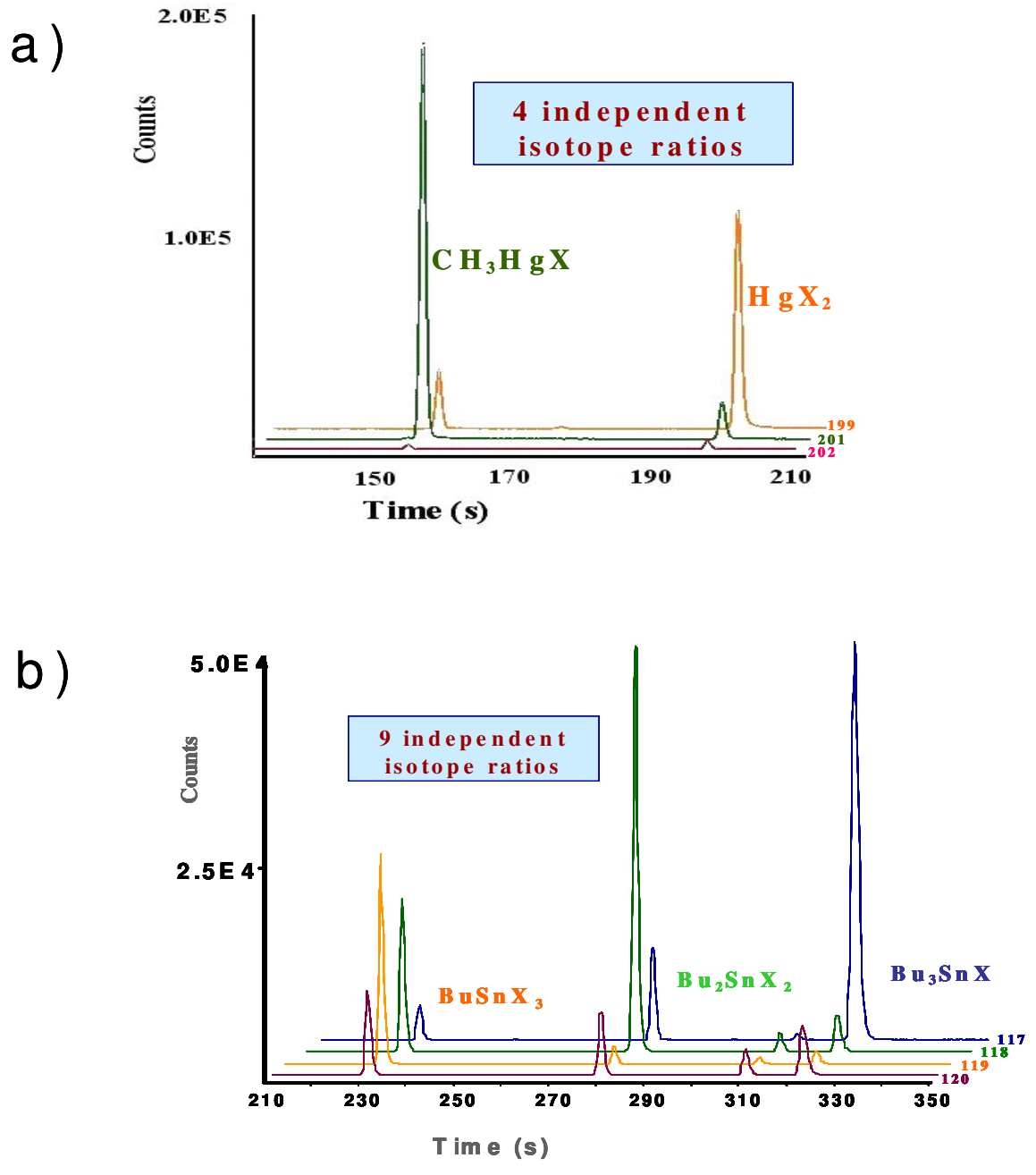


Figure 5.8: Isotope spikes for mercury and butyltin compounds

From figure 5.8 (a), there is a possibility of calculating four independent ratios for mercury compounds. R_m may be calculated for methylmercury in the form of 202Hg/201Hg or 202Hg/199Hg, the same applies for inorganic mercury. This allows for several ways of validating the method. In the case of organotin compounds, as shown on figure 5.8 (b), there are 9 independent ways of calculating R_m.

The other advantage is the possibility given of being able to monitor the interconversion reaction that may occur between species during the analysis.

In comparison with external calibration, instrumental sensitivity does not apply because there is no parameter related to it when isotopic dilution calibration is used. The response in external calibration curve is dependent on the concentration of the analyte hence a drift in signal from the instrument may affect results unnoticed. In isotope dilution the ration, $R_{m,,}$ corrects for any changes in sensitivity such as signal drift or matrix effect during analysis.

The second and the most important advantage of isotope dilution for speciation analysis is that once complete isotope equilibration between the sample and the spike has been achieved, possible loss of substance of the isotope-diluted sample will have no influence on the final result. This is due to the fact that any aliquot of the isotope-diluted sample will contain the same R_m, and therefore, there is no

need to know the pre-concentration or dilution factor of the sample or to take into account any non-quantitative separation or evaporation steps.

CHAPTER 6

APPLICATION OF DEVELOPED METHODS

In this chapter, the results obtained using the methods developed in chapter 5 are discussed. Different studies were done on speciation of tin, lead and mercury compounds. These results would help to know if the methods developed are successful when applied to the real environmental samples. They will also help to understand the pathways and transformation which may happen in environment.

6.1. Method validation

In chapter 4 the parameters needed for method validation were discussed. Note that the parameters and the instruments used can be found in the appendix 3.

A part of the study on the synthesis of magnesium tetraethylborate bromide and its applications were discussed in chapter 4; four other studies were conducted in this work. They are:

- Speciation of organotin in environmental samples: Application of supported liquid membrane probe for extraction and preconcentration of organotin compounds from environmental water samples.
- Organolead degradation in aqueous samples
- Organolead and organotin sorption on soil

- Simultaneous speciation of organotin and organomercury in water, sediment and plant samples.

In this section the "figures of merit" will be discussed for each method developed for the study mentioned above.

6.1.1. External calibration – SLMPE – GC-MS or GC-FID method

This external calibration with SLMPE method was used for the speciation of organotin in water samples using GC-MS and GC-FID. After optimisation of the SLMP extraction the same way as described in section 5.2.2, the method was validated. Other optimisation and validation done on SLMPE-GC-FID method are described in our publication in the appendix 6. The comparison of GC-MS and GC-FID separation and detection methods were also tested. The table below compiles the figures of merit for the two detection methods

Table 6.1: GC-FID and GC-MS comparison for organotin compounds speciation

Organotin	R^2		Repeatability		Reproducibility		LOD		LOQ	
	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS
$BuSnX_3$	0.9977	0.9987	11.2	8.9	6.3	2.2	4.2	3.6	10.2	5.3
Bu_2SnX_2	0.9954	0.9934	7.3	7.9	4.9	3.6	1.7	2.2	14.5	4.5
Bu_3SnX	0.9974	0.9979	8.8	7.7	11.8	1.4	2.1	1.3	11.9	3.6
Ph_3SnX	0.9982	0.9976	13.1	12.6	10.7	1.1	2.8	2.0	16.8	6.8

LOD: $\frac{3S}{mean}$, *LOQ:* $\frac{10S}{mean}$, *Reproducibility and Repeatability :* $\frac{SX100}{mean}$ *(for n = 7)*

This method was also used for study of organolead degradation. The optimum results for extraction were given in section 5.3.2. The table below shows the figures of merit for this analysis.

Table 6.2: Figures of merit of speciation of organolead

Organolead	Range	Equation	R^2	Repeatability	Reproducibility	LOD	LOQ
Me_4Pb	10-35	y=2775.9x + 551.1	0.9724	5.9	58.3	4.81	16.03
Me_3PbX	10-35	y=976x + 538.6	0.9939	9.6	74.3	4.7	15.95
Et_4Pb	10-35	y=2522.6x + 258	0.9832	6.3	52.3	4.76	15.86

LOD ($\mu g\ L^{-1}$): $\frac{3S}{mean}$, *LOQ ($\mu g\ L^{-1}$):* $\frac{10S}{mean}$, *Reproducibility and Repeatability :* $\frac{SX100}{mean}$ *(for n = 7)*

The reproducibility values shown in table 6.2 are not good. As the repeatability done by repeating the analysis in the same condition but at different period of time was good, several replicate analyses were performed to try to find out why the reproducibility gave bad results. After 3 replicates analyses there was fluctuation in signal which lead to different area count of the peak chromatogram. The phenyllead compounds are big molecules and with long period of analysis they conduct to the poor distribution onto the GC capillary column. To avoid this problem after each 3 injection the oven was heated (for 10 minutes) to the highest column temperature program and then next injections were done. Even so, the use of tetraethyllead as an internal standard helps to correct any fluctuation that may occur.

Table 6.3: SLMPE method validation for organolead compounds speciation

Parameters	Me_4Pb	Et_4Pb
Concentration spiked (µg L^{-1})	25.00	25.00
Concentration Determined (µg L^{-1})	21.31	23.02
Recoveries %	85.24	92.02
Coefficient Efficient %	6.82	7.37

The supported liquid membrane probe extraction can be used successfully for speciation of organolead. The table 6.3 gives good extraction recoveries ranging from 85 to 90 % and good coefficient efficient ranging between 6 and 7.

Flow diagram of method protocol

The step performed for organotin speciation in water sample and organolead degradation in water are drawn on the figure 6.1. The values given in the figure 6.1 are the optimum values.

Solutions of 5 % (m/v) of $NaEt_4B$ or $NaPh_4B$ were prepared in methanol, and few grams of anhydrous Na_2SO_4 were added. These solutions were stored under argon and kept in refrigerator at -4 °C.

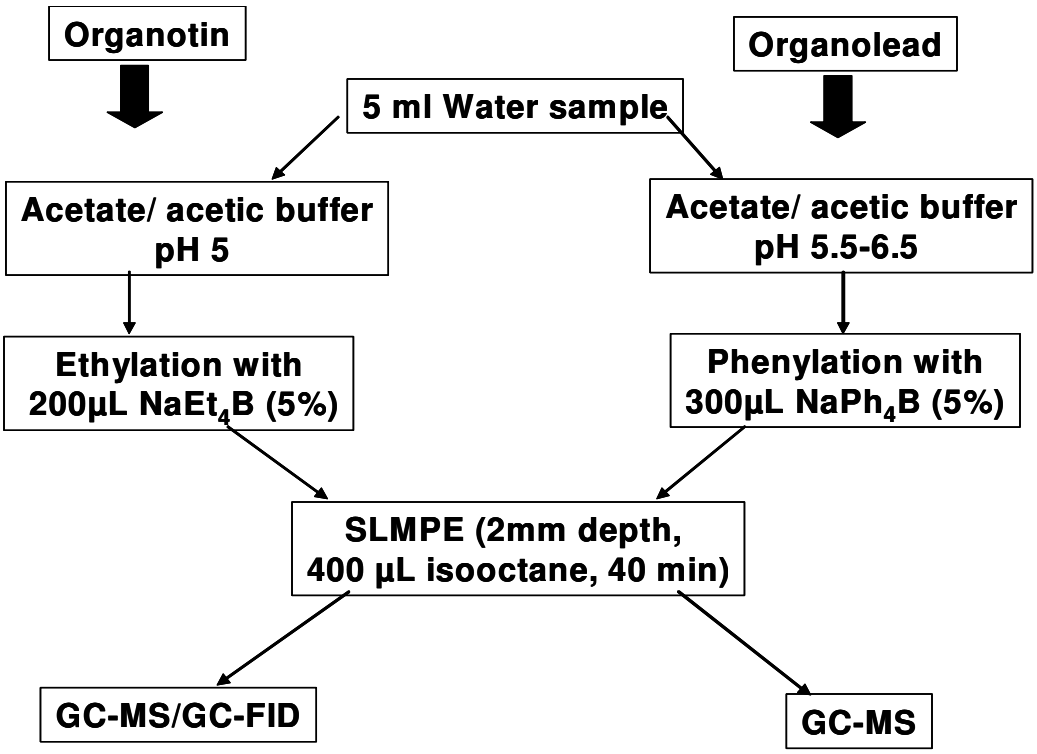


Figure 6.1: Schematic flow diagram of external calibration - SLMPE method

6.1.2. External calibration (EC) – LLE – GC-MS method or EC-ICP-OES

This external calibration with LLE method was used in two studies, the organolead and organotin adsorption process on soil samples

For organolead adsorption experiment, the external calibration methods were used after liquid-liquid extraction and GC-MS separation and detection method. In the case of organotin experiment the samples were analysed directly with ICP-OES without any extraction intermediate step.

Table 6.4 gives the extraction recoveries for tetramethyllead and tetraethyllead extraction using liquid-liquid extraction. High extraction recoveries were achieved. This is due to the non derivatisation step required for these two organolead compounds. They are at their non polar form.

Table 6.4: Liquid-Liquid Extraction (LLE) recoveries for organolead

Parameters	**Me_4Pb**	**Et_4Pb**
Concentration Spiked (µg L^{-1})	25	25
Determined (µg L^{-1})	23.7	23.9
Recovery %	94.8	95.6

Table 6.5 compiles the figures of merit for the method used to determine the tetraalkyllead. Good repeatability and reproducibility ranging below 10 and good limit of detection were obtained. The concentrations are in low values (5 µg L^{-1}).

Table 6.5: Analytical figures of merit for organolead speciation

Organolead	**Range**	**Equation**	**R^2**	**Repeatability**	**Reproducibility**	**LOD**	**LOQ**
Me_4Pb	10-45	y = 754.78x + 3315.4	0.9944	9.2	7.7	5.3	17.5
Et_4Pb	10-45	y = 506.56x + 2254.7	0.9845	9.4	8.1	4.9	16.2

LOD: $\frac{3S}{mean}$, *LOQ:* $\frac{10S}{mean}$, *Reproducibility and Repeatability :* $\frac{SX100}{mean}$ *(for n = 7)*

Flow diagram of method protocol

The steps performed for organotin and organolead speciation after adsorption experiment are drawn on the figure 6.2.

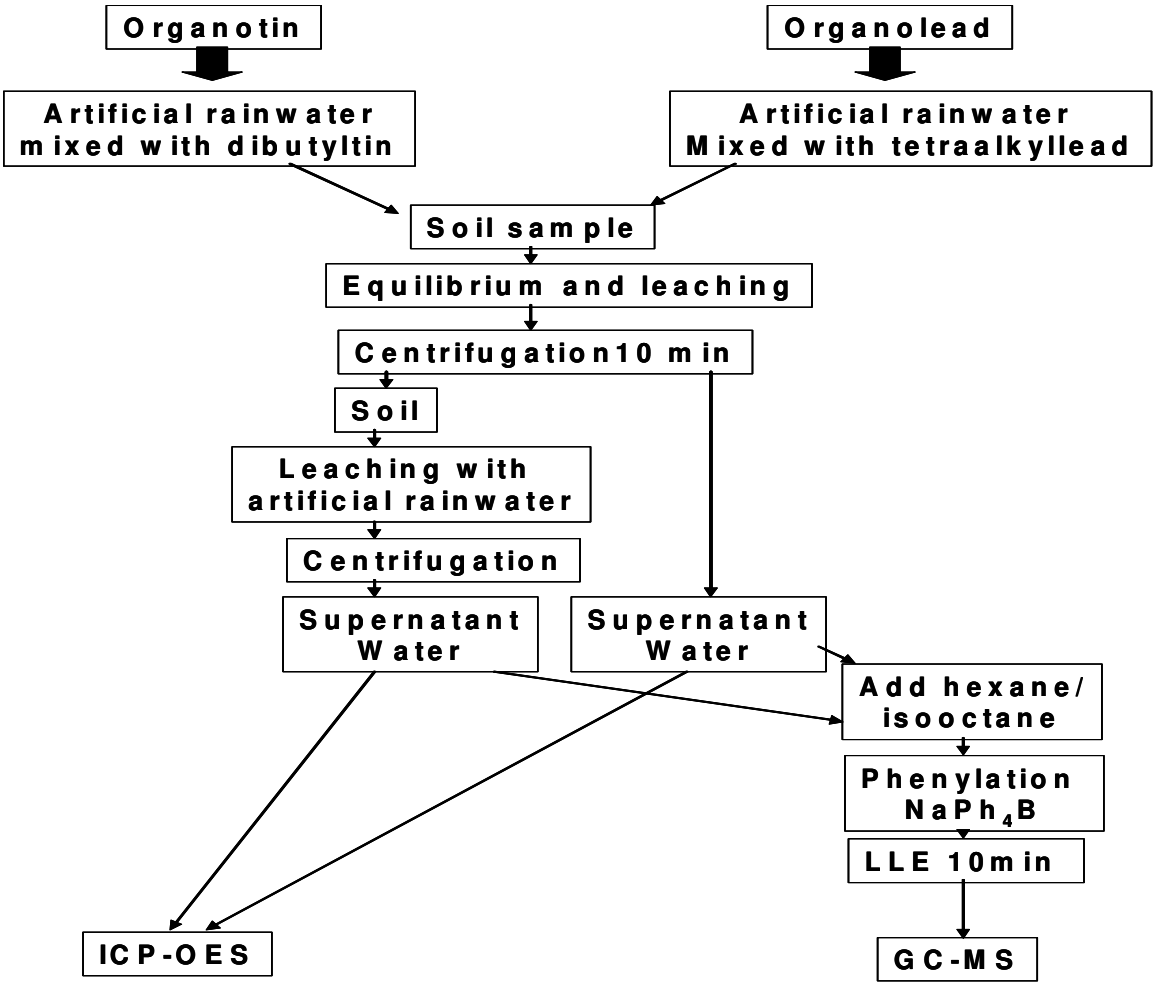


Figure 6.2: Schematic flow diagram of the adsorption experiment

In each experiment blank samples were analysed. They were obtained by soil leaching with non spiked artificial rainwater. Organolead compounds were not

found in soil. As the ICP-OES gives total metal concentrations, for dibutyltin adsorption experiment, a parallel experiment using non spiked artificial rain water was done and then dibutyltin concentrations were calculated by substracting Sn concentrations in blank samples from the obtained value.

For organolead experiment, the derivatisation step was included to monitor any degradation that may happen even if the whole experiments were done in the dark. The degradation of tetraalkyllead may convert to polar organolead such as trialkyl-, dialkyl- or inorganic lead that would require derivatisation to be able to analyse them on GC. There was no degradation noticed.

6.1.3. Isotope dilution – MAE and/or LLE – GC-ICP-MS method

This isotope with MAE and/or LLE method was used for the simultaneous speciation of mercury and organotin compounds in water, sediment and plant environmental samples.

The advantages of the isotope dilution were mentioned in section 5.4.3; though this method does not have so many parameters to control, some figures of merit have to be checked. Those figures includes, limit of detection and relative standard deviation.

Table 6.6: Limit of detection and relative standard deviation for organotin and organomercury speciation

Deion. water	**MeHgCl**	**$HgCl_2$**	**$BuSnCl_3$**	**Bu_2SnCl_2**	**Bu_3SnCl**
LOD (ng L^{-1})	0.004	0.053	0.030	0.023	0.020
RSD %	1.6	1.9	1.8	4.2	1.2

For solid sample that require MAE the spike was not used at the beginning of the microwave assisted extraction. These samples were also spiked at the same step as water sample. This means prior to the liquid-liquid extraction. To monitor the loss or other contamination that might occur prior the spiking with isotope species, we analysed the certified reference material the same way as real solid environmental samples (Table 6.7).

Table 6.7: Recoveries of the method for certified reference material

CRM	**Cert. MeHgX**	**Det MeHgX**	**Recovery %**	**Cert. HgX_2**	**Det. HgX_2**	**Recovery %**
IAEA 405 (µg/g)	5.49 (0.57)	4.81 (0.91)	87.61	0.81 (0.04)	0.80 (0.70)	98.76
BCR 279 (µg/g)	0.92 (0.67)	0.87 (0.10)	94.57	0.05 (0.11)	0.04 (1.11)	80.00

IAEA 405 is a sediment certified material and BCR 279 is a plant certified material for mercury. The table 6.7 gives good recoveries for the method.

Flow diagram of method protocol

Prior to MAE, dry frozen sediment and plant samples were ground and sieved. The grinding was done using a pestle and agate mortar.

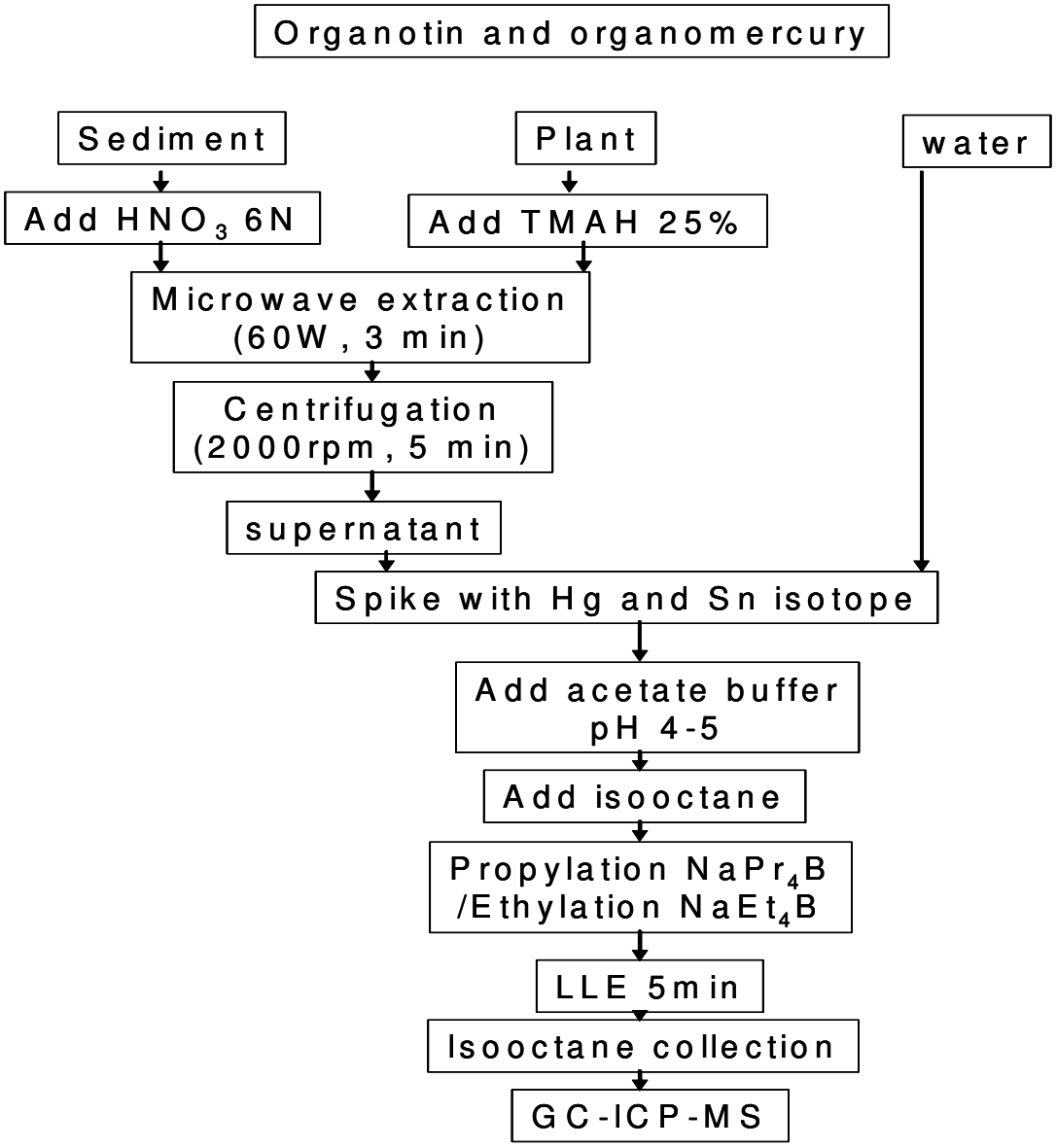


Figure 6.3: Schematic flow diagram of isotope dilution GC-ICP-MS method

The emulsion can be observed during liquid-liquid extraction for solid sample in general. When the emulsion occur the addition of 100 μL of HCl was enough to break it.

6.2. Speciation of organotin in water environmental samples

The speciation of organotin was done using the method described in section 5.4.1. The SLMP extraction method developed in our laboratory was used. Two different detection methods GC-FID and GC-MS were evaluated.

As the whole procedure and results using GC-FID are described in detail in our publication [Cukrowska et al., 2004] in the appendix 6, only the comparison data between GC-FID and GC-MS is discussed in this section.

The environmental water samples were from various areas in South Africa; surface water samples were from Rustenburg (R samples) and Germiston (G samples), sea water was from Port Elizabeth habor.

The analytical figures of merit were discussed in section 6.1.1. The table 6.8 gives the results for real environmental samples.

Table 6.8: Comparison between GC-FID and GC-MS for environmental water sample with RSD in bracket.

Samples	pH	ORP (mV)	Conductivity (µs cm-1)	MBT (FID)	MBT (MS)	DBT (FID)	DBT (MS)	TBT (FID)	TBT (MS)	TPT (FID)	TPT (MS)	Total Sn
Surface water sample												
R1	10.20	321	1003	10.4 (5.7)	11.2 (2.4)	17.7 (6.2)	19.1 (4.1)	ND	ND	ND	ND	35 (4.8)
R2	8.13	433	1038	11.8 (6.5)	13.1 (1.4)	19.3 (6.8)	19.7 (3.2)	ND	ND	ND	ND	98 (8.9)
R3	7.80	440	672	12.3 (7.1)	12.9 (2.2)	23.0 (7.2)	22.9 (2.2)	ND	1.2 (2.7)	1.5 (9.1)	2.1 (4.1)	105 (7.8)
R4	8.40	442	510	10.9 (7.5)	13.1 (3.3)	18.6 (6.7)	19.9 (1.5)	ND	ND	ND	ND	43 (10.4)
R5	8.22	428	1008	11.2 (6.8)	12.5 (0.9)	18.9 (5.5)	19.3 (3.1)	ND	ND	ND	1.1 (2.2)	82 (11.2)
G1	6.78	375	524	13.8 (8.4)	13.8 (2.2)	29.1 (7.1)	29.1 (2.1)	5.7 (6.3)	5.9 (3.3)	3.6 (4.9)	3.9 (1.7)	63 (8.9)
G2	6.37	312	722	14.3 (8.2)	15.5 (4.1)	33.9 (7.5)	34.5 (5.5)	6.8 (5.4)	7.3 (2.1)	4.5 (5.6)	4.5 (2.1)	70 (9.6)
G3	5.40	360	883	14.1 (7.9)	16.5 (1.9)	30.3 (6.8)	30.4 (0.8)	6.1 (5.7)	6.8 (3.1)	3.9 (4.3)	4.3 (2.8)	105 (8.6)
G4	2.90	691	1638	8.5 (6.9)	9.9 (3.1)	11.9 (7.0)	12.4 (3.2)	2.6 (5.1)	3.1 (1.1)	ND	1.3 (1.9)	125 (7.6)
G5	3.85	565	1418	13.9 (6.7)	14.4 (2.2)	29.5 (7.2)	30.1 (1.4)	5.9 (6.1)	6.6 (3.2)	3.8 (5.6)	4.3 (1.7)	117 (8.5)
Sea water												
S1	8.20	689	5468	2.3 (7.9)	2.3 (1.1)	4.6 (8.0)	4.9 (2.1)	10.4 (9.8)	10.9 (3.1)	6.3 (8.9)	6.8 (3.1)	113 (9.3)
S2	8.15	684	5519	2.4 (7.6)	2.4 (2.9)	4.5 (7.9)	4.8 (1.1)	10.9 (9.6)	11.1 (4.2)	6.2 (8.8)	6.8 (2.6)	120 (6.9)
S3	8.16	698	5575	2.8 (7.7)	3.1 (2.1)	4.8 (7.6)	4.8 (2.6)	11.5 (9.3)	11.9 (2.3)	6.9 (8.5)	7.4 (2.5)	134 (7.8)

Organotin compounds Concentrations in µg L^{-1}

The GC-MS gives the values which are slightly high than those from GC-FID. They are some samples in which tributyltin and triphenyltin were not detected by GC-FID but detected by GC-MS. Those are samples R3, R5 and G4. The other advantage of GC-MS is the easy peak identification; each organotin peak can be qualitatively identified. This give advantage when dealing with a complicated

environmental matrix such as solid and plant sample with a non selective extraction such as liquid-liquid extraction. Every compound derivatised and extracted will show a peak on the chromatogram, thus any change in carrier gas flowrate may lead to errors in identification in GC-FID (it uses retention time for identification).

In Rustenburg, where there is basic pH of water no tributyltin and triphenyltin compounds were observed while they were present in Germiston water (acidic pH). The trialkyl substituted tin might be stabilised in surface water by acidic conditions.

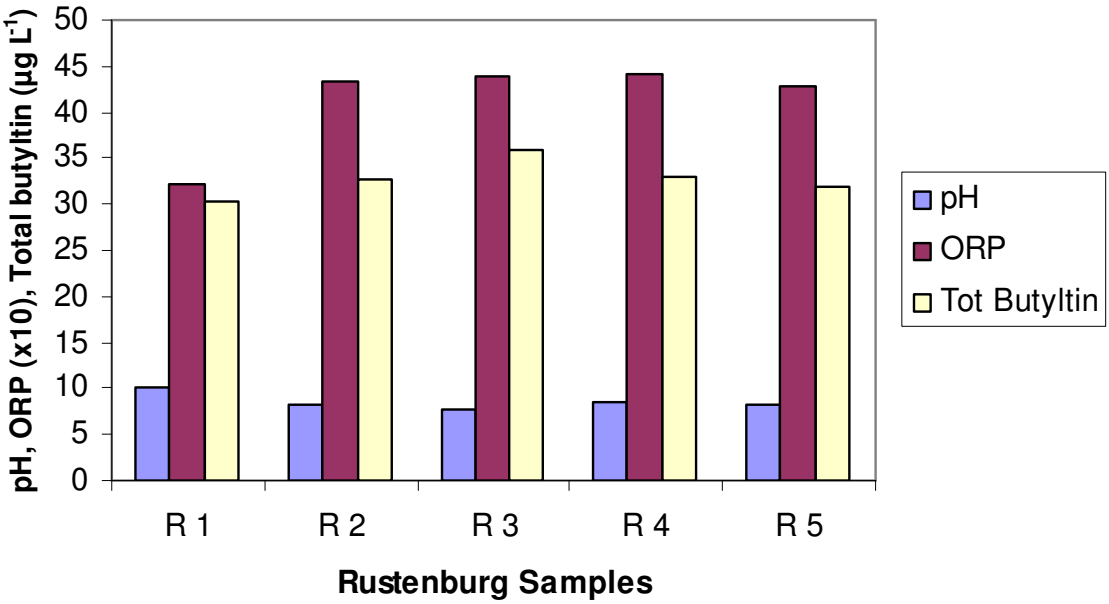


Figure 6.4: Butyltin variations with pH and ORP in Rustenburg sammple

The ORP of surface water in all samples were high, ranging from 312 to 698. In Rustenburg samples, it was found that the overall concentration of butyltin compounds vary with ORP, high ORP values are related to high butyltin concentrations. High ORP may favour the Sn (IV) that was found in organotin compounds, thus the stability of these compounds in these conditions (Figure 6.4).

In Germiston samples, the trend mentioned above is not clear, (figure 6.5) this is probably due to the acidic conditions of the samples, as at very low pH (sample G 4) is where the lowest butyltin compounds are found.

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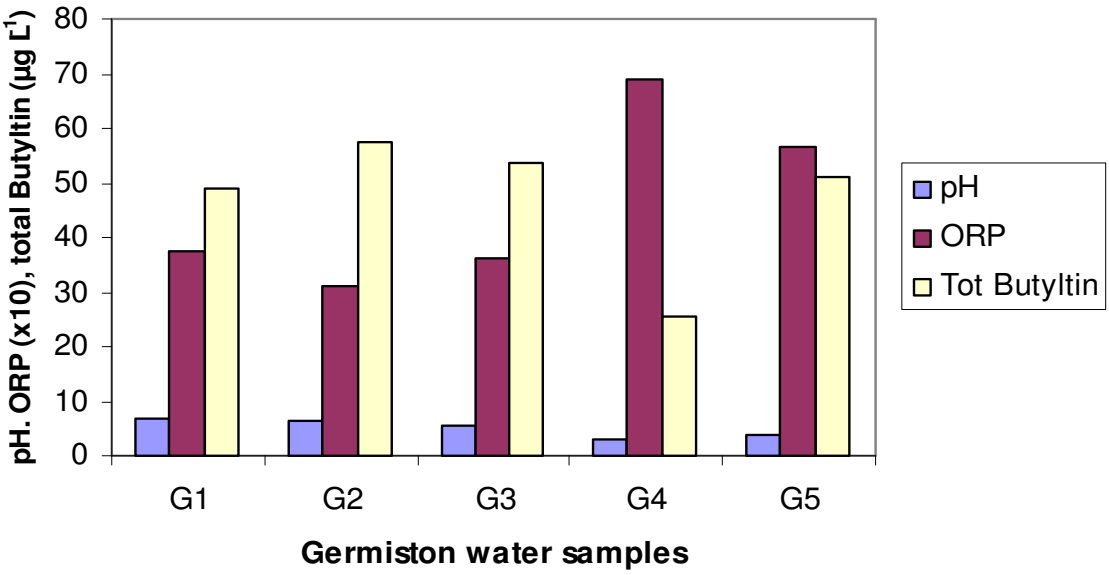


Figure 6.5: Butyltin variations with pH and ORP in Germiston samples

ORP and pH can not explain alone the environmental occurrence of organotin compounds. All biogeochemical transformations such as debutyration and butylation should be taken in account

Generally, we can conclude that both methods combined to a robust extraction method such as SLMP extraction used in this thesis can be used successfully.

6.3. Speciation of alkyllead compounds in aqueous samples

The external calibration with supported liquid membrane probe extraction method (section 5.4.1) was used to study the transformation of tetramethyllead in aqueous media at different concentration of major elements, K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}.

The environmental water samples were collected in Johannesburg city mainly in the high traffic area near car park stations and petrol stations.

Chemicals

Tetramethyllead (TML), Trimethyllead chloride (TiML), dimethyl dichloride (DML), tetraethyllead (TEL) and triethyllead (TiEL) used as internal standard were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium tetraphenylborate was from Across (Geel, Belgium). Sodium acetate–acetic acid buffer, hydrochloride acid, ammonium acetate – ammonia buffer and isooctane were from Sigma–Aldrich (Steinheim, Germany). All glassware used were soaked

in 2M nitric acid for at least 2 days and rinsed with Milli-Q water and ethanol immediately before use.

Degradation experiment

Solutions of artificial freshwater containing different concentration of major ions K^+ (1.20 – 1.7 mg L^{-1}), Na^+ (11.40 -18.40 mg L^{-1}), Ca^{++} (9.30 - 10.30 mg L^{-1}), Mg^{++} (4.80 - 5.20 mg L^{-1}), Cl^- (1.05 - 1.68 mg L^{-1}) and SO_4^{--} (45.21 - 46.71 mg L^{-1}) for soft fresh water and K^+ (4.20 - 5.60 mg L^{-1}), Na^+ (48.12 - 47.52 mg L^{-1}), Ca^{++} (26.93 - 27.23 mg L^{-1}), Mg^{++} (18.60 - 15.45 mg L^{-1}), Cl^- (4.09 - 4.99 mg L^{-1}) and SO_4^{--} (127.14 - 173.23 mg L^{-1}) for hard freshwater were prepared.

A glass container has been filled with soft or hard water then spiked with Me_4Pb (400 μg L^{-1}) and exposed to solar radiation. At different interval of time, the samples were collected and submitted to extraction and preconcentration followed by analysis of organolead compound using GC-MS. Tetraethyllead was used as internal standard.

This experiment was performed during the period of 3 months. The samples were divided into three groups: the blank sample containing only tetramethyllead, the artificial soft water and the artificial hard water as described previously. All samples were treated the same way during the whole process of analysis. For each group the aliquots for analysis were collected by intervals of time: 0 min, 15 min, 30 min, 60 min, 120 min, 480 min and 1440 min.

Sample preparation

Water samples were buffered to experimental pH 6 using acetic / acetate followed by the addition of derivatisation agent ($NaPh_4B$). 5 m L of this solution were placed into the SLM glass tube. The SLM probe was prepared by soaking into the organic solvent (isooctane) for 30 minutes. It was then flushed with deionised water both inside and outside to remove the excess of organic solvent on surface. The inside of the probe was filled with 400 µl of isooctane. The outside of the membrane was immersed at 2 mm depth into 5 ml of the stirred (at 6.5 level) sample solution (figure 5.4).

The extraction was allowed to proceed for 37 - 45 minutes. The extracted solution was allowed to equilibrate for 10 minutes and then removed from glass tube into a vial and then stored at 4ºC until analysed. Before the next extraction was performed, the inside of the probe was flushed with about 5 ml of fresh acceptor solution and the outside with deionised water.

Solubility of tetramethyllead

To be able to monitor the degradation of Me_4Pb we had to know its solubility in the three different water samples used. The samples have been spiked with Me_4Pb and analysed for its solubility without exposure to solar radiation.

Table 6.8 shows that as the concentration of major ions increases in the water the Me_4Pb becomes more soluble, the concentration vary from 80 μg L^{-1} (blank) to 140 μg L^{-1} (hard water).

Table 6.9: Solubility of tetramethyllead in water (RSD in brackets)

Sample	Tot.lead (μgL^{-1})	Me_4Pb (μgL^{-1})
Blank	92.23 (4.3)	84.49 (7.3)
Soft water	129.44 (8.5)	121.91 (11.2)
Hard water	148.81 (4.6)	142.08 (9.6)

Correlation component

The correlation of inorganic ions with organolead is shown in table 6.10. The concentrations of Me_3PbX and total lead (Pb) are highly correlated to exposure time. As the time of exposure increases the concentration of Me_4Pb degraded increases, thus the concentration of Me_3PbX and total lead (Pb) increases. Me_4Pb is highly correlated to pH, redox potential (Eh) and concentration of all major ions which explain why the harder the water the higher level of dissolved Me_4Pb.

Table 6.10: Correlation matrix of alkyllead and major parameters

Variable	Pb	Me_4Pb	Me_3PbX
Time	***0.88***	-0.06	***0.89***
pH	0.20	***0.97***	0.20
Eh	-0.22	***-0.97***	-0.20
Mg	0.20	***0.88***	0.12
K	0.22	***0.88***	0.14
Ca	0.23	***0.95***	0.17
Na	0.23	***0.88***	0.16
Cl	0.22	***0.87***	0.13
SO_4^{2-}	0.17	***0.90***	0.10

Degradation kinetics

Table 6.11. gives results that show the average of degradation of Me_4Pb at least to the first level of degradation in Me_3PbX during the period of study based on a daily degradation. The Me_4Pb is more degraded in hard freshwater (H1, H2) than in soft freshwater (S1, S2) or Blank (B1, B2). The whole amount of initial Me_4Pb would be degraded between 24 and 37 days in aqueous media.

Table 6.11: Degradation kinetic (RSD in brackets)

Sample	Init.Conc. (µg L^{-1})	Final Conc. (µg L^{-1})	Tot. degradation (day)
B1	84.49 (7.3)	82.13 (9.5)	37
B2	95.04 (6.7)	92.56 (10.6)	
S1	121.91 (11.2)	118.1 (7.4)	31
S2	128.76 (8.9)	124.51 (6.5)	
H1	142.08 (9.6)	136.25 (10.4)	24
H2	144.13 (12.4)	137.93 (7.7)	

It was found that in a period of 24 hours, the Me_2PbX_2 produced by degradation is not quantifiable, but the peak detected corresponds to Me_2PbX_2 (figure 6.6).

The tetraalkyllead is not stable in aqueous media, it undergoes degradation between 20 and 40 days, this period is an estimated one as our experiment were done in clean condition, no solid matter involved. The fact that they degrade fast in hard water may conduct to the degradation in short period time in real environmental sample with highly organic matter and particle content

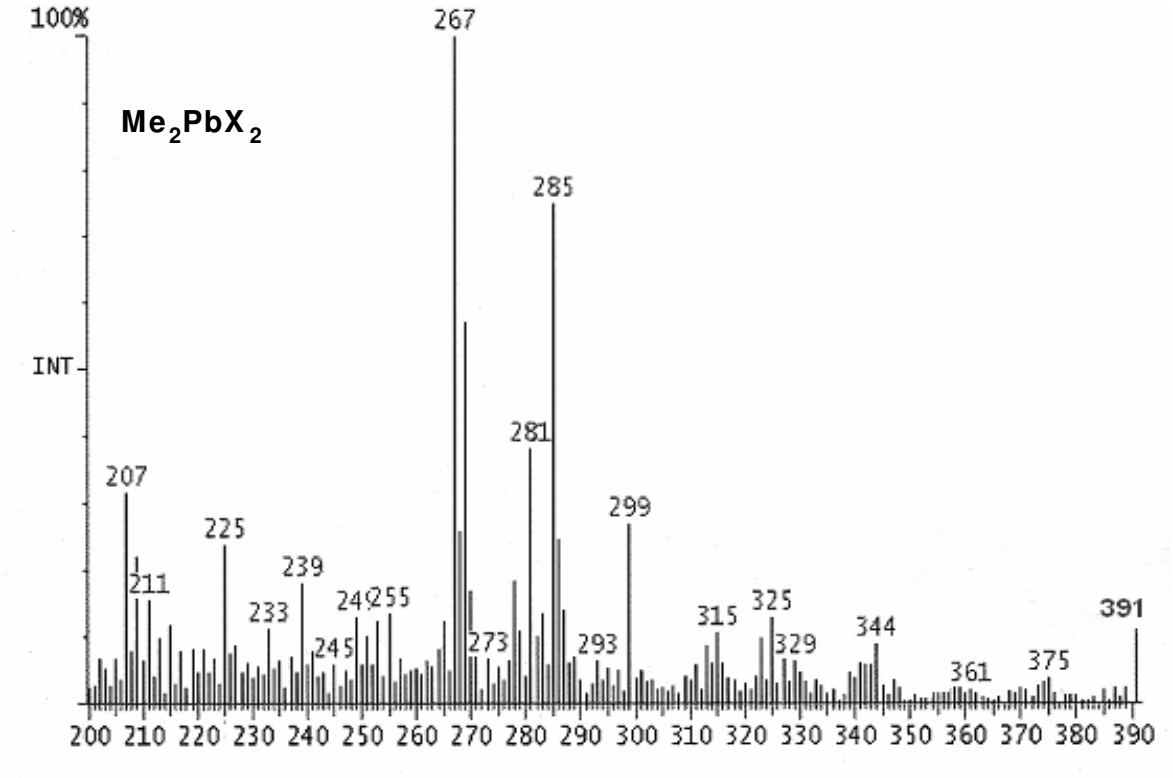


Figure 6.6: Dimethyllead mass spectrometry chromatogram

Environmental water sample analysis

The analytical results are given in table 6.12. The samples with neutral pH and low redox potential do not show any presence of the organolead. This observation support the existence of the Pb (II) ion opposed to Pb (IV) in this kind of environment. The other samples with alkaline pH and high oxidation potential contain at least one organolead. The dominance of Me_3PbX is due to its stability in aquatic media. Me_4Pb was only detected in highly alkaline samples which favorites its stability.

Table 6.12: Analysis of environmental samples (standard deviation of three replicates in brackets)

Sample	**pH**	**ORP (mv)**	**Total Pb (µg L^{-1})**	**Me_4Pb (µg L^{-1})**	**Me_3PbX (µg L^{-1})**	**Et_4Pb (µg L^{-1})**	**Et_3PbX (µg L^{-1})**
Brixton (Park station)	7.02	204	123.3 (9.9)	ND	ND	ND	ND
Smit (suburb)	7.92	684	57.8 (11.2)	ND	ND	ND	11.4 (4.5)
De Korte (petrol station)	8.53	724	48.9 (8.7)	ND	ND	9.4 (6.9)	17.9 (7.3)
Jorissen (petrol station)	7.01	234	204.4 (9.5)	ND	ND	ND	ND
Jan Smuts (highway junction)	7.52	712	98.2 (8.3)	ND	7 (6.7)	ND	14.3 (5.9)

The extraction of organolead compounds using SLMP in aqueous samples to organic acceptor solution by phenylation derivatisation was found to be successful.

We would recommend to the authority an investigation into the application and use of unleaded gasoline in Southern Africa.

6.4. Sorption of tetraalkyllead and dibutyltin compounds sorption

In chapter one the use and regulations of the organolead and organotin compounds were discussed. Whereas these regulations try to cut down their introduction into environment, we should also be looking to their pathways. This section discusses the adsorption and desorption of the tetraalkyllead (Me_4Pb and Et_4Pb) and dibutyltin (Bu_2SnCl_2). We chose to study tetraalkyllead due to their high toxicity compared to their homologues. They are also subject to other transformations in the environment. On the other hand the choice of dibutyltin is due to their use in domestic material, several studies were done on the tributyltin in marine and coastal environmentals. Looking at continental level the dialkyltin are the one which are more applied.

6.4.1. Sample characterisation

The sorption processes were performed on soil samples. The soil samples (S1, S2, S3 and S4) were collected randomly in various area in South Africa. Plastics bags (500g each) were used for collection, labelled and transported to the lab. They were dried in an oven at 70°C and sieved to 2 mm. Soil samples were afterwards ground, sorted to remove rock particles, stones and organic materials like grass. Samples were stored at -4°C prior to analysis.

Table 6.13: Soil samples properties

Parameters	S1	S2	S3	S4
Loss on ignition %	6.48	3.75	2.78	1.87
OM	11.15	6.45	4.78	3.21
CEC	652.6	500.15	258.45	224.15
Sand %	12.46	17.43	20.68	27.33
Silt %	55.19	63.32	68.60	67.55
Clay %	32.35	19.25	10.72	5.12
pH (soil)	6.55	6.82	7.07	6.72
pH (rainwater))	6.32	6.52	6.92	6.68
Al	215.500 (0.2)	162.600 (0.6)	149.100 (0.3)	0.847 (2.5)
Ca	430.300 (0.9)	336.900 (1.0)	88.800 (0.4)	4.509 (0.1)
K	289.700 (0.9)	207.000 (1.0)	182.800 (1.1)	221.511 (0.3)
Li	1.700 (0.1)	17.000 (0.1)	1.700 (0.1)	0.016 (0.1)
Mg	295.500 (1.1)	213.800 (0.5)	48.500 (0.3)	0.076 (0.5)
Pb	3.200 (8.4)	nd	nd	0.0170 (9.1)
Si	740.000 (0.9)	2.657 (1.6)	623.700 (0.4)	0.239 (1.1)
Sn	0.008 (1.3)	nd	0.006 (2.2)	0.004 (2.4)

CEC: cation exchange capacity, OM: organic matter

The sorption of organometallic on soil depends on its properties [Hoch, 2001, Berg, 2001]. The main parameters which influence the sorption were investigated (table 6.13).

The CEC is defined as the sum of the exchangeable cations of a soil. The calculation of the cation exchange capacity was done using the equation 6.1

CEC (meq/100g) = ex Ca + ex Mg + ex K + ex acidity (ex :exchangeable) (6.1)

The exchangeable acidity in the equation 6.1 was determined by titration. The soil was first leached by potassium chloride then titrated with sodium hydroxide (NaOH). The ex. Acidity calculated using the equation 6.2.

Ex. acidity (meq/100g) = (ml $NaOH_{sample}$ – ml $NaOH_{blank}$) X 10 (6.2)

Malvern mastersize 2000 was used for the determination of the soil texture (sand, silt and clay).

The combustion of soil at 450 °C gave the organic matter (carbon content) present in soil. The use of very high temperature can lead to a loss of some structural water from clay or loss of volatile minerals (500 °C), at too low temperature there is a risk of incomplete combustion. The combustion at 450 °C was used as an optimum. The organic matter was calculated based on loss on ignition (equation 6.3), which was multiplied by a convenient factor [Evangelou, 1998].

Loss on ignition (%) = (weight loss (g) X 100)/oven-dry weight (g) (6.3)

The cations found in table 6.13. were determined by ICP-OES. Lead was found in two soil samples, which conduct on the use of sodium tetraphenylborate to avoid the interference when sodium tetraethylborate is used for derivatisation. Tin concentration was substracted from the concentration obtained during dibutyltin sorption study.

6.4.2. Sorption experiment

Chemicals

Tetramethyllead (TML) 65%, tetraethyllead (TEL) 95%, trimethyllead (TiML) 95%, dibutyltin (DBT) 98%, sodium acetate, acetic acid buffer and hexane were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium tetraphenylborate was from Across (Geel Belgium).

Sample preparation

The sample was buffered to pH 6 with acetic acetate; hexane was added prior to the derivatisation agent ($NaPh_4B$). The mixture was manually shaken for 10 minutes and allowed to equilibrate for 5 minutes. The organic phase was then collected and stored at 4 ºC for further GC-MS analysis.

Adsorption and desorption experiment

The batch technique was used to determine the adsorption of tetraalkyllead and dibutyltin. Rainwater was used as leaching solution

A solution of artificial rainwater was prepared with major cations and anions in a similar composition of Southern Africa rainfall [Mphepya et al., 2004] but without organic components or particulates. The artificial rainwater were spiked with tetralkyllead solutions to yield concentrations ranging 50-250 µg L^{-1}, and then mixed with soil. The suspensions were shaken in the dark at room temperature for different time depending on the experiment. After shaking, the samples were

centrifuged for 10 min. 10-20 ml of supernatant were transferred to the vials for derivatisation and further analysis. The remaining adsorbent was stored in a refrigerator, for desorption experiments.

The same experiment was performed for dibutyltin sorption but no derivatisation step was needed (section 6.1.2). Dibutyltin was determined as total tin by ICP-OES. The final concentration was obtained by subtracting the initial concentration of tin found in blank samples.

In desorption experiments, the supernatant was poured off after centrifuging, and the remaining adsorbent was rinsed once with Milli-Q water for short time and the experimental vessel was refilled with rainwater. Sample preparation and further steps were carried out in the same way as described in adsorption experiment.

6.4.3. Adsorption kinetics

Equilibrium between solution and adsorbed or sorbed phases is a condition commonly used to evaluate adsorption or sorption processes in soils or soil-clay minerals [Evangelou, 1998]. The adsorption kinetics of tetraalkyllead and dibutyltin were studied on sample soil S1.

The figure 6.7 shows that the equilibrium was reached in all the tetraalkyllead compounds after 24 hrs.

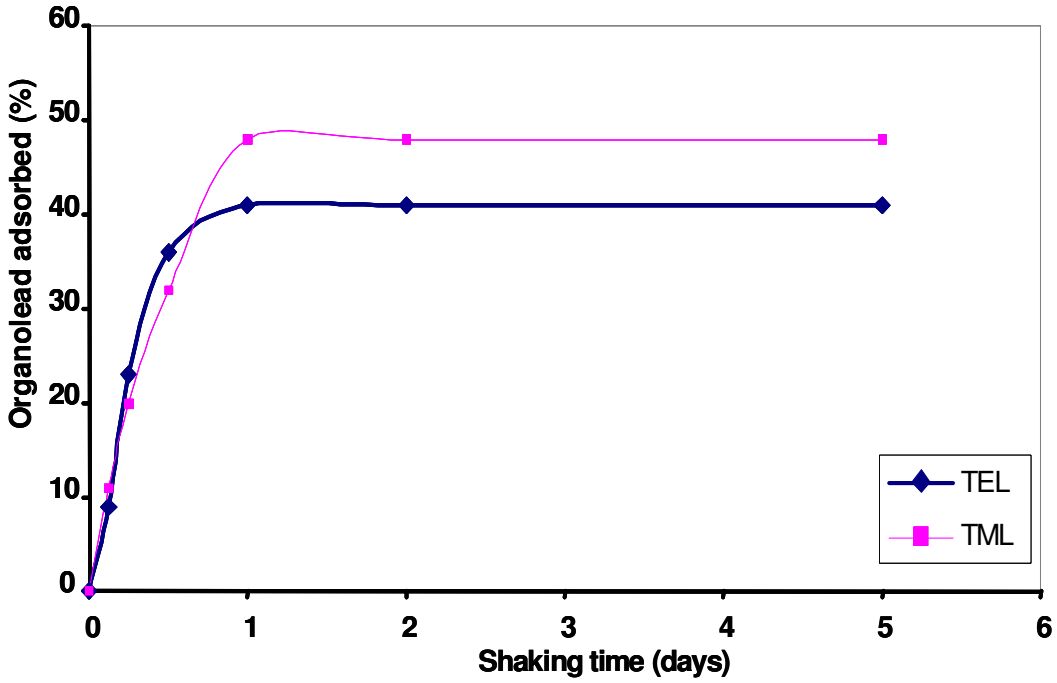


Figure 6.7: Adsorption kinetic of tetramethyllead and tetraethyllead

Figure 6.8. shows that dibutyltin adsorption equilibrium was reached at about 12 hrs.

The figures 6.7 and 6.8 indicate different adsorption equilibrium between dibutyltin (Bu_2SnX_2) and tetraalkyllead (Me_4Pb and Et_4Pb). Dibutyltin reached the equilibrium earlier than the tetraalkyllead. This could be influenced by the degree of polarity, the electrostatic attraction between the positively charged molecules and the negatively charged clay mineral surface that are responsible for the adsorption process, Hoch et al (2003) in their study on organotin compound found

that the adsorption was in their polarity order. Dibutyltin being much polar than tetraalkyllead might be adsorbed faster.

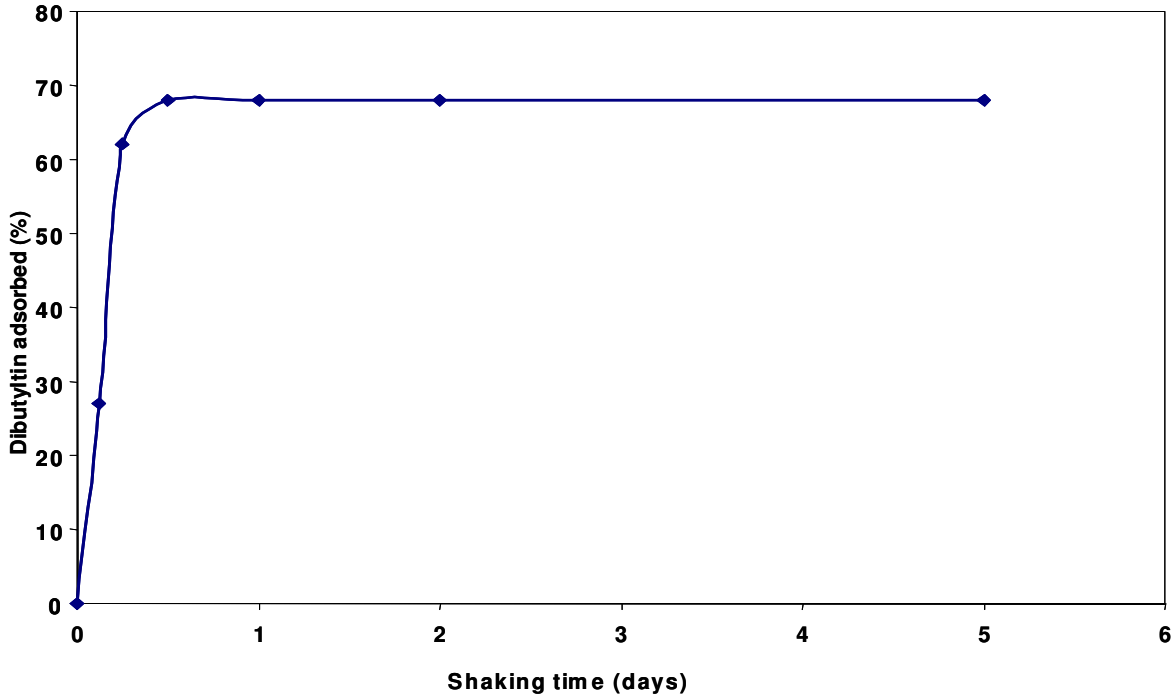


Figure 6.8: Adsorption kinetic of dibutyltin

Note that these equilibrium are not an indication of any saturation on the adsorbent site. The adsorption is a reversible reaction [Huang, 2003]. If the initial adsorbed layer can act as a substrate for further adsorption then, instead of the isotherm leveling off to some saturated value, it can expected to rise indefinitely. The most widely used isotherm dealing with multilayer adsorption was derived by Stephen Brunauer, Paul Emmet and Edward Teller, and is called the BET isotherm

[Knight, 1996, Gas Adsorption]. In this experiment, the adsorption reached an equilibrium.

Other observation is that dibutytin reaches equilibrium at high percentage than tetraalkyllead (TML reaching the higher percentage than TEL). This can be due to homovalent cation exchange which refers to the exchanging cations with similar valence [Evangelou, 1998]. As the sample S1 contains high amount of Ca and Mg, dibutyltin cation (Bu_2Sn^{2+}) may exchange with those cations at high yield than tetraalkyllead. Tetraalkyllead are more likely to be adsorbed by non-polar process, Donard and Weber (1985) obtained same results on methyltin adsorption study.

6.4.4. Adsorption as a function of pH

Figure 6.9 shows that tetraalkyllead are adsorbed strongly at pH 7. Figure 6.10 gives high adsorption of dibutyltin at pH 6.

At higher pH, weak acid metal commonly referred as bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+) can replace strong Lewis acid metals (Al^{3+},Fe^{3+}…),when coordinating cations of higher valence are replaced by cations of lower valence, a deficit of internal positive charge results or conversely a net negative charge on the mineral surface is generated [Evangelou, 1998]. This results in an increase of negative charge at the existing negative charge of soil surfaces. Thus, it would allow the non polar tetraalkyllead to adsorb strongly at higher pH.

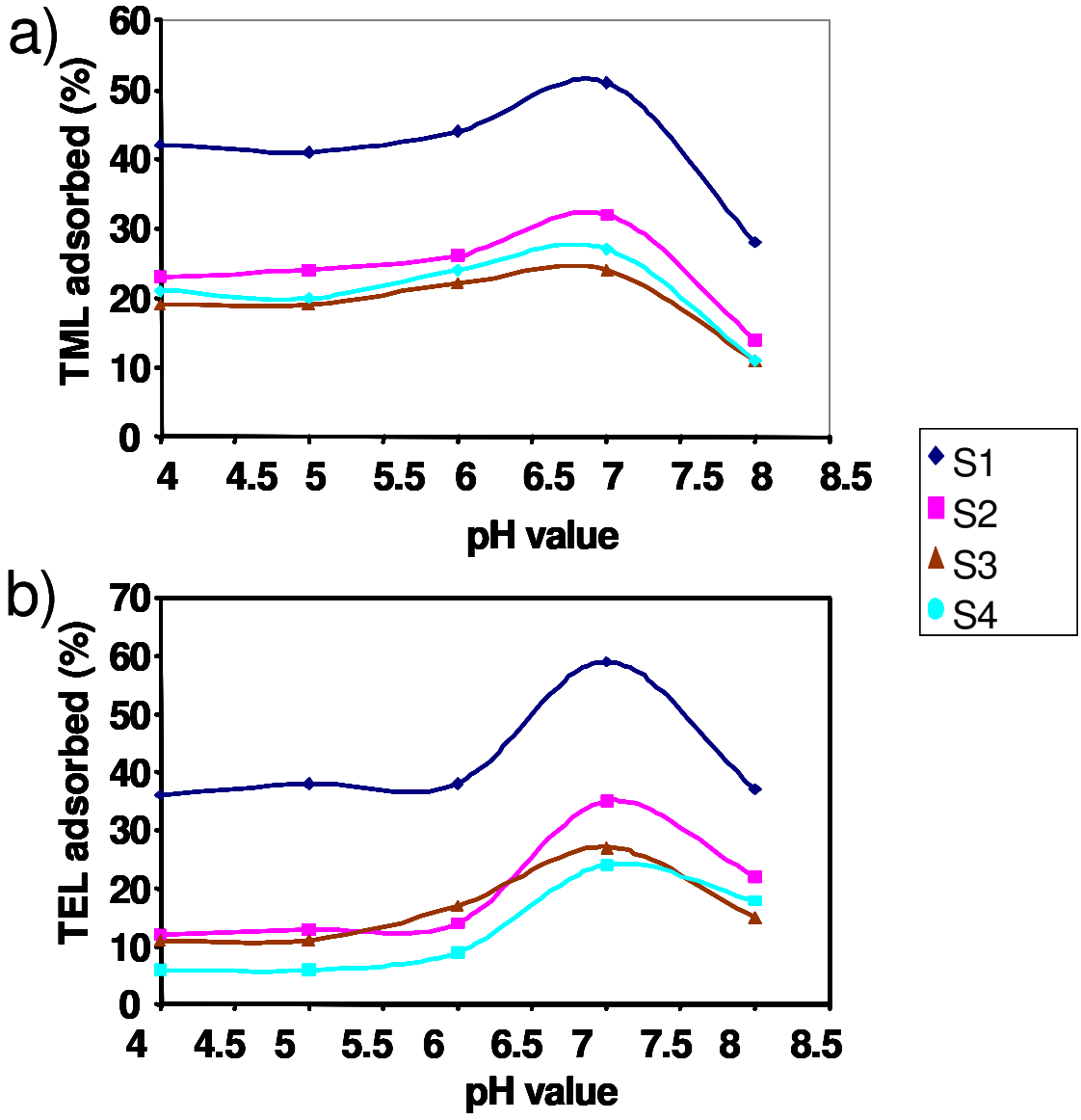


Figure 6.9: Tetramethyllead and tetraethyllead adsorption in function with pH

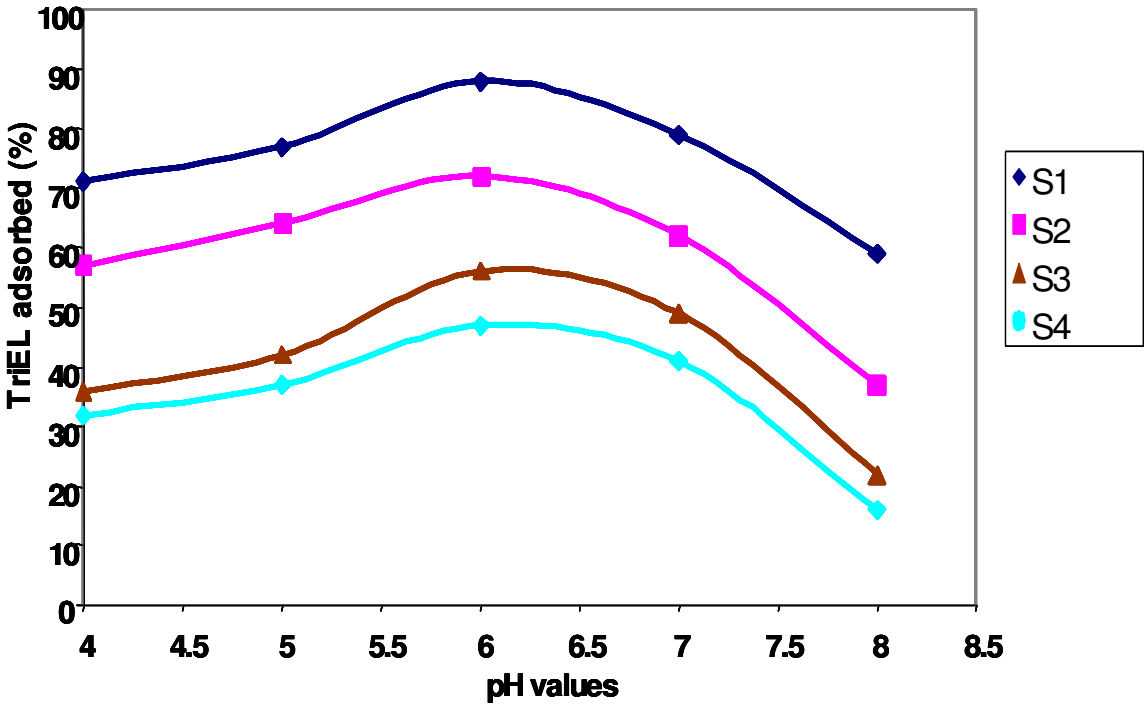


Figure 6.10: Adsorption of dibutyltin in function with pH

6.4.5. Adsorption Isotherms

All adsorption isotherms obtained were linear (figure 6.11 and 6.12). Generally, the linear isotherm describes partitioning, which suggests interaction between a generally hydrophobic adsorbate with a hydrophobic adsorbent [Evangelou, 1998].

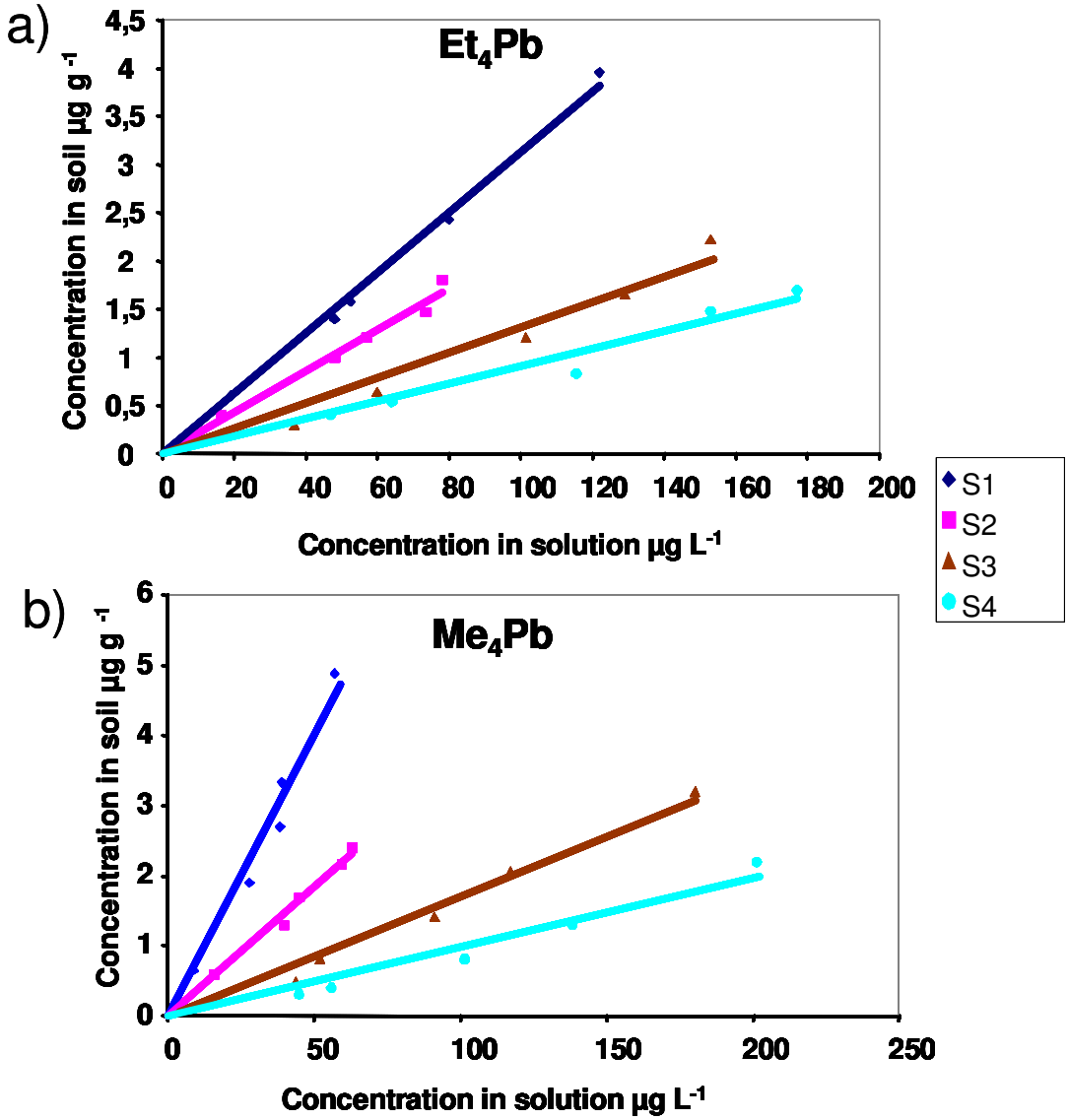


Figure 6.11: Adsorption of tetramethyllead and tetraethyllead on different type of soil

The adsorption coefficient K_d values of tetraalkyllead studied are in order of S1> S2> S3 > S4 (Table 6.14). The order of adsorption follows the trend of clay

content and CEC. Due to the high surface area and reactivity, clay minerals show high sorption capacity to organic contaminants [Hoch et al., 2003].

The K_d value of adsorption of dibutyltin gives an unexpected adsorption trend, S1 > S2 > S4> S3 (Table 6.14). The K_d value for the sample S4 was higher than S3. The S3 sample soil having higher CEC and clay content than S4, we expected the K_d value of S3 to be higher than S4. This could be due to pH variation, for S4 the pH is close to pH 6, at which dibutyltin is strongly adsorbed, while the pH of S3 is closer to natural pH where it was found a high affinity for non polar organometallic compounds.

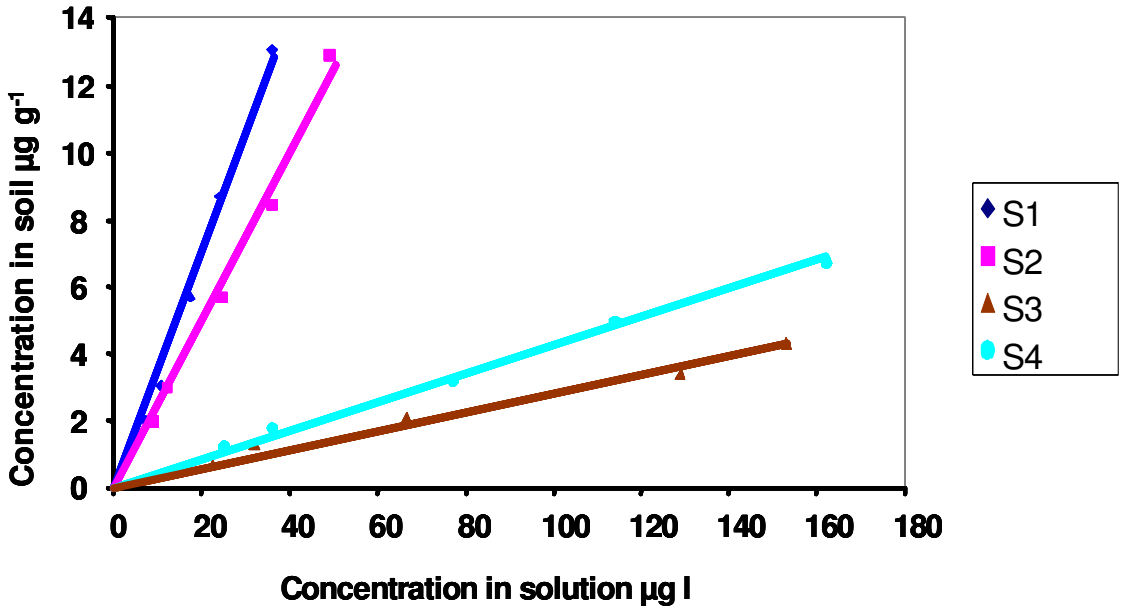


Figure 6.12: Adsorption of dibutyltin on different type of soil

6.4.6. Desorption Isotherms

All desorption isotherms were also linear (figure 6.13 and 6.14). In most cases, K_d values of desorption follow a reverse trend of their K_d values of adsorption except S3 and S4 for tetramethyllead (table 6.14). As the values of CEC are close for these two types of soil and organic matter content being low, the only parameters that might be influencing desorption are clay content and pH. The mineral surface components for adsorption are the so-called functional groups. In the case of minerals with permanent charge, the functional group is the siloxane or ditrigonal cavity, in the case of clay –mineral edges, the surface functional groups are -OH species capable of dissociating hydrogen. There are three such potential functional groups on clay minerals. The first group is the -Al-OH (octahedral) or aluminol with pKa of around 5, the second is silanol (-Si-OH) (tetrahedral) with a pKa of around 9, and the third is the intermediate –Si-Al-OH_2, or a Lewis acid site (OH is shared between a tetrahedral sheet and an octahedral sheet), with an apparent pKa of around 6-7 [Evangelou, 1998]. Hingston et al., 1972 in Evangelou (1998) indicate that these pKa are directly proportional to the pH of maximum adsorption. The sample S3 has a pH of 6.92 which is very close to the maximum adsorption pH of tetramethyllead. This sample (S3) contain high amount of silica than S2, and S2 has higher silica content than S4. Putting together all these properties we may suppose that the Si-Al-OH_2 (pKa 6-7) was responsible for the adsorption. Thus, tetramethyllead would be strongly adsorbed on S3 that make desorption on S3 difficult than on others.

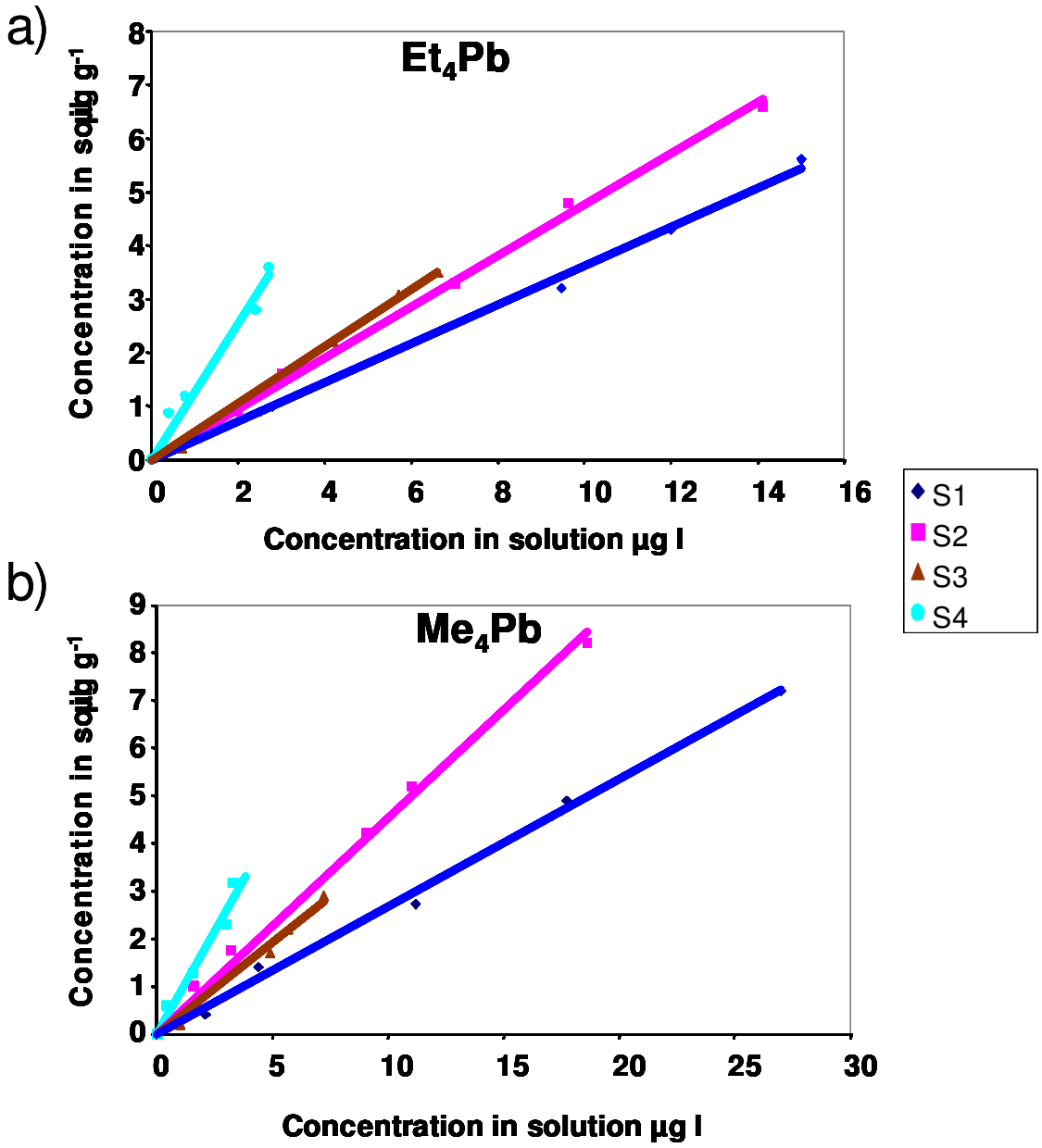


Figure 6.13: Desorption of tetramethyllead and tetraethyllead in different type of soil

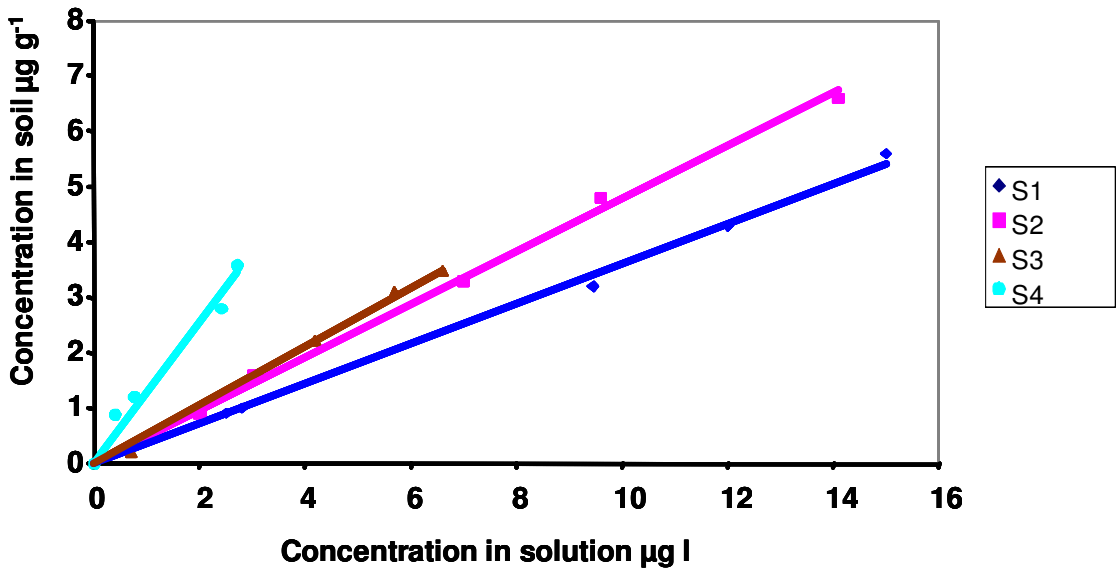


Figure 6.14: Desorption of dibutyltin on different type of soil

Desorption and adsorption comparison

Almost all K_d values for desorption are higher than the K_d adsorptions, which shows that the adsorption process is reversible (table 6.14). Dibutyltin is strongly adsorbed than the tetraalkyllead, the percentage adsorption ranges between 48.8 and 88.3, for Et_4Pb ranges between 9.1 and 38.3, and for Me_4Pb, the adsorption ranges between 24.9 and 44.2. The desorption of Bu_2SnX_2 was obtained between 9.4 and 23.7 %, for Et_4Pb ranges between 19.3 and 38.9 and for Me_4Pb it was found to be between 21.5 and 32.7%. These results show that the non-polar organolead can be easily leached than the ionic forms.

Although the organometallic compounds subject to these study (Et_4Pb, Me_4Pb and Bu_2SnX_2) have both metallic and organic characters, their adsorption coefficient in

soils were of similar magnitudes to those of other heavy metals in soil (270-217000 Pb, 400-7900 Cd and 200-13400 for Zn) [Sauvé et al, 2000], but significantly larger than some organic pesticides (e.g. 0.13-5.0 for mesotrione, 1.8-9.3 for 4-nitrobenzamide).

Table 6.14: Adsorption coefficient and adsorption / desorption percentage of Et_4Pb, Me_4Pb and Bu_2SnX_2

Sample	Kd (l/kg)	Adsorption (%)	Kd (l/kg)	Desorption (%)
S1	314	38.3	3618	19.3
S2	215	14.6	4783	23.8
S3	131	17.2	5302	31.3
S4	91	9.1	12835	38.9

Tetraethyllead

Sample	Kd (l/kg)	Adsorption (%)	Kd (l/kg)	Desorption (%)
S1	803	44.2	2675	21.5
S2	367	26.3	4536	27.3
S3	171	22.2	3864	22.8
S4	99	24.9	8717	32.7

Tetramethyllead

Sample	Kd (l/kg)	Adsorption (%)	Kd (l/Kg)	Desorption (%)
S1	3514	88.3	3007	9.4
S2	2500	72.8	4385	12.3
S3	281	56.5	7902	23.7
S4	425	48.8	5592	17.7

Dibutyltin

The percentages of adsorption are higher than the percentage of desorption almost in all soil samples studied. This explains the very low concentration of organometallic found in lower sediment profile and groundwater samples. These organometallic compounds are more likely to remain few meters from the surface. The sediment and soil would act as sink of these organometallic compounds thus all transformation discussed in chapter 1 may happen at water-sediment or water-soil interfaces, Fent (2004) reported the same observation**.**

6.5. Speciation of organomercury and organotin in sediment, water and plant

This section discuss the results obtained using the method described in section 5.4.3. Some chromatograms obtained are given in appendix 7. The water and sediment samples ware collected in Klipriver (Johannesburg-South Africa) and plants sample in its vicinity. (Figure 6.15)

6.5.1. Sampling and sample preparation

Sampling area

The Johannesburg area forms the watershed between the Crocodile River catchment in the north and the Klipriver catchment in the south [Tutu, 2005]. Thus, there is an interest of sampling in this river. According to the 2001 Statistics South Africa census, the population of the Greater Johannesburg Metropolitan Area is almost eight million. Johannesburg is the site of a large-scale gold mining due to its location on the mineral-rich Witwatersrand range of hills [Wikipedia,

2006, Johannesburg]. On the addition of mining activity that conducted to several dams, in highly populated metropolitan like Johannesburg many industrial activities occur and industrial usage water reachs the natural water system.



Figure 6.15: Klipriver sampling map (Google Earth, February 2007)

The global positioning system (GPS) positions are presented in table 6.15.

Table 6.15: Klipriver GPS data

Sample	GPS (South)	GPS (East)
K1	26º 19.544´	027º 59.258´
K2	26º 19.544´	027º 59.258´
K3	26º 19.586´	027º 59.320´
K4	26º 19.529´	027º 59.511´
G	26º 19.505´	027º 59.487´
S (0-30 cm)	26º 19.529´	027º 59.511´
S (31 – 60 cm)	26º 19.529´	027º 59.511´
S (60 – 85 cm)	26º 19.529´	027º 59.511´
S (85 – 100 cm)	26º 19.529´	027º 59.511´

The field measurement and other parameters measured could be found on the table 6.16. The field measurements taken were pH, temperature, oxidation reduction potential (ORP). K samples are surface water samples, G are ground water samples and S stands for sediment samples

Chemicals and material

All glassware and plasticware were cleaned with a biocide detergent (2% in hot tap water) and rinsed with tap water and then with milliQ water. They were then soaked for 3 days in a 10% (v/v) HNO_3 (technical grade) solution, and rinsed with MilliQ water. All vessels were finally cleaned with 10% (v/v) HCl (technical acid), rinsed with MilliQ water and dried under a laminar flow hood or an oven.

Natural Bu_3SnCl and enriched Bu_3SnCl (^{117}Sn) were obtained from LGC limited (Teddington, U.K). $BuSnCl_3$ and Bu_2SnCl_2 of natural isotope composition were from Aldrich. $HgCl_2$ and CH_3HgCl were obtained from Strem Chemicals. Enriched CH_3HgCl was prepared at LCABIE as described by Rodriguez Martin-Doimeadios et al. (2002a). Sodium acetate–acetic acid buffer, hydrochloride acid, ammonia and isooctane were from Sigma–Aldrich (Steinheim, Germany).

Sample preparation

Klipriver water samples were collected in 250 borosilicate glass bottles with gastight Teflon lined cap and then acidified at 1% (v/v) with ultra pure HCl acid. The bottles were stored in double plastic bag, in the dark at 4°C. Blank samples with milliQ water were also treated the same way to estimate the potential contamination due to the container and the storage.

Sediment samples were collected in polypropylene bottles at different profiles using an auger, then frozen and dry-frozen and stored at 4 °C until analysis

Plants samples were collected, rinsed with milliQ water and kept in polyethylene plastic bag, then frozen and dry-frozen at -50 °C and stored at 4 °C until analysis.

All samples were transported in cooler box by flight from Johannesburg (South Africa) to Pau (France). The analyses were done in LCABIE at Pau University.

Experimental

The analytical protocol was described in section 6.1.3. A sample was spiked with the organotin and organomercury internal standard species then buffered to pH 5. Isooctane was added prior to the derivatisation agent for the same reasons as discussed previously, and then sodium tetrapropylborate added. The mixture was shaken manually for five minute and allowed to equilibrate for 3 to five minute. The organic phase was then collected for separation and detection by GC-ICP-MS.

6.5.2. Mercury and butyltin compounds in water and plant sample

The results obtained are represented in table 6.16. These results indicate a lower range of mercury compounds in environment with inorganic mercury ranging in 1 – 7 ng L^{-1} and monomethylmercury ranging between 5-36 pg L^{-1}. There were no tributyltin compound. This compounds is usually found in marine and coastal water where there is a highly use in antifouling paint. Tributyltin was not detected in water samples. Only mono and dibutyltin were found. Without excluding tributyltin degradation in water column which may lead to the other forms of butyltin (mono- and di-), we could say that the reason of that high concentration is the use of PVC and other material such as pesticide containing mainly dibutyltin than tributyltin.

Table 6.16: Speciation of mercury and butyltin compound in water samples

Sample	pH	ORP	Temp°C	HgX_2	MeHgX	$BuSnX_3$	Bu_2SnX_2	Bu_3SnX
K1	7.23	407	15.8	0.85 (1.2)	15.65 (4.1)	7.32 (1.8)	36.05 (1.9)	ND
K2	7.49	408	18.3	6.89 (2.6)	5.55 (2.7)	10.28 (2.6)	21.44 (5.2)	ND
K3	7.54	466	17.2	2.64 (1.3)	24.71 (4.6)	19.7 (4.3)	9.83 (3.3)	ND
K4	7.48	423	18.0	4.65 (3.3)	36.16 (4.4)	17.79 (5.5)	11.50 (2.1)	ND
G	7.30	66	16.6	4.09 (2.4)	19.43 (1.1)	3.78 (0.9)	2.31 (1.2)	ND

Concentration are ng L^{-1} (pg L^{-1} for MeHgX), RSD in brackets

The samples were collected in April 2006, which is the beginning of winter period in South Africa. The concentrations of mercury or butyltin compounds might be different in summer where there is heavy rainfall that will discharge different particles in rivers. This can be a controversial discussion as long as it is known that in winter time there is preconcentration of pollutant due to the absence of rain. But we should take into account the fact that these organometallic are adsorbed strongly to sediment and soil and don't reach the groundwater which serve as the tributary of rivers and streams. Therefore the only main source of the organometallic compounds might be a direct spillage in water, or rainfall discharge and seasonal streams discharge. A slight seasonal variation with a total

mercury concentration minimum in May and maximum in August was also observed by Baker et al., (2002).

On the other hand in plant samples (Table 6.17), tributyltin was in detectable concentrations. Its concentration might be too low, but it gives indication of the presence of tributyltin in the Klipriver. This may due to bioaccumulation of tributyltin.

This presence of tributyltin in plant sample is also a good indication of bioconcentration. As there is no tributyltin detected in water, the plant has uptaken the tributyltin at a long period.

Table 6.17: Speciation of mercury and butyltin in plant samples

Plants	**HgX_2**	**MeHgX**	**$BuSnX_3$**	**Bu_2SnX_2**	**Bu_3SnX**
P1	9.76 (2.9)	0.50 (8.8)	12.07 (2.5)	26.45 (4.4)	0.67 (5.3)
P2	68.37 (7.2)	0.48 (9.5)	9.65 (4.3)	11.2 (5.1)	0.23 (5.5)
P3	26.39 (5.9)	0.52 (7.7)	4.78 (3.3)	14.07 (6.6)	ND
P4	95.64 (6.9)	4.91 (6.5)	2.02 (7.3)	32.48 (1.2)	0.87 (7.2)

Concentration in ng g^{-1} dw

6.5.3. Mercury and butyltin compounds in sediment sample

The trend of butyltin compound in sediment is shown by the figure 6.16. The same observation as discussed previously for water and plant sample can be made. As you move away from the surface the concentration of dibutyltin (DBT) reduces

and the concentration of monobutyltin (MBT) increases. This could be explained by the degradation of dibutyltin in monobutyltin compounds. As there is very low concentration of tributyltin the same observation can be made for the tributyltin degradation.

Table 6.18: Sediment samples field measurement for depth profile

Samples	**pH**	**Temp. ºC**	**ORP**
S (0 – 30 cm)	6.93	17.5	82
S (30 – 60 cm)	7.04	17.4	2
S (60 – 85 cm)	7.01	17.4	-11
S (85 – 100 cm)	6.84	17.6	-111

The high concentration is observed in the first 30 cm and it reduces below that depth. This confirms the high adsorption capacity of organotin on the sediment, the later acting as a sink of these organometallics. Monobytyltin and dibutyltin were found to be strongly adsorbed on organic and mineral sediment, but the tributyltin has a small mobility in the mineral sediment. The maximum adsorption of organotin compounds in the sediment was found in samples when the pH of the sediment is close to the pKa of organotin compounds [Huang and Matzner, 2004]. The pKa of organotin compounds are comprised between 6 and 7 and the pH of the sediments were between 6.8 – 7.0. (Table 6.18). The remobilisation of these

butyltin becomes difficult, that explains the low concentration found below the 30 cm.

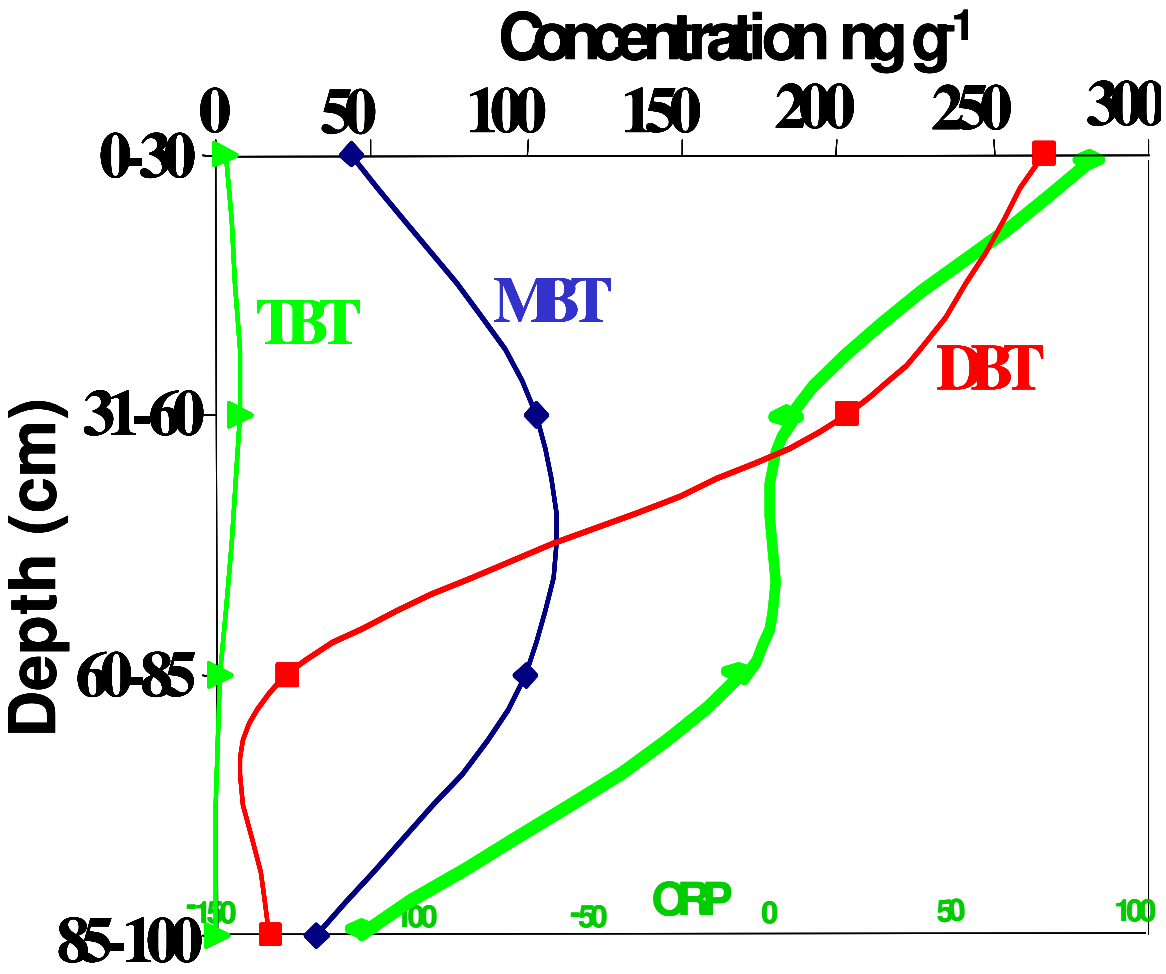


Figure 6.16: Speciation of butyltin in sediment samples

It was also found that the variation of ORP goes with the debutylation transformation (Figure 6.16). Assuming that the process of debutylation happen

between 60 and 85 cm there is an increase in monobutyltin and a decrease in tributyltin, simultaneous the ORP does not change.

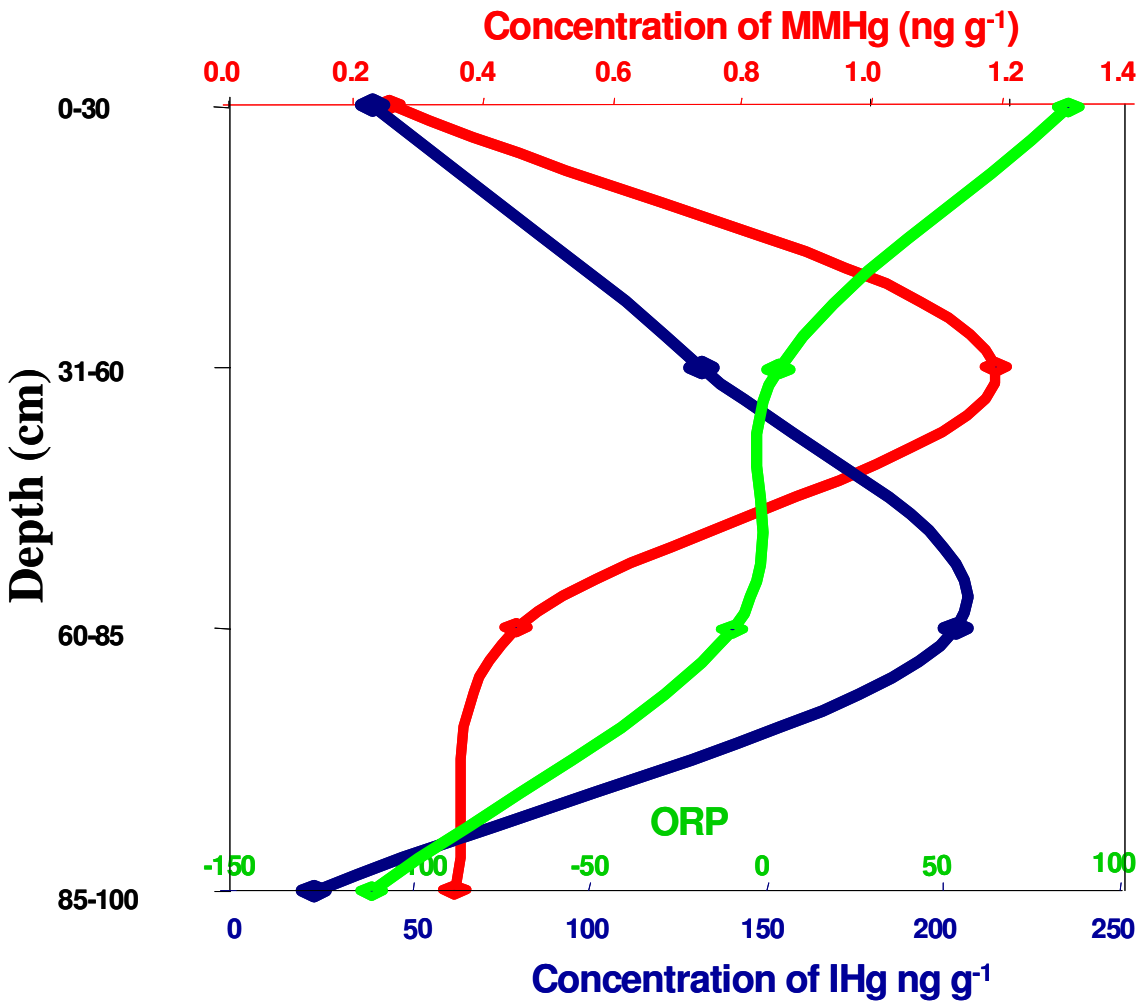


Figure 6.17: Speciation of mercury compounds in sediment samples

The mercury compounds have been found in sediment at lower level, MeHgX being in the range of 0.2-1.2 ng g^{-1} and HgX2 in the range of 20 – 200 ng g^{-1}. Looking at the trend shown on the figure 6.17, a change was observed in

methylmercury and inorganic mercury concentrations at static reducing redox potential between 30 and 60 cm depth. At 30 cm there is a high concentration of monomethylmercury and lower concentration of inorganic mercury, and the opposite observation can be noticed at 60 cm. In chapter one, the methylation processes were discussed, moving away from the surface the redox potential becomes low, favorising reducing processes. Thus the inorganic mercury could be reduced to monomethylmercury conducting to the lowering in its concentration. It looks like this process happens in thin layer with reducing conditions. This trend was also observed by Gagnon et al (1996).

CHAPTER 7

SUMMARY AND CONCLUSIONS

The speciation of organometallic compounds of tin, lead and mercury in environmental samples was achieved using different analytical methods.

Development of new methods in analytical chemistry is for a high interest especially when you have to deal with speciation of chemical compounds in real environmental samples.

A speciation method using a new derivatisation agent (Et_4BMgBr) was developed. This method was tested with Pb, Sn, Hg organometallics. It allows *in situ* derivatisation of environmental samples. Four organoboron compounds can be now used for derivatisation purposes; those are $NaEt_4B$, $NaPr_4B$, Pr_4BMgBr and Et_4BMgBr.

A new extraction method was developed and optimised. This is a miniaturised Supported Liquid Membrane Probe extraction (SLMPE). Good recoveries and extraction efficiency were obtained. It was applied to organotin compounds [Cukrowska et al, 2004] and organolead compounds [Nsengimana et al, 2004] successfully.

Several analytical procedures were developed using either the existing sample preparation method (derivatisation by $NaEt_4B$ or $NaPr_4B$ and extraction by liquid-

liquid extraction or microwave extraction) or the new sample preparation using Et_4BMgBr or/and supported liquid membrane probe extraction.

In addition to these methods, a number of applications were done in environmental conditions for speciation of tin, lead and mercury compounds in different matrix.

Tetraalkyllead were found to be degradable in aqueous media and their degradation depend strongly to the water composition. They were found to degrade faster in hard water than in soft water. In a period of a month the tetraalkyllead can undergo degradation at least till trialkyllead.

During organotin speciation study in water and sediment samples taken from different area in South Africa, low or nil concentrations of Bu_3SnX were generally found. Bu_2SnX_2 compounds were found to be high than other butyltin. The reason being may be their pathways such as the use of material containing Bu_2SnX_2 in the area or debutylation / butylation processes. Bu_2SnX_2 may be a result of Bu_3SnX degradation or $BuSnX_3$ butylation. Although, Bu_2SnX_2 compounds can be produced that way, on the other hand the study on organotin sediment indicated possible degradation of dibutyltin to monobutyltin which reduces their concentration in environment.

It was also found that both organotin and organolead compounds are strongly adsorbed on soil. The soil will act as a sink the same way as sediments do.

Mercury compounds speciation indicated lower amount of these species in Klipriver (South Africa) water and sediment samples, and in plant samples taken from its vicinity. Different distribution trend of inorganic mercury and methylmercury was found in sediment samples. This could be a result of possible methylation processes or a simple different migration of mercury compounds in sediment. These analyses were done in LCABIE (Pau-France) using GC-ICP-MS hyphenated technique. It was a success with very low LOD. This instrumental technique is planed to be developed at University of the Witwatersrand-Johannesburg.

This thesis has added it own to the poor literature found in South Africa on speciation of organometallic compounds. Still many studies have to be done in South African environment such as the processes concerning natural alkylation and dealkylation of tin and mercury compounds and speciation of organolead compounds and their environmental stability.

PUBLICATIONS

Cukrowska, E.; Chimuka, L.; Nsengimana, H. and Kwaramba, V. (2004). Application of supported liquid membrane probe method for Extraction and preconcentration of organotin compounds in Environmental water sample, Analytica Chimica Acta, 523, 141 - 147

Nsengimana, H.; Cukrowska, E.; Dinsmore, A. and Chimuka, L., Speciation of alkyllead in aqueous samples with Application of Supported Liquid Membrane Probe for extraction and preconcentration, submitted to Journal of Separation Science.

CONFERENCE PRESENTATIONS AND SEMINARS

Hermogène Nsengimana, In situ derivatisation of organotin and organomercury species in sediment and plant samples by tetraethylborate magnesium bromide-Determination by GC-ICP-MS, the 38th Convention of the South African Chemical Institute, Durban, 3-8 december 2006. *Oral presentation*

Hermogène Nsengimana, Speciation of organomercury and organotin compounds by isotope dilution GC-ICP-MS: Application to water, soil and biological samples Analitika 2006 Conference, South africa, Pilanesberg 10th - 13th September 2006. *Oral presentation*

Hermogène Nsengimana, GC coupled to ICP-MS: Application to speciation of organomercury and organotin compounds in water, sediment and biological samples, SPECTRO, South Africa, Pretoria, 14th September 2006. *Oral presentation*

Hermogène Nsengimana, Organometallic compounds: Sample preparation, modern extraction technologies as sample preparation for environmental, pharmaceutical, biomedical and food speciation analysis, University of the Witwatersrand, Johannesburg, 7 – 8 September 2006, , *Oral presentation*

Ewa M. Cukrowska, Luke K. Chimuka, Hermogène Nsengimana, , Supported liquid membrane extraction probe (SLM EP) for study of heavy metals speciation in environmental matrices, 34th International Symposium on Environmental Analytical Chemistry, June 4-8, 2006 Hamburg, Germany. *Poster presentation*

Hermogène Nsengimana, Ewa Cukrowska, Andy Dinsmore, Speciation of Alkyllead after degradation of tetramethyllead in aqueous media, the 37th Convention of the south African Chemical institute, Pretoria 4-9 July 2004. *Oral presentation*

Hermogène Nsengimana, J. B. Rwagaju Rulinda, Contribution aux paramètres physico-chimiques des Eaux du bassin du Nil et du Congo au Rwanda. Etat de Recherche au Rwanda, UNR, 10-16 July 2001. *Oral presentation*

REFERENCES

Aguerre, S., Lespes, G., Desauziers, V., Potin-Gautier, M. **(2001)**. Speciation of organotins in environmental samples by SPME-GC: comparison of four specific detectors: FPD, PFPD, MIP-AES and ICP-MS, J. Anal. At. Spectrom. 16, 263–269.

Adelman, D., Hinga, K. R. and Pilson, M. E. Q. **(1990).** Biogeochemistry of Butyltins in an Enclosed Marine Ecosystem, Environ. Sci. Technol. 24, 1027.

Alexander, M., (2005), www.southafrica.info/ess_info/sa_glance/ sustainable/ ethanol-120905.htm: Ethanol Africa: clean maize fuel.

Ali, I. and Aboul- Enein, H. Y. (2006). Instrumental Methods in Metal Ion Speciation (Chromatographic Science), New York, John Wiley, USA..

Allafrica. (2006), www.baselpretoria. org.za/Jan06.htm: slow progress on phase out of leaded fuel in SADC.

Allard, B. and Arsenie, I. (1991). Abiotic reduction of mercury by humic substances in aquatic system - an important process for the mercury cycle water, Air and Soil Pollution, 56, 457.

Amouroux, D., Tessier, E. and Donard, O.F.X. **(2000).** Volatilization of organotin compounds from estuarine and coastal environments. Environ. Sci. Technol., 34, 988-995.

Amouroux, **D.,** Tessier, E., Pécheyran, C. and Donard, O.F.X., **(1998).** Sampling and probing volatile metal(oid) species in natural waters by in situ purge and trapping

followed by gas chromatography and inductively coupled plasma mass spectrometry (P-CT-GC-ICPMS). Anal. Chim. Acta, 377, 241-254.

Amouroux, D., Wasserman, J.C., Tessier, E. and Donard, O.F.X., **(1999)**. Elemental mercury in the atmosphere of a tropical Amazonian forest (FrenchGuiana). Environ. Sci. Technol., 33, 3044-3048.

Anderson, B., Unger, M. and Moore, K.A. **(2002).** Fate of tributyltin in a created wetland. Environ. Tox. and Chem.,1176-1183.

Anton Paar GmbH. (2002), microwave 3000

Arnold, C. G., Berg, M., Muller, S. R., Dommann, U. and Schwarzenbach, R. P. **(1998)b.** Determination of Organotin Compounds in Water, Sediments, and Sewage Sludge using Perdeuterated Internal Standards, Accelerated Solvent Extraction, and Large-Volume-Injection GC/MS. Anal. Chem. 70, 3094-3101

Arnold, C.G. (1998)a. Triorganotin compounds in natural waters and sediments: aqueous speciation and sorption mechanisms. Ph.D. thesis, Swiss Federal Institute of Technology (ETH), Zűrich.

Ashby J. R. and Craig P. J. **(1989).** New method for the production of volatile organometallic species for analysis from the environment; some butyl tin levels in U.K. sediments, The Science of The Total Environment, 78, 219-232

Astruc M., Pinel, R. and Astruc A. **(1992).** Determination of tributyltin in sediments

by hydride generation/GC/QFAAS, Mikrochim. Acta, 109, 73.

ATSDR (Agency for Toxic Substances and Disease Registry), **(1992)**. Toxicological profile for Tin, TP-91/27.

Baena, J. R., Gallego, M., and Valcarcel, M. **(2001).** Comparison of Three Coupled Gas Chromatographic Detectors (MS, MIP-AES, ICP-TOFMS) for Organolead Speciation Analysis , Anal. Chem, 73, 3927-3934.

Baena J.R., Cárdenas S, Gallego M, Valcárcel M **(2000).** Speciation of inorganic lead and ionic alkyllead compounds by GC/MS in prescreened rainwaters, Anal Chem 72,1510–1517.

Baker, P.G.L.; Brunke E.G; Slemr, F. and Crouch A.M. **(2002).** Atmospheric mercury measurements at Cape Point, South Africa, Atmospheric Environment, 36, 2459–2465.

Barkay, T., Miller, S. and Summers A.O. **(2003).** Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27, 355-384.

Barnard, T. E. (1995). Environmental sampling. In: Chemometrics in Environmental Chemistry – Statistical Methods. Edited by Einax J., Springer Verlag, German, Vol 2. Part G, 1- 47.

Barnes, R. D., Bull, A. T. and Poller R. C. **(1973)**. Studies on the persistence of the organotin fungicide fentin acetate (triphenyltin acetate) in the soil and on surfaces exposed to light. Pestic. Sci. 4, 305–317.

Batley, G.E. (1991). Trace element speciation: Analytical Methods and Problems, CRC Press.Inc, United State.

Berg, M., Arnold, C.G., Müller, S.R., Mühlemann, J., Schwarzenbach, R.P. **(2001).** Sorption and desorption behavior of organotin compounds in sediment-pore water systems. Environ. Sci. Technol., 35, 3151-3157.

Bergan, T. and Rodhe, H. (1999). Mercury in the Global Troposphere: A Three Dimensional Model Study, in J. Barbosa, R. Melamed and R. Villas Bôas (eds), Mercury as a Global Pollutant–5th International Conference, May 23–27, 1999, Rio de Janeiro, Brazil. CETEM-Center for Mineral Technology, Rio de Janeiro, Brazil, p. 104.

Bergmann K. and Neidhart B., (2001). In situ propylation of ionic organotin and organolead species in water samples-Extraction and determination of the resulting tetraorganometallic compounds by gas chromatography-atomic absorption spectrometry, J. Sep. Sci. 2001, 24, 221-225

Bloom, N.S. and Fitzgerald, W.F. (1988). Determination of volatile mercury species at the picogram level by low temperature gas chromatography with cold-vapor atomic fluorescence detection. Analytical Chimica Acta, 208, 151-161.

Blunden, S.J. and Chapman A. (1986). Organotin compounds in the environment. In: Organometallic compounds in the environment. Principles and Reactions; Craig, P.J., ed; Longman, London, 111-159.

Blunden, S.J., Hobbs, L.A., Smith, P.J. **(1984).** The environmental chemistry of organotin compounds, Bowen H.J.M. (Ed), Environmental chemistry. The royal society of chemistry, London, pp 49 – 77

Bouyssiere, B., Szpunar, J., Lobinski, R. **(2002)**. Gas chromatography with inductively coupled plasma mass spectrometric detection in speciation analysis, Spectrochimica Acta Part B., 57, 805–828.

Bowles, K. C., Apte S. C. and Hales, L. T. **(2003).** Determination of butyltin species in natural waters using aqueous phase ethylation and off-line room temperature trapping. Anal. Chim. Acta, 477, 103 - 111.

Brown, H.C. and Racherla, U.S. (1986)a. An unusual solvent effect in the reaction of ethylmagnesium bromide with triethylborane in ethyl ether or tetrahydrofuran.A simple direct route to tetraorganylborate complexes, organometallics, 5, 391-393.

Brown, H.C. and Racherla, U.S. (1986)b, organoborones.43. A convenient, highly efficient synthesis of triorganylboranes via a modified organometallic route, J. Org. Chem.,51, 427-432

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., Burt, G.R. **(1986)**. The decline of the gastropod Nucella lapillus around south-west England : Evidence for the effect of tributyltin from antifouling paints. J. Mar. Biol. Assoc. UK, 66, 611-640.

Budavari, S. ed. (1989). The Merck Index: an encyclopedia of chemicals, drugs and biologicals, 11th ed. Rahway, New Jersey, Merck and Co., Inc.

Bulten, E. J. and Meinema, H.A. (1991). Tin. In: Merian E, ed. Metals and their compounds in the environment. Weinheim, Germany: VCH, 1243-1259.

Burger, J. (2003). Effect of deep-frying fish on risk from mercury Journal of Toxicology and Environmental Health, Part A, 66, 817–828

Bushong, S.J., Hall, L.W., Hall, W.S.; Johnson, W.E., Herman, R.L. **(1988)**. Acute toxicity of TBT to selected Chesapeake Bay fish and invertebrates. Water Res., 22, 1027-1032.

Cai, Y., Monsalud, S., Jaffe, R. and Jones, R.D. **(2000).** Gas chromatographic determination of organomercury following aqueous derivatization with sodium tetraethylborate and sodium tetraphenylborate-Comparative study of gas chromatography coupled with atomic fluorescence spectrometry, atomic emission spectrometry and mass spectrometry, J. Chromatogr. A. 876, 147–155.

Cai Y., Rapsomanikis, S. and Andrea, M. O. **(1993).** Determination of butyltin in river sediment samples by gas chromatography - atomic absorption spectrometry following in situ derivatisation with sodium tetraethylborate, Journal of analytical atomic spectrometry, 8, 119-125.

Cassi, R., Tolosa, I., Bartocci, J. and De Mora, S. **(2002).** Organotin speciation analyses in marine biota using sodium tetraethylborate ethylation and gas chromatography with flame photometric detection." Appl. Organometal Chem. 16, 355-359.

CEE, (1998). Directive 98/83/CE du Conseil du 3 novembre 1998 Communauté Européenne.

CEE, législation communautaire en vaguer, document, 391L015, **(1999)** In : http://www.admi.net/eur/loi/leg_euro/fr_391L0157.html

Centineo, G., Rodríguez – González, P., González B. E.,García Alonso J. I. and Sanz-Medel A. **(2004).** Simultaneous determination of mono-, di- and tributyltin in environmental samples using isotope dilution gas chromatography mass spectrometry J. Mass Spectrom., 39: 485–494.

Centineo, G., Rodríguez-González, P., González B. E., García Alonso J. I., Sanz-Medel A, Cardona, N. F., Aranda Mares, J. L. and Nebot S. B. **(2006)**. Isotope dilution GC-MS routine method for the determination of butyltin compounds in water Anal Bioanal Chem 384, 908–914.

Champ, M. A. (1996). Organotin Environmental Fate and Effects, Chapman & Hall.

Chao, W.S. and Jiang, S.J. (1998). Determination of organotin compounds by liquid chromatography-inductively coupled plasma mass spectrometry with a direct injection nebulizer. J. Anal. Atom. Spectrom. 13, 1337.

Chemaly, S.M, (2002). The link between vitamin B-12 and methylmercury: a review. South African journal of science, 568-572.

Compeau, G.C. and Bartha, R., (1985). Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol., 50, 498-502.

Cossa, D., Martin, J.M. and Sanjuan, J. **(1994).** Dimethylmercury formation in the Alboran Sea. Mar. Pollut. Bull., 28, 381-384.

Costa, M. and Liss P. S. (1999). Photoreduction of mercury in sea water and its possible implication for Hg^0 air-sea fluxes. Mar. Chem., 68, 87–95

Covelli, S., Faganeli, J., Horvat, M. and Brambati, A. **(1999).** Porewater distribution and benthic flux measurements of mercury and methylmercury in the Gulf of Trieste (northern Adriatic Sea). Estuar. Coast. Shelf Sci., 48(4), 415-428.

Craig, P.J. (1986). Organometallic compounds in the environment, principles and reaction, 1st ed, Longman, U.K.

Craig, P.J, (2003). Organometallic compounds in the environment, principles and reaction, 2nd ed, John Wiley & sons, U.K.

Craig, P.J. and Rapsomanikis, S. (1985), Reactions of methylcobalamin with tin and lead compounds, Inorg. Chim. Acta 107, 39-43.

Craig, P. J. and Rapsomanikis, S. (1985). Methylation of tin and lead in the environment - oxidative methyl transfer as a model for environmental reactions. Environ. Sci. Techno. 19, 726-730.

Crouch, A. (2006). SAMA-Mercury fact, http://www.waternet.co.za/samercury/ res_frame.html

CSIR, Workshop: 7-8 March **(2006).** South African Mercury Assessment Programme; www.hgtech.com/Coal%20Hg%20Emissions/Coal% 20Hg%20 China. htm, Mercury Emissions from Coal Combustion, China

Cukrowska, E., Chimuka, L., Nsengimana, H. and Kwaramba, V. **(2004).** Application of supported liquid membrane probe for extraction and preconcentration of organotin compounds from environmental water samples. Anal. Chim. Acta. 523, 141-147.

Dabek-Zlotorzynska, E., Laib, E. P. C., Andrei R. and Timerbaev A. R. **(1998).** Capillary electrophoresis: the state-of-the-art in metal speciation studies, Analytica Chimica Acta, 359, 1-26.

Dadfarnia, S., Green, I. and McLeod, C. W. **(1994).** Anal. Proc., 31, 61.

Dastoor, A. and Larocque, Y. (2004). Global circulation of atmospheric mercury: a modeling study. Atmospheric Environment. 38(1),147-162.

De Smaele, T., Moens, L., Dams, R. and Sandra, P. **(1996).** Capillary gas chromatography ICP mass spectrometry: A powerful hyphenated technique for the determination of organometallic compounds. Fresenius J Anal Chem 355, 778–782.

De Smaele, T., Moens, L., Dams, R, Sandra, P., Van der Eycken, J. and Vandyck **(1998).** Sodium tetra(n-propyl)borate: a novel aqueous in situ derivatization reagent for the simultaneous determination of organomercury, -lead and -tin compounds with

capillary gas chromatography inductively coupled plasma mass spectrometry. J. Chromatogr A, 793, 99–106.

DeJonghe, W. R. A., and Adams F. C. (1986). Biogeochemical cycling of organic lead compounds. Adv Environ Sci Technol 17, 561-594.

Dietz, C., Camara, C. and Madrid, Y. **(2000).** Spanish Patent P200001603, priority date June 28th.

Dirkx, W.M.R., Van Cleuvenbergen, R.J.A. and Adams, F.C. (1992). Speciation of organotin compounds in water and sediments by gas-chromatography atomic-absorption spectrometry (gc-aas). Mikrochim. Acta, 109, 79.

Donard, O.F.X. and Quevauviller, Ph. (Editors), **(1992).** Proceedings of the Workshop on Improvements of Speciation Analyses in Environmental Matrices, Mikrocbim. Acta, 109,1-4.

Donard, O.F.X., Short, F.T. and Weber, J.H. **(1987)**. Regulation of tin and methyltin compounds by the green alga Enteromorpha under simulated estuarine conditions. Can. J. Fish. Aquat. Sci., 44, 140-145.

Donard, O.F.X. and Weber, J.H. (1988). Volatilization of tin as stannane in anoxic environments. Nature, 332, 339-341.

Duhamel, K., Blanchard, G., Dorange G. and Martin, G. **(1987).** Recovery of all species from photolytic degradation of tributyltin compounds TBTX (X = Cl, OSn Bu3). Appl. Organometal. Chem. 1, 133.

Dunemann, L., Hajimiragha, H. and Begerow, J. **(1999)**. Simultaneous determination of Hg(II) and alkylated Hg, Pb, and Sn species in human body fluids using SPME-GC/MS-MS. Fresenius J Anal Chem 363:466–468.

Eisenreich, S. J., Looney, B. B. and Thornton, J. D. **(1981).** Airborne organic contaminants in the Great Lakes ecosystem. Environ. Sci. Technol., 15,30-38

Emteborg, H., Snell, J., Jin Qian and Frech, W. **(1999).** Sources of systematic errors in mercury speciation using grignard reagents and capillary gas chromatography coupled to atomic spectrometry, Chemosphere,39 (7), 1137-1152.

Evangelou, V. P. (1998). Environmental soil and water chemistry: principles and applications, John Wiley & Sons Inc., New York, USA.

Fairman, B. and Wahlen, R. **(2001).** Speciation analysis of organotin compounds by HPLC –ICP- MS, Spectrocopy Europe 13/5.

Fent, K. and Hunn, J. **(1996).** Cytotoxicity of organic environmental chemicals to fish liver cells (PLHC-1). Mar. Environ. Res. 42 (1–4), 377–382.

Fent K. (2004). Ecotoxicological effects at contaminated sites, Toxicology 205, 223–234.

Fent, K. and Looser, P.W. (1995). Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids.Water Res. 29, 1631–1637.

Fent, K. and Meier, W., **(1992).** Tributyltin-induced effects on early life stages of minnows Phoxinus phoxinus. Arch. Environ. Contam. Toxicol. 22, 428–438.

Fitzgerald, W.F., Engstrom, D.R., Mason, R.P. and Nater, E.A., **(1998).** The case for atmospheric mercury contamination in remote areas. Environ. Sci. Technol., 32, 1-7.

Förstner, U. and Wittmann, G. T. W. **(1993).** Metal pollution in the Aquatic Environment, Springer, Berlin, German.

Fromme, H., Mattulat, A., Lahrz, T. and Ruden, H. **(2005).** Occurrence of organotin compounds in house dust in Berlin (Germany), Chemosphere 58, 1377–1383.

Gallert, C. and Winter J. **(2004).** Degradation of alkyllead compounds to inorganic lead in contaminated soil. Water Research 38, 4204–4212

Gagnon, C., Pelletier, E., Mucci, A.. and Fitzgerald, W.F. **(1996).** Diagenetic behavior of methylmercury in organic-rich coastal sediments. Limnol. Oceanogr. 41, 428-434.

García Alonso, J. I., Ruiz Encinar, J., Rodríguez González, P. and Sanz-Medel, A. **(2002).** Determination of butyltin compounds in environmental samples by isotope-dilution GC–ICP–MS. Anal. Bioanal. Chem. 373, 432–440.

Gardfeldt, K., Munthe, J., Strömberg, D. and Lindqvist, O., **(2003)**. A kinetic study on the abiotic methylation of divalent mercury in the aqueous phase. Sci. Total Environ., 304, 127-136.

Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A. and Stordal, M.C. **(1998).** Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochem., 40, 327-345.

Gomez-Ariza, J.L., Morales E., Giraldez I., Sanchez-Rodas D. and Velasco A. **(2001).** Sample treatment in chromatography-based speciation of organometallic pollutants Journal of Chromatography A, 938, 211–224

Grandjean, P. (1984). Biological Effects of Organolead Compounds"; CRC Press: Boca Raton, FL.

Gray, B.H., Porvaznik, M., Flemming, C. and Lee, L.H., **(1987).** Tri-n-Butyltin: a membrane toxicant. Toxicology, 47, 35-54.

Grinberg, P., Campos, R.C., Mester, Z. and Sturgeon R. E., **(2003).** A Comparison of alkyl derivatisation methods for speciation of mercury based on solid phase microextraction gas chromatography with furnace atomization plasma emission spectrometry detection. J. Anal. At. Spectrom., 18, 902–909

Guard, H. E., Cobet, A.. B., Coleman, W. M., **(1981).** Methylation of trimethyltin compounds by estuarine sediments, Science 213, 770 -771.

Harris, D.C, (1998), Quantitative chemical analysis, 5th edition, Freeman&company.

Harrison, G. F, Branica, M. and Konrad, Z. **(1980).** Lead in the marine environment, Pergamon, Oxford.

Harrison, R. and Rapsomanikis, S. (Editors). **(1988).** Environmental analysis using chromatography interfaced with atomic spectroscopy, Ellis Horwood, Chichester, UK, 189.

Harrison, R. M., Hewitt, C. N. and Radojevic, M. **(1986).** Decomposition chemistry of organolead compounds in the environment In: Proc. Int. Con., "Chemicals in the Environment" Lisbon S 110-116.

Harvey, B. E., Robert, F. B. and Baoshan, Z. **(2006).** Mercury Emissions from Coal Combustion, China, In: http://www.hgtech.com/Coal%20Hg%20Emissions /Coal%20Hg %20m China.htm

Heisterkamp, M., De Smaele, T., Candelone, J. P., Moens, L., Dams, R., Adamas, F. C. **(1997)**. Inductively coupled plasma mass spectrometry hyphenated to capillary gas chromatography as a detection system for the speciation analysis of organolead compounds in environmental waters. J. Anal. At. Spectrom. 12, 1077–1081

Hempel, M., Kuballa, J. and Jantzen, E. **(2000).** Discovery of a transalkylation mechanism – Identification of ethylmercury at a tetraethyllead-contaminated siteusing sodiumtetrapropylborate, GC-AED and HPLC-AFS. Fresenius J. Anal. Chem., 366, 470–475

Hewitt, C. N. and Harrison, R. M. **(1986).** Formation and decomposition of trialkyllead compounds in the atmosphere. Environ. Sci. Technol. 20(8),797-802.

Hewitt, C. N. and Harrison, R. M. **(1987).** Atmospheric concentrations and chemistry of alkyl lead compounds and environmental alkylation of lead. Environ Sci Technol 21, 260-266.

Hoch, M. (2001). Organotin compounds in environment – an overview. Applied geochemistry 16, 719-743

Hoch, M., Alonso-Azcarate, J. and Lischicka, M. **(2003).** Assessment of adsorption behavior of dibutyltin (DBT) to clay-rich sediments in comparison to the highly toxic tributyltin (TBT). Environmental Pollution, 123, 217–227

Hoch, M. and Schwesig, D. **(2004).** Parameters controlling the partitioning of tributyltin (TBT) in aquatic systems. Appl. Geochem. 19, 323-334

Honeycutt, J. B. and Jr.; Riddle, J. M. **(1961).** Preparation and reactions of sodium tetraethylboron and related compounds. J. Am. Chem. Soc., 83, 369-373

Horiguchi, T., Shiraishi, H., Shimizu, M., and Morita, M., **(1997)**. Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, Thais clavigera. Environ. Pollut. 95, 85–91.

Horvat, M., Jereb, V., Fajon, V., Logar, M., Kotnik, J., Faganeli, J., Hines, M.E., and J.C. Bonzongo **(2002).** Mercury distribution in water, sediment and soil in the Idrija and Soca river systems. Geochemical Exploration and Environmental Analysis 2(2): 287-296.

Huang J.-H. Ilgen G. and Matzner E. **(2003).** Simultaneous extraction of organotin, organolead and organomercury species from soils and litter , Analytica Chimica Acta 493, 23–34.

Huang, J. H. and Matzner, E. (2004), Biogeochemistry of trimethyllead and lead in a forested ecosystem in Germany. Biogeochemistry 71 (1), 125-139.

IARC (International Agency for Research on Cancer). (2005). Overall evaluations of carcinogenicity to humans: As evaluated in IARC Monographs volumes 1-82 (at total of 900 agents, mixtures and exposures). Lyon, France: International Agency for Research on Cancer. In: http://www-cie.iarc.fr/monoeval/crthall.html.

ICMM, (2007). ICCM position statement on mercury, www.icmm.com

Ikonomou, M. G., Fernandez, M. P., He T. and Cullon D. **(2002).** Gas chromatography-high-resolution mass spectrometry based method for the simultaneous determination of nine organotin compounds in water, sediment and tissue. J. of Chromatogr. A., 975, 319-333.

INERIS (Institut National de l'environnement industriel et des risques). **(2003).** Mercure et ses dérivés. Fiche de données toxicologiques et environnementales des substances chimiques. Institut National de l'Environnement industriel et de Risques. http://www.ineris.fr/recherches/fiches/fiches.htm.

Irwin, R. J, VanMouwerik, M., Stevens, L., Seese, M. D., and Basham, W. **(1997).** Environmental Contaminants Encyclopedia: Jet Fuel 8. National Park Service, Water Resources Division, Fort Collins, Colorado.

Jarvie, A. W. P., Markell R. N. and Potter, H. R. **(1981)**. Decomposition of organolead compounds in aqueous systems. Environ. Res., 25, 241-249)

Jarvie, A. W. P., Whltmore, A. P., Markall, R. N. and Potter, H. R. **(1983).** The sulfide assisted decomposition of trimethyllead chloride in aqueous systems. Environ. Pollut., Ser. B, 6, 81.

Kannan, K., Corsolini, S., Focardi, S., Tanabe, S. and Tatsukawa, R., **(1996).** Accumulation pattern of butyltin compounds in dolphin, tuna, and shark collected from Italian coastal waters. Arch. Environ. Contam. Toxicol. 31, 19–23

Kannan, K., Senthilkumar, K., Loganathan, B.G., Takahashi, S., Odell, D.K. and Tanabe, S. **(1997).** Elevated accumulation of tributyltin and its breakdown products in bottlenose dolphins (Tursiops truncatus) found stranded along the US Atlantic and Gulf coasts. Environ. Sci. Technol. 31, 296–301.

Kannan, K., Tanabe, S., Iwata, H. and Tatsukawa, R. **(1995).** Butyltins in muscle and liver of fish collected from certain Asian and Oceanian countries. Environ. Pollut. 90, 279–290.

Kegley S. E. and Andrews, J. **(1996).** The Chemistry of Water, University Science Books.

KEMI (Swedish National Chemicals Inspectorate), **(2000).** Organotin stabilisers in PVC. Assessmemt of risks and risk reduction measures. Stockholm, Sweden.

King, J.K., Kostka, J.E., Frischer, M.E. and Saunders, F.M. **(2000)**. Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. Appl. Environ. Microbiol., 66, 2430-2437.

King , J.K., Kostka, J.E., Frischer, M.E., Saunders, F.M. and Jahnke, R.A., **(2001)**. A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria. Environ. Sci. Technol., 35, 2491-2496.

Knight, 1996, Gas Adsorption http://www.chem.ufl.edu/~itl/4411L_f00/ads/sample/knight.html

Ko, M. M. C., Bradley, G.C., Neller A.H. and Broom, M.J. **(1995).** Tributyltin Contamination of Marine Sediments of Hong Kong. Marine Pollution Bulletin 31(4-12): 249-253.

Lalonde, J.D., Amyot, M., Kraepiel, A.M.L. and Morel, F.M.M., **(2001)**. Photooxidation of Hg (0) in artificial and natural waters. Environ. Sci. Technol., 35, 1367-1372.

Lamborg, C.H., Fitzgerald, W.F., O'Donnell, J. and Torgersen, T. **(2002).** A non-steady state compartmental model of global-scale mercury biogeochemistry with interhemispheric atmospheric gradients. Geochim. Cosmochim. Acta, 66, 1105-1118.

Larsen E. H. and Hansen S. H., **(1992)**. Separation of arsenic species by ion-pair and ion-exchange high-performance liquid-chromatography. Mikrochim. Acta, 109, 47.

Leermakers, M., Galletti, S., De Galan, S., Brion, N. and Baeyens, W. **(2001).** Mercury in the southern North Sea and Scheldt estuary. Mar. Chem., 75, 229-248.

Liu W. and Lee, H. K. **(1997).** Simultaneous analysis of inorganic and organic lead, mercury and selenium by capillary electrophoresis with nitrilotriacetic acid as dramatization agent, Journal of Chromatography A 796,385-395

Liu W. and Lee, H. K. **(1999).** Chemical modification of analytes in speciation analysis by capillary electrophoresis, liquid chromatography and gas chromatography, Journal of Chromatography A, 834, 45–63

Looser, P.W., Bertschi, S., Fent, K., **(1998).** Bioconcentration and bioavailability of organotin compounds: influence of pH and humic substances. Appl. Organomet. Chem. 12, 601–611.

Lu, J., Guibin Jiang, Qunfang Zhuo and Ziwei Yao, **(2001).** Comprehensive-trace level determination of Methyltin compounds in aqueous samples by cryogenic purge-and-trap gas chromatography with flame photometric detection. Analytical Sciences 17, 1279 - 83.

Maguire, R.J., (1991). Aquatic environmental aspects of non-pesticidal organotin compounds. Water Pollut. Res. J. Can., 26, 243-360.

Maier E.A., Quevauviller Ph. and Griepink B., **(1994).** Interlaboratory studies as a tool for many purposes - proficiency testing, learning exercises, quality-control and certification of matrix materials. Anal. Chim. Acta, 283, 590.

Mailhot, G., Astruc, M. And Bolte, M., **(1999).** Degradation of TBT in water photoinduced by iron (III). Appl. Organomet. Chem., 13, 53-61.

Manceau A., Boisset M.C., Sarret G., Hazemann, J.L., Mench, M., Cambier, Ph. and Prost, R. **(1996).** Direct determination of lead speciation in contaminated soils by EXAFS spectroscopy. Environmental Science & Technology, 30, 1540-1552

Marshall, D. J. and Rajkumar, A., (2003). Imposex in the indigenous Nassarius kraussianus (Mollusca : Neogastropoda) from South African harbours. Marine Pollution Bulletin. 46, 1150-1155.

Martin, F.M., Tseng, C.-M., Belin, C. Quevauviller Ph. and Donard, O.F.X. **(1994).** Interferences generated by organic and inorganic-compounds during organotin speciation using hydride generation coupled with cryogenic trapping, gas-chromatographic separation and detection by atomic-absorption spectrometry. Anal. Chim. Acta, 286, 343.

Marvin-Dipasquale, M., Agee, J., McGowan, C., Oremland, R.S., Thomas, M., Krabbenhoft, D. and Gilmour, C.C, **(2000).** Methylmercury degradation pathways: a comparison among three mercury-impacted ecosystems. Environ. Sci. Technol., 34, 4908-4916.

Mason, R.P. and Fitzgerald, W.F., (1990). Alkylmercury species in the equatorial Pacific. Nature, 347, 457-459.

Mason, R.P. and Sullivan, K.A. (1999). The distribution and speciation of mercury in the south and equatorial Atlantic. Deep-Sea Res. II, 46, 937-956.

Matilainen, T. (1995). Involvement of bacteria in methylmercury formation in anaerobic lake waters. Wat. Air Soil Pollut., 80, 757-764.

Mauro, J.B.N, Guimaraes, J.R.D., Hintelmann, H., Watras, C.J., Haack, E.A. and Coelho-Souza, S.A., **(2002).** Mercury methylation in macrophytes, periphyton, and

water: comparative studies with stable and radiomercury additions. Anal. Bioanal. Chem., 374, 983-989.

Mbendi, (2000): South Africa - Oil and Gas: Petroleum and Petroleum Products Wholesalers. In: http://www.mbendi.co.za/indy/oilg/ogds/af/sa/p0015.htm#20

McCarty, L.S. and Borgert C.J., **(2006).** Review of the toxicity of chemical mixtures: Theory, policy, and regulatory practice. Regulatory Toxicology and Pharmacology 45, 119–143

Meinema, H. A., Burger-Wiersma, T., Versluis-De-Haan, G. and Gevers, E. Ch. **(1978)**. Determination of trace amounts of butyltin compounds in aqueous systems by gas chromatography/mass spectrometry. Environ. Sci. Technol., 12 (3): 288-293.

Mester Z. and Sturgeon, R. E. **(2002).** Detection of volatile organometal chloride species in model atmosphere above seawater and sediment. Environ. Sci. Technol., 36,1198–1201.

Michel, P. and Averty, B. **(1999).** Distribution and fate of tributyltin in surface and deep waters of the northwestern Mediterranean. Environ. Sci. Technol., 33, 2524-2528.

Moens, L., De Smaele, T., Dams, R., Van Den Broeck, P. and Sandra, P. **(1997).** Sensitive, simultaneous determination of organomercury, -lead, and -tin compounds with headspace solid phase microextraction capillary gas chromatography combined with inductively coupled plasma mass spectrometry. Anal Chem 69:1604–1611

Monperrus, M., (2004). Utilisation de traceurs isotopiques stables pour la speciation du mercure et des butyletains application à l'etude de la biomethylation du mercure dans les environnements aquatiques, l'Université de Pau et des Pays de l'Adour, France.

Monperrus, M., Guyoneaud, R., Tessier, E., Duran, R., Goni, M., Amouroux, D., Donard, O.F.X. and Caumette, P., **(2003)a.** Mercury methylation rates in coastal sediments versus microbial diversity and specific activity. J. phys., IV., 107, 883-886.

Monperrus, M., Krupp, E., Amouroux, D., Donard, O.F.X. and Rodriguez Martin-Doimeadios, R.C. **(2004).** Potential and limits of speciated isotope-dilution analysis for metrology and assessing environmental reactivity. Trends Anal. Chem., 23, 261-272.

Monperrus, M., Rodriguez Martin-Doimeadios R. C., Scancar J., Amouroux D., and Donard O. F. X. **(2003)c.** Simultaneous Sample Preparation and Species-Specific Isotope Dilution Mass Spectrometry Analysis of Monomethylmercury and Tributyltin in a Certified Oyster Tissue. Anal. Chem., 75, 4095-4102

Monperrus, M., Zuloaga, O., Krupp, E., Amouroux, D., Wahlen, R., Fairman, B. and Donard, O. F. X. **(2003)b**. Rapid, accurate and precise determination of tributyltin in sediments and biological samples by species specific isotope dilution-microwave extraction-gas chromatography-ICP mass spectrometry. J. Anal. At. Spectrom., 18, 3, 247-253.

Morabito, R., Massanisso, P. and Quevauviller, Ph. **(2000).** Derivatisation methods for the determination of Organotin compounds in environmental samples, trends in analytical chemistry, vol. 9,nos 2+3, 113- 119

Mphepya, J. N., Pienaar, J. J., Galy-Lacaux, C., Held, G. and Turner, C.R. **(2004).** Precipitation Chemistry in Semi-Arid Areas of Southern Africa: A Case Study of a Rural and an Industrial Site, Journal of Atmospheric Chemistry, 47, 1–24

Ndungu K. and Mathiasson, L. (1999). Microporous membrane liquid liquid extraction technique combined with Gas chromatography mass spectrometry for the determination of organotin compounds, Anal Chem (404), 319-328

Negri, P., Hales, L. T., Battershill, C., Wolff, C. and Webster, N. S. **(2004).** TBT contamination identified in Antarctic marine sediments. Mar. Pollut. Bull., 48, 1142-1144.

Nielsen, O. J, O'Farrell, D. J. and Treacy JJ **(1991).** Rate constants for the gas-phase reactions of hydroxyl radicals with tetramethyllead and tetraethyllead. Environ Sci Technol 25(6), 1098-1103.

Nriugu JO, (1990). The rise and fall of leaded gasoline. Sci. Total Environ. 92,13-28

Nsengimana H., Cukrowska, E. and Dinsmore, A. **(2004).** Speciation of Alkyllead after degradation of tetramethyllead in aqueous media, the 37th Convention of the south African Chemical institute, Pretoria 4-9 July 2004.

O'Halloran, K., Ahokas, J.T. and Wright, P.F.A., **(1998)**. Response of fish immune cells to in vitro organotin exposures. Aquat. Toxicol., 40, 141-156.

Omae, I. (1999). Applications of organometallic compounds, John Wiley& Sons, Chechester, England, 91-107

Omerland, R.S., Culbertson, C.W. and Winfrey, M.R., **(1991)**. Methylmercury decompositions in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. Appl. Environ. Microbiol., 57, 130-137.

OSHA. (2005)a. Air contaminants. Occupational safety and health standards for shipyard employment. Washington, DC: Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1915.1000. http://www.osha.gov/comp-links.html.

OSHA. (2005)b. Gases, vapors, fumes, dusts, and mists. Safety and health regulations for construction. Washington, DC: Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.55, Appendix A. http://www.osha.gov/comp-links.html.

OSHA. (2005)c. Limits for air contaminants. Occupational safety and health standards. Washington, DC: Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1000. http://www.osha.gov/comp-links.html. February 15, 2005.

OSHA. (2005)d. Toxic and Hazardous Substances. Occupational safety and health standards. Washington, DC: Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1025. http://www.osha.gov/comp-links.html.

Ou L. T., Thomas, J. E. and Jing, T. W. **(1994)**. Biological and chemical degradation of tetraethyl lead in soil. Bull Environ Contam Toxicol 52, 238-245.

Pacyna, E.G., Pacyna J.M. and Pirrone, N. **(2001).** European emissions of atmospheric mercury from anthropogenic sources in 1995, Atmos. Environ., 35, 2987-2996.

Pacyna, E. G. and Pacyna, J.M., **(2002).** Global emission of mercury from anthropogenic sources in 1995. Wat. Air Soil Pollut., 137, 149-165.

Pacyna, J.M., Wilson, F. Steenhuisen and Pacyna, E. G. **(2005).** Spatially Distributed Inventories of Global Anthropogenic Emissions of Mercury to the Atmosphere. (www.amap.no/Resources/HgEmissions/)

Pacyna, J.M., (1986). Atmospheric trace elements from natural and anthropogenic sources, Advances in Environmental Science and Technology, 17, 33-52.

Pak, K.R. and Bartha, R., (1998)a. Mercury methylation and demethylation in anoxic lake sediments and by strictly anerobic bacteria. Appl. Environ. Microbiol., 64, 1013-1017.

Pak, K.R. and Bartha, R., (1998)b. Mercury methylation by interspecies hydrogen and acetate transfer between sulfidogens and methanogens. Appl. Environ. Microbiol., 64, 1987-1990.

Parker J. L. and Bloom, N. S. (2005). Preservation and storage techniques for low-level aqueous mercury speciation Science of the Total Environment 337, 253– 263

Parkinson D.R.; Bruheim I.; Christ I. and Pawliszyn J. **(2004).** Full automation of derivatization-solid-phase microextraction-gas chromatography-mass spectrometry with a dual-arm system for the determination of organometallic compounds in aqueous samples. Journal of Chromatography A, 1025, 77–84

Pawliszyn J., (2002). Sampling and sample preparation for field and laboratory, Elsevier, Amsterdam.

Pereiro, I. R., Wasik, A. And Lobinsky, R. **(1998).** Determination of mercury species in fish reference materials by isothermalmulticapillary gas chromatography with atomic emission detection after microwave-assisted solubilization and solvent extraction. J. Anal. At. Spectrom., 13, 8, 743-747.

Pirrone, N., Keeler, G.J. and Nriagu, J.O. **(1996).** Regional differences in worldwide emissions of mercury to the atmosphere. Atmos. Environ., 30, 2981-2987.

Pongratz, R., Heumann, K.G**. (1998).** Production of methylated mercury and lead by polar macroalgae – a significant natural source for atmospheric heavy metals in clean room compartments. Chemosphere, 36,1935-1946.

Postgate, J. R. (1965). Recent Advances in the study of the sulfate-reducing bacteria Bacteriological Reviews, Dec., Vol. 29, No. 4

Poulain, A.J., Amyot, M., Findlay, D., Telor, S., Barkay, T. and Hintelmann, H. **(2004)**. Biological and photochemical production of dissolved gaseous mercury in lakes. Limnol. Oceanogr., 49, 2265-2275.

Prange, A. and Jantzen E (1995). Determination of organometallic species by gas-chromatography inductively-coupled plasma-mass spectrometry. J Anal At Spectrom 10,105–109

Quevauviller Ph., (1998)a. Method Performance Studies for Speciation Analysis, The Royal Society of Chemistry, Cambridge.

Quevauviller, Ph., Astruc, M., Ebdon, L., Desauziers, V. Sarradin, P.M., Astruc, A., Kramer G.N. and Griepink, B. **(1994).** Certified reference material (crm-462) for the quality-control of dibutyl-tin and tributyl-tin determinations in coastal sediment. Appl. Organomet. Chem., 8, 629.

Quevauviller, Ph., Ebdon, L., Harrison, R. and Wang, Y. **(1998)b.** Certifying the contents of trimethyllead in an artificial rainwater reference material. Analyst 123, 971.

Quevauviller, Ph., (2000), Harrison, R., Adams, F. and Ebdon, L., An overview of methods for the determination of trimethyllead in rainwater and urban dust reference materials. Trends in analytical chemistry, vol. 19, 195-199

Quevauviller, Ph., (1996). Improvement of quality control of speciation analysis using hyphenated techniques A decade of progress within the European Community, Journal of Chromatography A, 750, 25-33

Randall, L. and Weber, J.H., (1986). Adsoprtive behavior of butyltin compounds under simulated estuarine conditions. Sci. Total Environ., 57, 191-203.

Rapsomanikis, S., Donard, 0. F. X. and Weber J. W. **(1986).** Speciation of Lead and Methyllead Ions in Water by Chromatography/Atomic Absorption Spectrometry after Ethylation with Sodium Tetraethylborate. Anal. Chem, 58, 35-38

Rapsomanikis, S., CieJka, J. J. and Weber, J. H. **(1984).** Methylation of lead(ii) and methyllead(iv) compounds in water by a trans-dimethylcobalt(ii) complex. Inorg. Chim. Acta 179-183.

Reuther R., Jaeger, L. and Allard, B. **(1999).** Determination of organometallic forms of mercury, tin and lead by in situ derivatization, trapping and gas chromatography - atomic emission detection. Anal. Chim. Acta, 394, 259

Ritsema R., (1992). Speciation of organotin and organoarsenic in water samples. Mikrochim. Acta, 109, 61.

Rodrìguez Martìn-Doimeadios R.C., Tessier, E., Amouroux, D., Guyoneaud, R. , Duran, R., Caumette, P. And Donard, O.F.X. **(2004).** Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species-specific enriched stable isotopes, Marine Chemistry, 90, 107– 123

Rodríguez Martín-Doimeadios, R. C., Krupp, E., Amouroux, D., and Donard, O. F. X. **(2002a).** Application of Isotopically Labeled Methylmercury for Isotope Dilution Analysis of Biological Samples Using Gas Chromatography/ICPMS Anal. Chem., 74, 2505-2512

Rodríguez Martín-Doimeadios, R. C., Monperrus, M., Krupp, E. Amouroux, D., and Donard O. F. X. **(2003).** Using Speciated Isotope Dilution with GC-Inductively Coupled Plasma MS To Determine and Unravel the Artificial Formation of Monomethylmercury in Certified Reference Sediments, Anal. Chem., 75, 3202-3211

Rodriguez Martin-Doimeadios, R. C., Stochiev, T., Krupp, E., Amouroux, D., Holeman, M. and Donard, O.F. X., **(2002b).** Micro-scale preparation and characterization of isotopically enriched monomethylmercury. Appl. Organom. Chem. 16, 610 - 615

Rodriguez Pereiro I. and Carro Díaz A. (2002). Speciation of mercury, tin, and lead compounds by gas chromatography with microwave-induced plasma and atomic-emission detection (GC–MIP–AED) AED) Anal Bioanal Chem 372, 74–90

Rosenkranz, B., Bettmer, J., Buscher, W., Breer, C. and Cammann, K., **(1997)**. The behaviour of different organometallic compounds in the presence of inorganic mercury(II): Transalkylation of mercury species and their analysis by the GC-MIP-PED system. Appl. Organomet. Chem., 11, 721-725.

Rubio R., Padro A., Alberti J. and Rauret G., **(1992).** Speciation of organic and inorganic arsenic by HPLC-HG-ICP. Mikrochim. Acta, 109, 39.

Ruiz, J.M., Bachelet, G., Caumette, P. and Donard, O.F.X., **(1996).** Three decades of tributyltin in the coastal environment with emphasis on Arcachon Bay, France. Environ. Pollut., 93, 195-203.

Sauvé, S., Hendershot, W. and Allen, H. E. **(2000).** Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden and organic matter, Environ. Sci. Tech. 34, 1125–1131.

Sax, N. I. and Lewis, R. J. (1989). Dangerous Properties of Industrial Materials, 7th ed. New York, Van Nostrand Reinhold

Schroeder, W.H. and Munthe, J., (1998). Atmospheric mercury - an overview. Atmos. Environ., 32, 809-822.

SDSU (San Diego State University), department of geological sciences. **(2007),** Sediment sampling, http://www.geology.sdsu.edu/classes/geol552/ sedsampling. Htm

Seigneur, C., P. Karamchandani, K. Lohman, and K. Vijayaraghavan **(2001).** Multiscale modeling of the atmospheric fate and transport of mercury, J. Geophys. Res., 106, 27,795-27,809.

Sellers, P., Kelly, C.A., Rudd, J.W.M. and Machutchon, A.R. **(1996)**. Photodegradation of methylmercury in lakes. Nature, 380, 694-697.

Selwyn, M.J. (1976). Triorganotin compounds as ionophores and inhibitors of ion translocating ATPases. In: Organotin Compounds: New Chemistry and Applications; Zuckerman, J.J., ed.; American Chemical Society, Washington D.C., 204-226.

Seyferth, D., (2003)a. The Rise and Fall of Tetraethyllead. 1. Discovery and Slow Development in European Universities, 1853-1920, Organometallics, 22, 346

Seyferth, D., (2003)b. The rise and fall of tetraethyllead. 2., Organometallics 22, 5154-5178

Sheu, G.-R. and R. P. Mason **(2001)**. An examination of methods for the measurements of reactive gaseous mercury in the atmosphere. Environ. Sci. Technol., 35, 1209-1216

Siciliano, S. D., O'Driscoll, N. J. and Lean, D. R. S. **(2002).** Microbial reduction and oxidation of mercury in freshwater lakes. Environ. Sci. Technol., 36, 3064-3068.

Simon, S. (2002). Transfer of organotins from water to plants development of a pretreatment process before analysis, Villenave d'Ornon Cedex, France, 1-18

Soares H. M. V. M., Machado C. M. M. and Barros, G. R. T. **(2002).** Electrochemical determination of methyltin compounds, mikrochimica acta (138), 43-48

Song X., Zapata A., May L. and Eng G. **(2005),** Effects of pH on the speciation of several triorganotin compounds in Anacostia River sediments using Mössbauer spectroscopy, Taylor & Francis, 4 (1), 39-48

Stäb, J.A., Traas, T.P., Stroomberg, G., van Kesteren, J., Leonards, P., van Hattum, B., Brinkman, U.A.T. and Cofino, W.P. **(1996).** Determination of organotin compounds in the foodweb of a shallow freshwater lake in the Netherlands. Arch. Environ. Contam. Toxicol. 31, 319–328.

Stein, E.D., Cohen, Y. and Winer, A.M., **(1996).** Environmental distribution and transformations of mercury compounds. Crit. Rev. Environ. Sci. Technol., 26, 1-43.

Stoichev T., Amouroux D., Wassermanc J.C., Pointa D., De Diegod A., Bareillea G. and Donard O.F.X. **(2004).** Dynamics of mercury species in surface sediments of a macrotidal estuarineecoastal system (Adour River, Bay of Biscay) Estuarine, Coastal and Shelf Science 59, 511 - 521

Stoichev T., Rodriguez Martin-Doimeadios R.C., Tessier E., Amouroux D. and Donard O.F.X. **(2004).** Improvement of analytical performances for mercury speciation by on-line derivatization, cryofocussing and atomic fluorescence spectrometry Talanta 62, 433–438

Sullivan, J. J. (1991). Screening in gas-chromatography with atomic emission detection. Trac-Trends Anal Chem 10:23–26

Sunil, J. K., Tesfalidet, S., Snell, J. P., Nguyen Van, D., and Frech, W. **(2004)**. A simple method for synthesis of organotin species to investigate extraction procedures in sediments by isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry Part 2.Phenyltin species, J . Anal. At . Spectrom., 19 , 368 – 372

Takahashi, S., Tanabe, S., Takeuchi, I. and Miyazaki, N. **(1999).** Distribution and specific bioaccumulation of butyltin compounds in a marine ecosystem. Arch. Environ. Contam. Toxicol. 37, 50–61.

Takeuchi, M., Mizuishi, K. and Hobo T. **(2000).** Determination of organotin compounds in environmental samples. Analytical Sciences 16, 349-359.

Themelis, N. J. and Gregory A. (2002). Mercury Emissions from High Temperature Sources in the Hudson Basin. Proceedings of 10th North American Waste To Energy Conference, Philadelphia, PA, May 2002.

Tessier A. and Turner, D.R. (1996). Metal speciation and bioavailabilty in aquatic systems, John Wiley& Sons.

Tessier, (2004). Etude de la reactivite et du transfert du tributyletain et du mercure dans les environnements aquatiques, theses, Université de Pau et des Pays d'Adour, France.

Tessier, E., Amouroux, D. and Donard, O.F.X. **(2002).** Volatile organotin compounds (butylmethyltin) in three European estuaries (Gironde, Rhine, Scheldt). Biogeochem., 59, 161-181.

Thornton, (2002). Environmental Impacts of Polyvinyl Chloride (PVC) Building Materials, Health building network In: http://www.healthybuilding.net/pvc/ ThorntonPVCSummary.html

Tolosa I., Dachs J. and Bayona J.M. **(1992).** Tributyltin speciation in aquatic matrices by cgc-fpd and cgc-ms confirmation, Mikrochim. Acta, 109, 87.

Tseng C. M., De Diego A , Martin F. M. and Donard OF..X **(1997).** Rapid and Quantitative Microwave assisted Recovery of Methylmercury from Standard Reference Sediments, Journal of Analytical Atomic Spectrometry, 12, 629–635

Tseng C. M., De Diego A., Wasserman J. C., Amouroux D. and Donard O.F.X. **(1999).** potential interferences generated during mercury species determination using acid leaching, aqueous ethylation, cryogenic gas chromatography and atomic spectrometry detection techniques Chemosphere, Vol. 39, No. 7, pp. 1119-1136,

Tutu, H. (2005). Determination and geochemical modeling of the dispersal of uranium in gold- mine polluted land on the Witwatersrand, University of Witwatersrand, Johannesburg, South Africa.

UNEP, (2003). Inclusion of the chemicals tetraethyl lead and tetramethyllead and adoption of the draft decision guidance document In: http://www.pic.int/incs/icrc4/s15Add2)/English/ICRC4.15add.2_FINAL.pdf

Unger, M.A., MacIntyre, W.G. and Huggett, R.J., **(1988).** Sorption behavior of tributyltin on estuarine and freshwater sediments. Environ. Toxicol. Chem., 7, 907-915.

UNEP (United Nations Environment Programme). **(2003).** Pacific Islands regional report: regionally based assessment of persistent toxic substances, United Nations, Geneva.

Unob, F., Hagege, A., Lakkis, D. and Leroy M. **(2003).** Degradation of organolead species in aqueous solutions by electron beam irradiation, Water Research 37, 2113–2117

USEPA, (1997)a. Fate and transport of mercury in the environment. Mercury study report to Congress. United States Environmental Protection Agency. EPA-452/R-97-005. http://www.epa.gov.

USEPA. (1997)b. Health effect of mercury and mercury compounds. United States Environmental Protection Agency. EPA 452/R-97-007. http://www.epa.gov.

USEPA. (2003). Ambient aquatic life water quality criteria for tributyltin (TBT). United States Environmental Protection Agency. EPA 822-R-03-031. http://www.epa.gov.

USEPA. **(1986).** Quality criteria for water, EPA 440/5-86-001

USEPA. (1985). Controls applicable to gasoline refiners and importers. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 80.20

USEPA. (1996)a. Bioavailability of lead in soil samples from the New Jersey Zinc NPL Site Palmerton, Pennsylvania. U.S. Environmental Protection Agency Region 8. Document Control No. 04800-030-0162.

USEPA. (1996)b. U.S. Environmental Protection Agency. Fed Regist 61:3

USEPA. (2004)b. Hazardous air pollutants. Washington, DC: U.S. Environmental Protection Agency. United States Code 42 USC 7412. http://www.epa.gov/ttn/atw/orig189.html. February 15, 2005

USEPA. (2005)a. Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act. Washington, DC: U.S. Environmental Protection

Agency. Code of Federal Regulations. 40 CFR 116.4. http://www.epa.gov/ttn/atw/orig189.html. February 15, 2005.

USEPA. (2005)b. National primary and secondary ambient air quality standards for lead. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 50.12. http://www.epa.gov/epacfr40/chapt-I.info/chi-toc.htm. February 15, 2005.

USEPA. (2005)c. Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 117.3. http://www.epa.gov/epacfr40/chapt-I.info/chi-toc.htm. February 16, 2005.

USEPA. (2005)d. Superfund, emergency planning, and community right-to-know programs. Designation, reportable quantities, and notifications. Washington, DC: U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 302.4. http://www.epa.gov/epacfr40/chapt-I.info/chi-toc.htm. February 15, 2005.

USEPA. (2005)f. Toxic chemical release inventory reporting forms and instructions: Revised 2004 version. Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986). U.S. Environmental Protection Agency. Office of Environmental Information. EPA260B05001.

Wang, Y., Allen A. G. and Harrison, R. M. **(1996).** Determination of Octanol-Water Partition Coefficients, Water Solubility and vapour Pressures of Alkyl-lead Compounds, Appl. Organomet. Chem. 10, 773-778.

Wasik, A., Rodriguez Pereiro, I., Dietz, C., Szpunar, J. and Lobinsky, R. **(1998).** Speciation of mercury by ICP-MS after on-line capillary cryofocussing and ambient temperature multicapillary gas chromatography. Anal. Commun., 35, 10, 331-335.

Weber, J. H. (1999). Volatile hydride and methyl compounds of selected elements formed in the marine environment. Mar. Chem., 65, 67-75.

Weidenhaupt, A., Arnold, C., Müller, S.R., Haderlein, S.B. and Schwarzenbach, R.P., **(1997).** Sorption of organotin biocides to mineral surfaces. Environ. Sci. Technol., 31, 2603-2609.

WHO (World Health Organization), **(1977).** Lead. Environmental Health Criteria No 3, Geneva, WHO

WHO (World Health Organization), **(1990).** IPCS. Environmental health criteria 116. Tributyltin Compounds. Geneva.

WHO (World Health Organization), **(1999)a**. Triphenyltin compounds. Concise International Chemical Assessment Document 13. Geneva.

WHO (World Health Organization), **(1999)b.** Tributyltin oxide. Concise International Chemical Assessment Document 14. Geneva.

WHO IPCS, (1989). Environmental Health Criteria 86: Mercury environmental aspects. World Health Organization, Geneva. htpp://www.inchem.org.

WHO IPCS, (1990). Environmental Health Criteria 101: Methylmercury. World Health Organization, Geneva. htpp://www.inchem.org.

WHO IPCS, (1990). Environmental Health Criteria 116: Tributyltin compounds. World Health Organization, Geneva. htpp://www.inchem.org.

WHO IPCS, (1991). Environmental Health Criteria 101: Mercury, inorganic. World Health Organization, Geneva. htpp://www.inchem.org.

WHO IPCS, (1996). Guidelines for drinking-water quality. World Health Organization, Geneva. htpp://www.inchem.org.

WHO. (2000). Air quality guidelines. 2nd edition. Geneva, Switzerland: World Health Organization. http://www.euro.who.int/air/Activities/20050104_1. February 15, 2005.

WHO. (2004). Guidelines for drinking-water quality. 3rd edition. Geneva, Switzerland: World Health Organization.

Wikipedia, (2006)a. Tin In:www.en.wikipedia.org/wiki/Tin

Wikipedia, (2006)b. Lead In: www.en.wikipedia.org/wiki/Lead

Wikipedia, (2006)c. Mercury In: www.en.wikipedia.org/wiki/Mercury

Wikipedia, (2006)d. Bioaccumulation In: www.en.wikipedia.org/wiki/ Bioaccumulation

Wikipedia, (2006)e. Cyanocobalamin, In: www.en.wikipedia.org/wiki/ Cyanocobalamin

Won Y., Pyong Gil Jung, Chung M. ; Kim B., Yim Y. ; So H. and Yong Seong Kim, **(2004).** Application of a gas chromatography/mass spectrometric method for the determination of butyltin compounds in sediment, Bulletin of the Korean Chemical Society 25 (10), 1508-1512

Yozenawa, Y., Fukui, M., Yoshida, T., Ochi, A., Tanaka, T., Noguti, Y., Kowata, T., Sato, Y., Masunaga, S. and Urushigawa, Y., **(1994)**. Degradation of tri-n-butyltin in Ise bay sediment. Chemosphere, 29, 1349-1356.

Yu, X. and Pawliszyn, J. (2000). Speciation of alkyllead and inorganic lead by derivatization with deuterium-labeled sodium tetraethylborate and SPME-GC/MS, Anal Chem 72, 1788–1792

Zhang, H, and Lindberg. S.E. (2001). Sunlight and iron(III)-induced photochemical production of dissolved gaseous elemental mercury in fresh water. Envir. Sci. Technol. 35, 928-935.

Zhou Q., Liu, J., Cai Y., Liu, G. and Jiang, G. **(2003).** Micro-porous membrane liquid–liquid extraction as an enrichment step prior to nonaqueous capillary electrophoresis determination of sulfonylurea herbicides , Microchemical Journal 74, 157–163

Zufiaurre, R., Pons, B. and Nerín, C. **(1997)**. Speciation of trimethyllead in rainwater by gas chromatography mass spectrometry after ethylation with sodium

tetraethylborate - Comparison with other alkylation methods, J Chromatogr A 779: 299–306

Appendix 1: Lead in children's toys

The article below was published in "Sunday times on the 23 October 2005". The Sundaytimes is a newspaper in south Africa. It can be accessed on this website www.sundaytimes.co.za.

Lead in children's toys

Toxic toys shock

Lead in paint on playthings up to 1300 times higher than international limit

ROWAN PHILIP and ILSE FREDERICKS

CHILDREN'S toys coated with paint containing poisonous amounts of lead are being pulled off the shelves of leading supermarkets and shops around the country. The withdrawal was sparked by a three-month-long Sunday Times investigation into toxic toys.

Laboratory tests done for the newspaper, and the Medical Research Council, have identified wooden toddler toys painted with levels of lead up to 1300 times higher than international safety limits.

Startled managers at Checkers, Toys R Us, Green Busters and Kidz-A-Peal, where the Sunday Times bought its samples, have removed hundreds of toys after seeing the test results. But thousands of harmful toys have already been sold.

Andrew Smith, laboratory manager of one of Britain's government-accredited toy-safety testing agencies, said: "These figures are amazing; horrifying." The worst toy

seen in the past five years from Europe would have been about 20 times over the safety limit, he said.

South Africa's Department of Health said the test results were "disturbing". Three sets of tests, done by the National Institute for Occupational Health, on 30 toys from stores in Johannesburg and Cape Town found 15 with toxic levels of lead. Most were made in South Africa.

Long exposure — by children chewing or sucking on paint containing lead — can lead to lethargy, reduced IQ, aggression, anaemia, hyperactivity or delayed puberty. South Africa has no laws restricting lead levels for toys or paint. But the Global Toy Safety Standard recommends that no toy be sold with more than 90 parts of lead per million parts of paint.

A "Blocks in Lorry" toy — made by Dovetail Products in Cape Town, and sold by Toys R Us in Sandton — tested over 120000 parts per million.

Health Department spokesman Solly Mabotha said: "Clearly there are manufacturers who are not adhering to their code [to limit levels of lead in paint]. Due to lack of regulations it makes it difficult to take action on the transgressors."

Smith said of toddlers who chewed or sucked on a toy measuring more than 40000 parts per million for extended periods: "The child's behaviour and learning abilities will be adversely affected and there will be health impacts in the long term."

Most lead-caused disorders go undetected, because lead builds up gradually and the effects resemble minor illnesses or show up later in life.

At least two paint manufacturers — Duram and Optica Coat Masters — have sold lead-based paint directly to toy companies, without warnings. Directors of both companies conceded that the lead pigment they used could be “harmful”.

Two of the toxic toys identified by the tests were made by Truly Toys in Johannesburg. Confronted with the results, managing director Ivan Shutte said the lead levels “can’t be possible” as he had been assured his paints were lead-free.

But Ian Burke, general manager of Shutte’s paint supplier, Duram, said: “Oops.” He admitted that “many” manufacturers, including Duram, used lead pigments to give durability and brightness to red and yellow colours. Burke said Duram decided a month ago to stop using lead because “it’s harmful stuff” and legislation to limit its use was “surely coming, and is long overdue”. He has offered to swop all lead-based paint sold by Duram for lead-free paint.

Another three toxic toys were made by Dovetail Products in Cape Town, supplied with paint by Optica Coat Masters. On Wednesday, five days after Dovetail was presented with the test results, it was still using lead-based Optica enamel on new toys. Optica removed its lead-based paint from Dovetail’s factory on Friday and offered to replace it with an alternative.

The Sunday Times commissioned the tests after the Medical Research Council’s environmental health director, Angela Mathee, said the council’s own tests in July had shown eight out of 11 painted wooden toys to be way over safety limits.

Compared with the danger limit of 90 parts per million (ppm), the Sunday Times tests found:

•45778 ppm on “Coloured Random Blocks”, made by Truly Toys;

•104048 ppm on a red and blue “Pull Tortoise”, made by the Jewish Shelter charity;

•87075 ppm on an “Iron and Ironing Board” set, made by Dovetail Products, and 120135 ppm on the company’s “Blocks in Lorry” toy;

•41997 ppm on a “Blocks in a Cart” toy, made by Truly Toys;

•2453 ppm on a “Mallet Box” toy, by foreign maker Hos-team; and

•86619 ppm on “Coloured Building Blocks”, made by Dovetail.

Appendix 2: Instruments pictures

This appendix shows some picture of instrument used.

Grinding instrument

Figure A2.1 shows the Fritsch GmbH Planetary Mono Mill used for soil grinding. Agate mortar was used to grind sediment and plant.



Figure A2. 1: Fritsch GmbH Planetary Mono Mill "Pulverisette 6

Particle size measurement instrument

Figure A2.2 shows Malverns mastersizer instrument used for soil particle size determination.

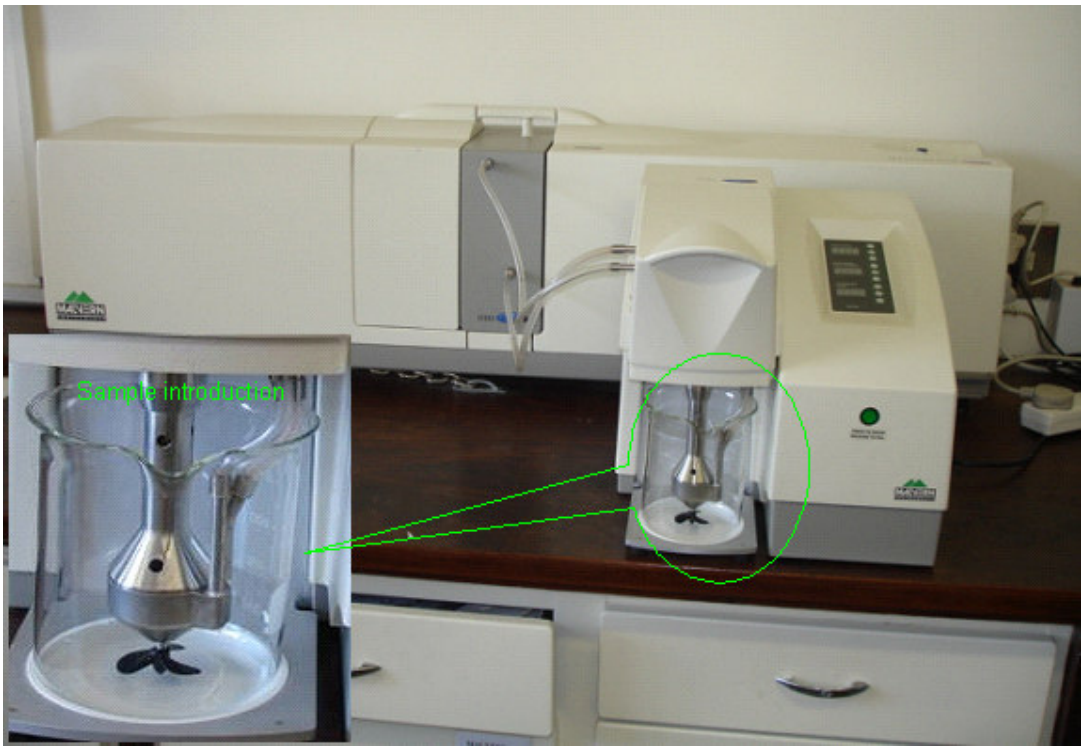


Figure A2. 2: Malverns Mastersizer S instrument

This instrument is connected to the computer which controls all parameters.

Microwave instruments



Figure A2. 3: Microdigest 301 (left), Multiwave 3000 (right)

GC-ICP-MS schematic diagram

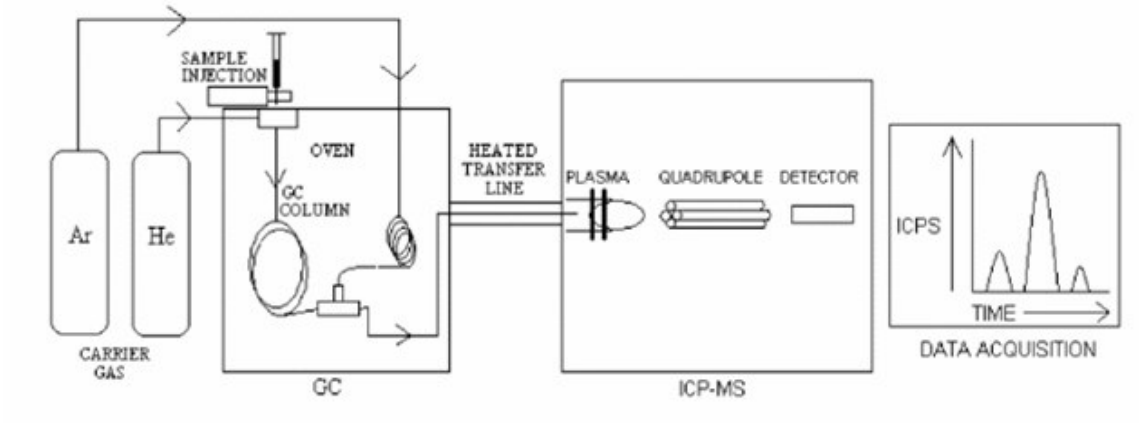


Figure A2. 4: Schematic diagram of GC-ICP-MS (Thermo Electron Corporation, Ref. n °: P G40694_E08/04C GC- ICP-MS Getting Started Guide)

Supported Liquid membrane probe extraction

Figure A2.5 shows the supported liquid membrane probe extraction for several simultaneous extractions.

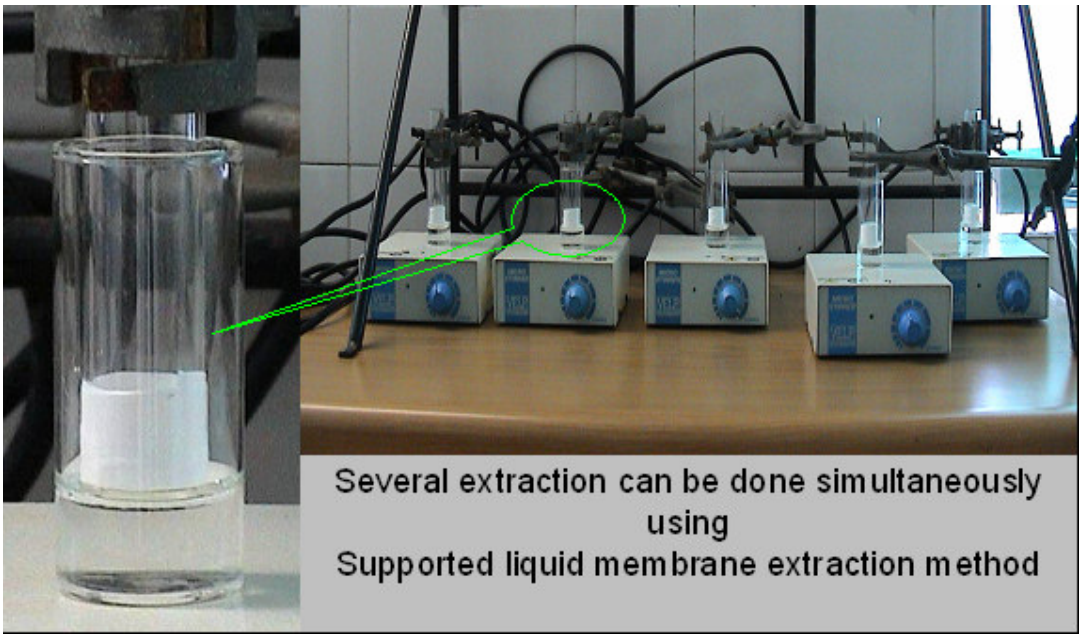


Figure A2. 5: Supported liquid membrane probe extraction setup

Appendix 3: Analytical parameters

This appendix gives the instrumental analytical parameters used in this thesis for GC coupled to FID, TCD, MS and ICP-MS detectors; IC; ICP-OES, VA and CHNS.

Table A3. 1: Instrumental analytical parameters

Parameters	**Settings**
GC-FID	
Injection port	Splitless mode
Injection volume	1µL
Injection port temperature	250°C
Detection temperature	300°C
Carrier gas-helium flow	1.44 mL min^{-1}
Column	Equity-5 30m x 0.32mm x 0.1µm film thickness
Oven program	100°C for 2 min the 10°C min^{-1} to 145°C for 10 min then 15°C min^{-1} to 280°C for 8 min
GC-TCD	
Injection port	Splitless mode
Injection volume	10µL
Injection port temperature	35°C
Carrier gas	Argon
Column	60/80 Carboxen-1000,15Ft, 1/8In S.S

Oven program	60 °C
GC–MS	
Injection port	Splitless mode
Injection volume	1µL
Injection port temperature	250°C
Detector temperature	200°C
Carrier gas–helium flow	40cm s^{-1}
Column	ZB–5ms,30m x 0.25mm x 0.25µm film thickness
Oven program	100°C for 2 min the 10°C min^{-1} to 145°C for 10 min then 15°C min^{-1} to 280°C for 8 min
GC–ICP–MS	
Injection port	Splitless
Injection volume	**2** µL
Injection port temperature	250 °C
Carrier gas–helium flow	25 mL min^{-1}
Make up gas–Argon flow	300 mL min^{-1}
Column	MXT Silcosteel 30m x 0.53 mm x 1µm
Oven program	60°C for 0 min the 60°C min^{-1} to 250
Transfer line column	Silcosteel Inner: 1 m x 0.53 mm x 0.28 mm Outer: 1 m x. 1/16 in x 1.0 mm
Transfer line temperature	280 °C

Plasma power	1 200 W
Coolant flow	15 L min^{-1}
Auxiliary gas flow	0.9 L min^{-1}
Nebulizer gas flow	0.6 L min^{-1}
Isotopes / dwell time	Hg : 202. 201.199, 30 ms Sn : 117. 120, 30 ms Tl : 203. 205, 5 ms Sb : 121. 123, 5 ms
IC	
Column	A SUPP 5, 4 mm ID x 150 mmL 0.7 mL min^{-1}
Detector	Suppressed CD
Pressure	8 MPa
ICP–OES	
Plasma power	1 300 W
Coolant flow	13.0 L min^{-1}
Auxiliary gas flow	1.0 L min^{-1}
Nebulizer gas flow (cross flow)	1.0 L min^{-1}
Sample uptake rate	2.0 mL min^{-1}
Torch injection diameter	2 mm
Spray chamber	Water-cooled single pass spray
Chamber temperature	4°C
VA	
Reference electrode	Ag/AgCl, 3M
Working electrode	Metrohm 6.1246.020 mercury electrode
Auxiliary electrode	HMDE
Method	Metrohm platinum 6.0343.30 ASV with differential pulse stripping step
CHNS	
Analysis constants	Channel # 1
Analysis element	CHN
Linearization	Cubic
Oxidation Furnace Temp	1000

Reduction Furnace Temp	650
Oxi. Fur. Standby Temp	600
Sample drop delay time	10
Conservation flow (cc. min^{-1})	35
Analysis flow (cc. min^{-1})	100
Carbon minimum timeout	100
Carbon delay time	6
Carbon comparator level	1.00
Carbon calibration	0.5268
Carbon blank	0.0
Oxygen dose #1 (cc. min^{-1})	10
Delay dose #2 (sec)	5
Oxygen dose #2 (cc. min^{-1})	0
Delay dose #3 (sec)	0
Oxygen dose #3 (cc. min^{-1})	0
Delay dose #4 (sec)	0
Oxygen dose #4 (cc. min^{-1})	0
IR cell Pressure	638 mm Hg
System Pressure	718 mm Hg

Appendix 4: NMR results

This appendix gives some details on how the identification of magnesium tetraethylborate bromide was done, using NMR. This includes the theoretical chemical shift of boron, proton and carbon NMR. The hydrolysis experiment done on magnesium tetraethylborate bromide is also presented.

Table A4. 1: Approximative ^{11}B NMR chemical shift

Compound	Chemical Shift	References
$Li^+[B(CH=CH_2)_4]^-$	-16.1 (Et_2O)	Thompson, R. J.; Davis, J. C. Jr. *Inorg. Chem.* **1965**, *4*, 1464.
$Li^+[B(CH_2CH=CH_2)_4]^-$	-16.8	Thompson, R. J.; Davis, J. C. Jr. *Inorg. Chem.* **1965**, *4*, 1464.
$Li^+[BEt_4]^-$	-17.5 (Et_2O)	Thompson, R. J.; Davis, J. C. Jr. *Inorg. Chem.* **1965**, *4*, 1464.
$Li^+[(n-Pr)_4B]^-$	-17.5 (Et_2O)	Thompson, R. J.; Davis, J. C. Jr. *Inorg. Chem.* **1965**, *4*, 1464.
$Li^+[n-Bu_3BC\equiv C-n-Bu]^-$	-18.8 (THF)	Midland, M. M. Ph. D. Thesis, Purdue University, 1972.
$Li^+[n-Bu_4B]^-$	-19.3 (THF)	Buhler, J. D. Ph. D. Thesis, Purdue University, 1973.
$Li_2^+[i-Bu_3BC\equiv C-i-Bu]^-$	-19.4 (THF)	Midland, M. M. Ph. D. Thesis, Purdue University, 1972.
$Li^+[BMe_4]^-$	-21.1 (Et_2O)	Nöth, H.; Vahrenkamp, H. *Chem. Ber.* **1966**, *99*, 1049
$Li^+[B(C\equiv CMe)_4]^-$	-22.5	Sinclair, J. A.; Brown, H. C. *J. Org. Chem.*, **1976**, *41*, 1078.
$Li^+[B(C\equiv CPh)_4]^-$	-31.2	Sinclair, J. A.; Brown, H. C. *J. Org. Chem.*, **1976**, *41*, 1078.
$NaBPh_4$	-6.2 (H_2O)	Nöth, H.; Vahrenkamp. H. *Chem. Ber.* **1966**, *99*, 1049.
$NaBEt_4$	-16.6 (Et_2O)	Nöth, H.; Vahrenkamp, H. *Chem. Ber.* **1966**, *99*, 1049
$MgBr^+[n-Bu_3BEt]^-$	-19.1 (THF)	Buhler, J. D. Ph. D. Thesis, Purdue University, 1973.
$MgBr^+[n-Bu_3BMe]^-$	-19.9 (THF)	Buhler, J. D. Ph. D. Thesis, Purdue University, 1973.
$MgBr^+[BEt_4]^-$	-16.5 (THF)	Brown, H. C.; Rachelia U. S., organometallics, 1986,

Table A4.1 gives boron NMR chemical shift found for different tetralkylboron compounds. Generally the chemical shift of those compounds range between -19 and -16. By boron NMR analysis a chemical shift of -16.973 was found for magnesium tetraethylboron bromide. Brown H. and Rachelia U. S. (1986) have found a chemical shift of -16.5 for the same compound. Figure A4.1 shows the boron NMR spectrogram obtained with the reference peak of $BF_3.OEt_2$ at about zero chemical shift.

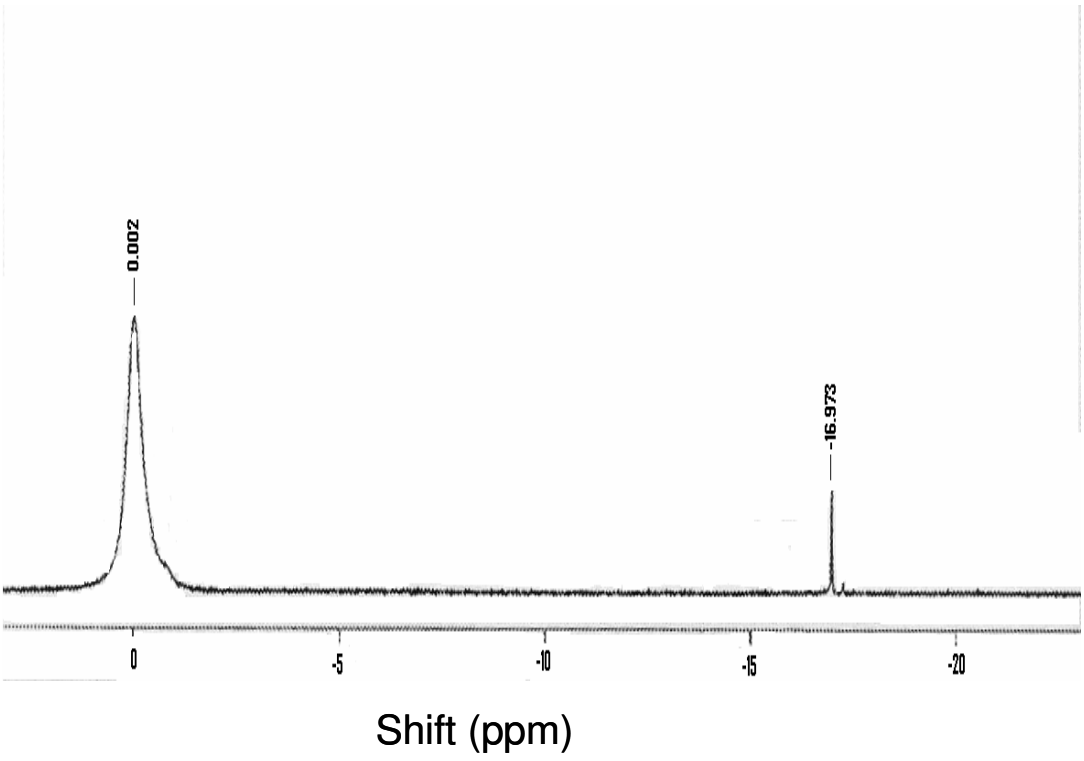


Figure A4. 1: ^{11}B NMR spectrogram

Figure A4.2 shows the approximate proton NMR chemical shift. The chemical shift of compounds such as RC**H$_3$** is ranged between 0.8 - 1.2 and 1.1 – 1.5 for R_2C**H$_2$.**

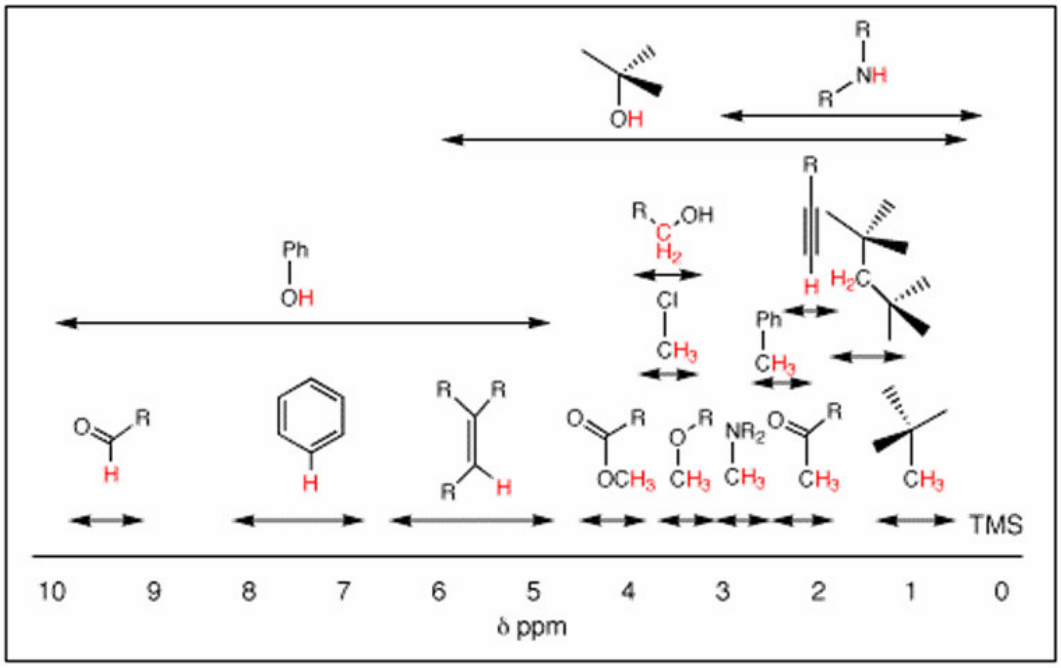


Figure A4. 2: Approximative ^{1}H NMR chemical shift

Figure A4.3 represents the spectrogram obtained by proton NMR. The red callout box shows the RC**H$_3$** group between 0.5- 1ppm and R_2C**H$_2$** between 1 – 1.5**.** These values are in good agreement with the values on the figure A4.2. The sample was dissolved in dimethyl sulfoxide.

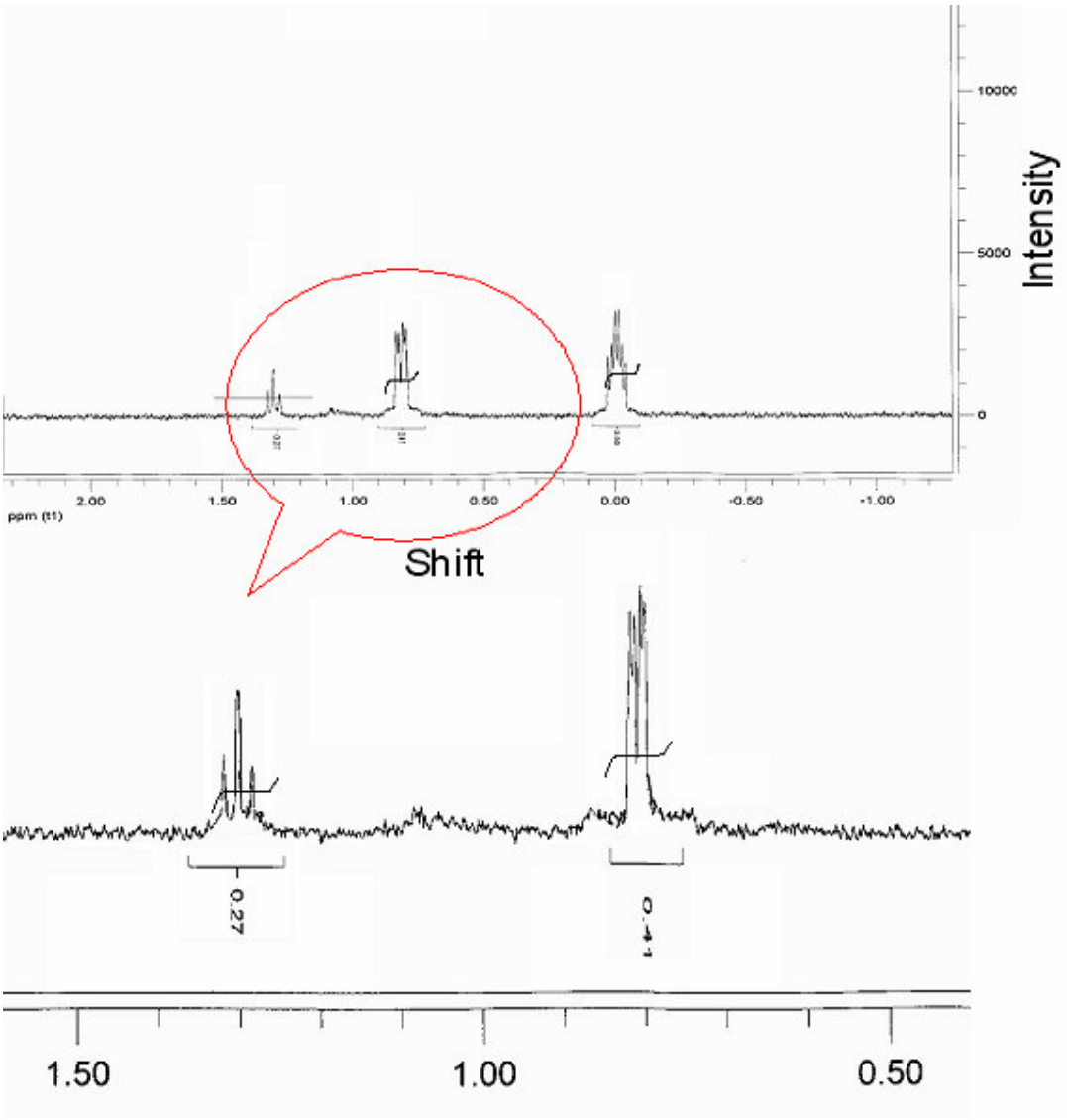


Figure A4. 3: ^{1}H NMR spectrogram

Figure A4.4 gives the approximate carbon NMR chemical shift. The alkanes chemical shift are found in the range of 0 – 40 ppm.

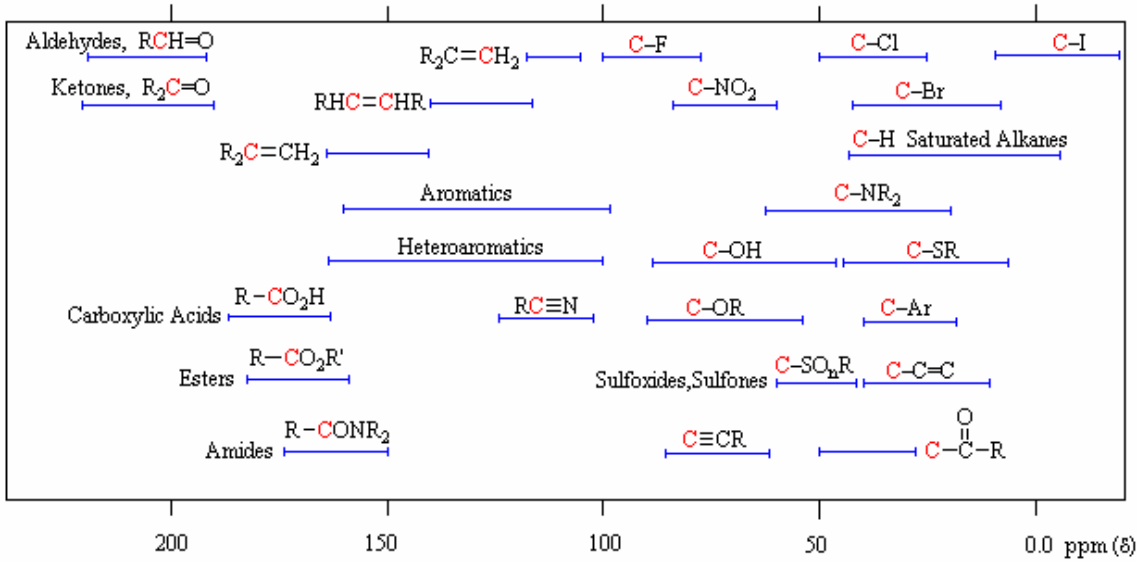


Figure A4. 4: Approximate ^{13}C NMR chemical shift

The NMR spectrogram (figure A4.5) found by dissolving the magnesium tetraethylborate bromide in deutorated chloroform gives the chemical shift in range between 10 and 30 ppm.

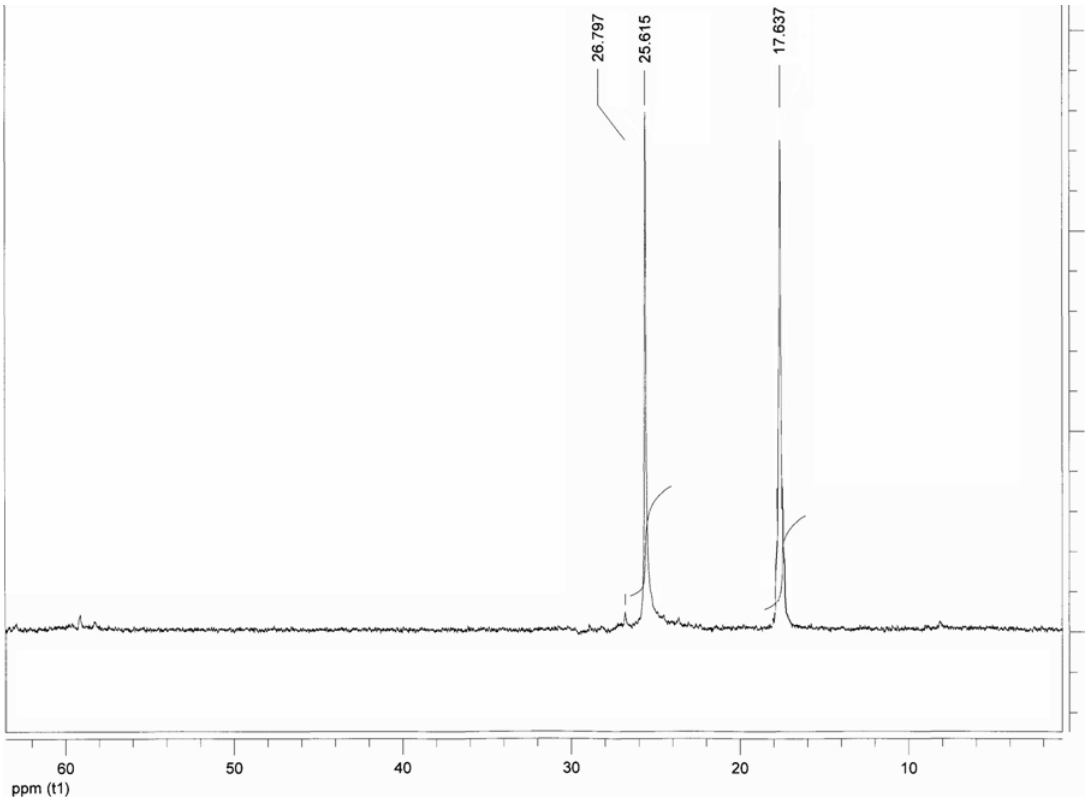


Figure A4. 5: ^{13}C NMR spectrogram

Hydrolysis of magnesium tetraethylborate bromide

The hydrolysis test was done using water and acetic acid. The reactions were performed by reacting 1g of magnesium tetraethylborate bromide with water or acetic acid. The sample was introduced in a 100 ml flask then water or acetic acid added. The flask was closed immediately with a rubber. The reaction in acetic acid was monitored for 1 hour by heating in water bath (50-60) while the reaction in water was left to continue for 12 hours in ambient temperature. The low heating temperature for acetic acid reaction was used to avoid interference from acetic acid vapour during GC-TCD

analysis. Acetic acid has a boiling point of 118.1 ◦C and vapour pressure of 11 mmHg at 20◦C [Wikipedia (2006), Acetic acid]

Gas chromatographic analyses were done by collecting the gas phase through the flask rubber lid. A gas syringe was used for collection, and then the injection to the gas chromatography was preformed immediately.

Figure A4.6 shows the chromatogram obtained for gas analysis during the acetic acid reaction. GC-TCD was used.

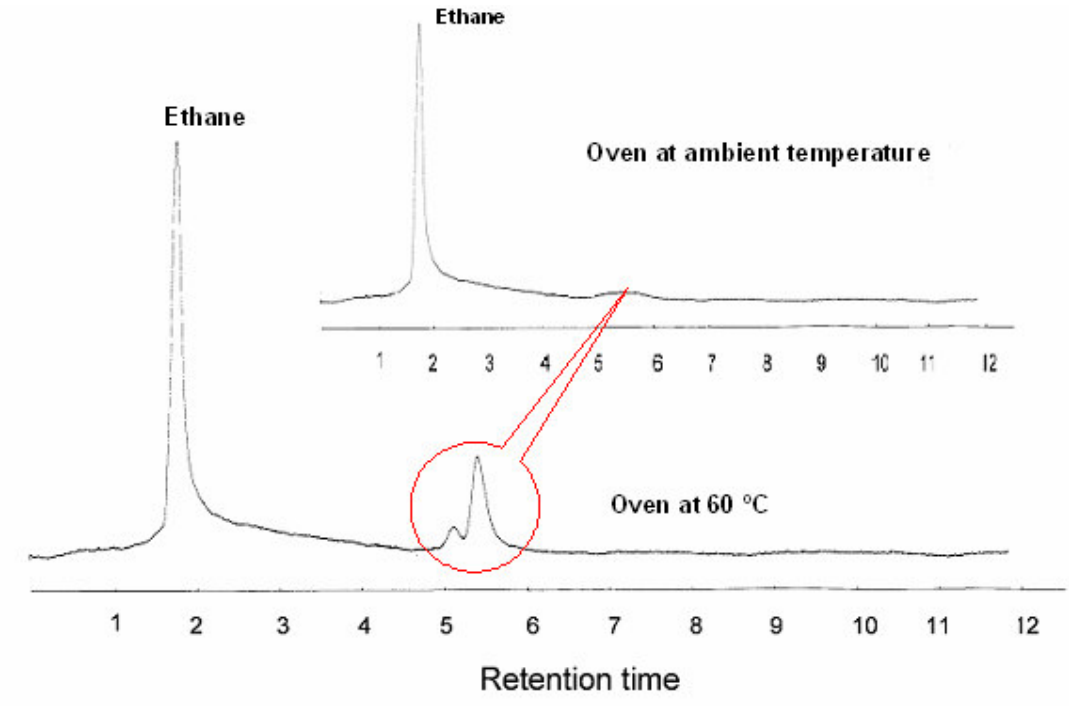


Figure A4. 6: Chromatogram of gas analysis for acetic acid reaction

The analysis done at oven ambient temperature gives one peak. That peak was identified as ethane. When the oven was programmed at 60 degree we obtained two small peaks as shown by the red callout on the figure A4.6. one of the small peak is acetic acid the other one might be from some impurity.

Damico (1974) postulated that the hydrolysis of these compounds involves rapid protonation and loss of alkane, followed by a slow hydrolysis of the resulting trialkylboranes (equation A4.1 and A4.2).

$$MBR_4 + AcOH \xrightarrow{Fast} MOAc + RH \quad \textbf{(A4. 1)}$$

$$R_3B + AcOH \xrightarrow{Slow} 3RH + B(OAc)_3 \quad \textbf{(A4. 2)}$$

There is no reaction when magnesium tetraethylborate bromide was added to water at ambient temperature. This shows the stability of magnesium tetraethylborate bromide in water.

Appendix 5: Phenylation derivatisation

This appendix presents some chromatogram obtained by GC-MS analyses.

Simultaneous analysis of tin and lead by phenylation

Simultaneous speciation of organotin and organolead in the same sample using deivatisation by phenylation was not possible as mentioned early in section 5.2.1. The chromatograms obtained were not resolved. The change in oven program failed to reach any resolved chromatogram. The figure A5.3 shows the chromatogram while trying to analyse tributyltin chloride, trimethyllead chloride and triethylchloride simultaneously by phenylation using sodium tetraethylborate. The chromatogram on the left (figure A5.3) shows unresolved peak of organotin and organolead in the mixture. On the right there is a chromatogram with well resolved peaks of organolead (trimethyllead and triethyllead). The first peak around 12 minutes is the solvent peak, the peak at 16 minutes is for trimethyllead and the one at 19 minutes is for triethyllead.

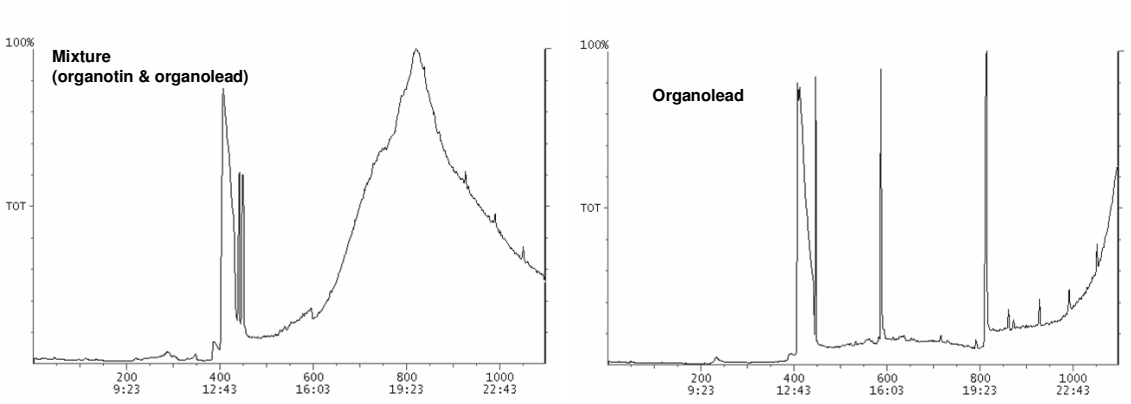


Figure A5. 1: Chromatogram of organotin and organolead derivatisation by phenylation

Figure A5.4 gives mass spectrometry chromatogram of dimethyllead and trimethyllead. While analysing the organolead products of tetramethyllead degradation in water by solar radiation, one of the products was dimethyllead but it couldn't be quantified. The figure A5.4 gives similar fragments as what found in literature for trimethyllead and dimethyllead [Zufiaure R., 1997]. The phenylation was used for derivatisation.

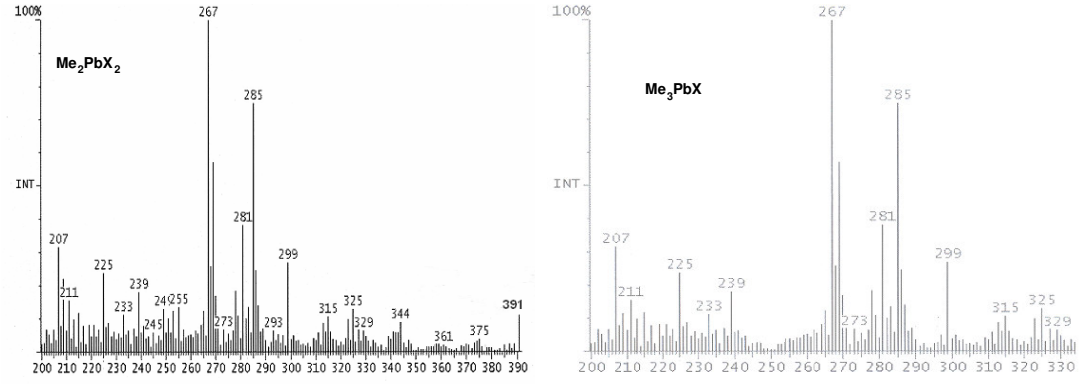


Figure A5. 2: Mass spectrometry chromatogram of organolead after degradation

Appendix 6

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Application of supported liquid membrane probe for extraction and preconcentration of organotin compounds from environmental water samples

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Abstract

The new supported liquid membrane (SLM) extraction probe (SLMP) was developed and optimized for extraction and preconcentration of organotin (OT) compounds from environmental water samples. SLMP is much simpler configuration than traditional SLM systems. The use of stirring allows the same sample to be extracted many times with higher extraction efficiency from smaller sample size. The SLMP was used to extract organotin compounds from aqueous solution to organic solvent (isooctane) followed by determination of organotin species by gas chromatography. The SLM probe extraction was optimized for extraction time, stirring rate, the geometry of the system, and ionic strength of the sample. The extraction efficiencies obtained were 63–94% for deionised water and 52–89% for sea water. The detection limit of the method ranged from 0.5 μg for triphenyltin to 1.5 μg for monobutyltin. The optimized procedure was applied for the speciation study of OT compounds in environmental water samples.
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Keywords: Supported liquid membrane extraction; Membrane probe; Organotin compounds speciation; Preconcentration

1. Introduction

Among the many concerns of environmental lists, the levels of heavy metals (tin, lead, mercury and arsenic) present in environmental systems are of high priority since they have been recognised as significant pollutants. A lot of data has been reported from speciation studies of various heavy metal ions [1–6]; far less has been reported on the organometallic forms of these metals. Organic derivatives of these metals are the most widely distributed organometallic ecotoxicants in the environment [7]. The reason for this is that the presence of the organic moiety renders, these compounds more accessible to biological systems through membranes and allows them to bioaccumulate therein [7–13].

Therefore, identification and quantification of these organometallic compounds is of vital importance as they are generally more toxic than their inorganic analogues. There has been a great deal of public attention focused on the toxicological and ecotoxicological aspects of organotins (OT) recently [8]. Organotin compounds have various industrial applications [14–16], which include use as stabilising agents in polymers [17], as pesticides [18,19], wood treatment [19] and as antifouling agents in paints [9,20–22]. There is a special concern over the use of tributyltin (TBT) because of its biocidal properties [11,23].

The biological effects of organotin ecotoxicants are mainly dependant on the number and nature of the constituent alkyl and aryl groups [24]. Due to the intensive use of tributyl- and triphenyltin in antifouling paints for ships, the most significant effects have been observed in the marine environment [15,16,21]. Trialkyl and triaryl derivatives of tin have been found to exert a powerful toxic action on the central ner-

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vous system and to suppress the thymus-dependent immune responses [7].

The toxic effects that the TBT pose results in strict regulations that can lead to its complete ban. The high toxicity of organotin compounds is confirmed by the extremely low LC_{50} lethal dose values of 1–4 $\mu g\,l^{-1}$ [11,15]. However, the world production of OT compounds has multiplied almost 10-fold in the past 40 years [15].

Analytical procedures for OT evaluation in environmental samples are usually cumbersome and involve several steps. One of the most critical steps of speciation analysis is the extraction of the sample from its matrix, its enrichment and clean up, before it is injected into a chromatographic column [25].

Two conflicting issues have to be balanced during extraction. These are: obtaining adequate recovery rates and preventing losses or the destruction of species [26].

The methods for pretreatment/extraction from matrices (water, soil, sediments and biological material) can be categorized according to the choice of solvent (polarity), sample pH, and the use of enzymes or chelating agents [27]. These are referred to classic extraction techniques. More recently, new approaches are becoming more common, which include microwave assisted extraction (MAE) [28,29], supercritical fluid extraction (SFE) [28,30–32], pressurized liquid extraction (PLE) [25,28,32], solid phase extraction (SPE) [28,33–37], and solid phase microextraction (SPME) [33–37]. These are known as fast extraction methods (FME) and are accompanied by a possible loss of chemical stability of the analyte species during the extraction steps, particularly in those methods, which employ high temperature or pressure, such as SFE, PLE, and MAE [27].

The aim of this project was to develop and optimized a sensitive and selective method that will allow analysis of OT species at low concentrations in very complicated matrices of environmental samples.

The supported liquid membrane probe (SLMP) device had been previously developed in our laboratory and used for the speciation of chromium and manganese in water and biological samples [38]. Its use as an extraction and preconcentration procedures had never been applied to the speciation analysis of OT compounds, which involve the extraction from aqueous donor solution to organic solvent acceptor. In this paper, we investigate this system and the effect of factors that influence the extraction efficiency of OT extraction by SLMP technique.

2. Experimental

2.1. Chemicals

Monobutyltin trichloride (MBT), dibutyltin dichloride (DBT), tributyl chloride (TBT), and triphenyltin chloride (TPhT) were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium tetraethylborate ($NaBEt_4$) was purchased in 1 g sealed aliquot from Merck (Darmstadt, Germany). Sodium acetate–acetic acid buffer and isooctane were from Sigma–Aldrich (Steinheim, Germany). All glassware used were soaked in 2 M nitric acid for at least 2 days and rinsed with Milli-Q water and ethanol immediately before use.

2.2. Solutions

1000 $mg\,l^{-1}$ stock solutions were prepared by dissolving the appropriate amount of the OT compound in methanol and were stored at 4 °C in the dark. A solution of 5% (m/v) of sodium tetraethylborate ($NaBEt_4$) in methanol was prepared and a few grams of anhydrous sodium sulfate (Na_2SO_4) were added. The solution was stored under argon and kept in the refrigerator at −4 °C. All solutions were prepared from analytical grade reagents and high-purity water was obtained from Milli-Q-67120 (Molsheim, Millipore, South Africa).

2.3. Environmental water samples

The environmental water samples were collected from industrially polluted areas in South Africa. The sea water was from the Port Elizabeth harbor. The river and dam water samples were from the areas around Johannesburg affected by mine tailings dumps. The Rustenburg region, north of Johannesburg, has clay soils and is loaded with waste material after PGMs production. The Germiston region, south of Johannesburg, has mixed sandy/clay soils and is affected by tailings dumps from gold production.

2.4. Instrumentation

2.4.1. Membrane probe unit

The new miniaturised-supported liquid membrane probe was made from polypropylene tube with dimensions; 13 mm i.d., 16 mm o.d. and 92 mm length. In this configuration, one end of a polypropylene tube was closed with the porous Millipore filter sealed with PTFE tape and soaked with organic solvent (isooctane) (Fig. 1). The inside of this probe served as the acceptor phase. The probe was then immersed in a polypropylene tube containing the stirred sample. Compared to traditional SLM configurations, this has many advantages. It is the simplest configuration to be reported to our knowledge, inexpensive since no pumps are needed, possible to perform many probe extractions simultaneously, an important aspect in routine analysis and drug screening process. Since the sample is stirred, it allows the same sample to be extracted many times thus, giving higher extraction efficiency. This means that only small sample volumes are needed. Depending on the matrix in the sample, the SLM probe can be used many times. It is also very easy to regenerate.

2.4.2. Gas chromatography equipment

All samples were analysed using HP 5890 gas chromatograph with capillary column ZB-5 from Phenomenex (Torrance, CA, USA).

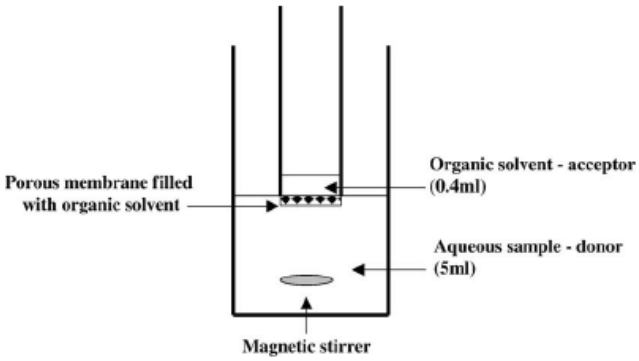


Fig. 1. Supported liquid membrane extraction probe unit used in the study.

2.4.3. Other equipment

The pH was measured using a Φ50 pH meter with glass electrode from Beckman (Fullerton, USA). The magnetic stirrer used was from Velp Scientitia (Europe).

2.5. Procedures

2.5.1. Sample preparation

Water samples for optimization of the extraction procedure were spiked with known concentrations of each OT compound. The pH was adjusted to 5.0 using sodium acetate–acetic acid buffer. Then, 200 μl of a 5% $NaBEt_4$ solution was added and the mixture was stirred for 5 min. The environmental water samples were treated in the same way.

Calibration standards were prepared by mixing 1 ml of isooctane with 10 ml of the derivatized OT compounds. The mixture was shaken for 5 min and after phase separation, the organic phase was collected and used for GC analysis. In determining the extraction efficiency, blank water samples were extracted first followed by spiked ones. Any concentrations of OT compounds present in the blank was quantified and subtracted from the spiked ones. In quantifying the concentrations of the OT compounds in real samples, however, standard addition method was used. In this case, the derivatized sample was split into four or three 5 ml portions. These were spiked with different but known concentrations of OT compounds except in one and extracted in the same way.

2.5.2. Preparation of the membrane units and extraction procedures

The SLM probe was soaked into the organic solvent (isooctane) for 30 min. It was then flushed with deionised water both its inside and outside to remove all the excess of organic solvent on the surface.

The inside of the probe served as the acceptor phase and was filled with 400 μl of isooctane. The outside of the membrane probe was immersed into 5 ml of the stirred sample solution (containing OT compounds which were derivatized using tetraethylborate solution). The extraction was allowed to proceed for 40 min. The probe was then removed from the sample solution and the acceptor phase (isooctane) was collected into a polypropylene vial, analysed immediately or closed and stored at 4 °C until analysed. Before next extraction was performed, the inside of the probe was flushed with about 5 ml of fresh acceptor solution and the outside with deionised water.

2.5.3. GC analysis

The analysis of sample was performed using gas chromatography. Details of the chromatographic measurement conditions are shown in Table 1.

2.5.4. Extraction efficiency

The extraction efficiency (E), i.e., the fraction of analyte in the extracted sample that is found in the acceptor phase is given by equation below [39–42]:

$$E = \frac{C_A V_A}{C_D C_D} \quad (1)$$

where C_A is the concentration in the collected acceptor fraction and C_D is the concentration in the extracted sample. V_A is the collected acceptor volume while V_D is the volume of the sample that has been extracted.

Table 1
GC–FID analytical parameters

Parameters	Setting
Injection port	Split/splitless mode: splitless
Injection volume	1 μl
Injection port temperature	280 °C
Detector temperature	310 °C
Carrier gas–helium flow	1.44 ml min^{-1}
Column (capillary column)	ZB-5 (5% phenyl, 95% dimethylsiloxane), diameters: 30 m × 0.32 mm × 0.1 μm film thickness
Oven program	40 °C for 2 min then 10 °C min^{-1} to 100 °C, 20 °C min^{-1} to 280 °C for 8 min
Detector type	Flame ionization detector (FID)
Detector temperature	300 °C

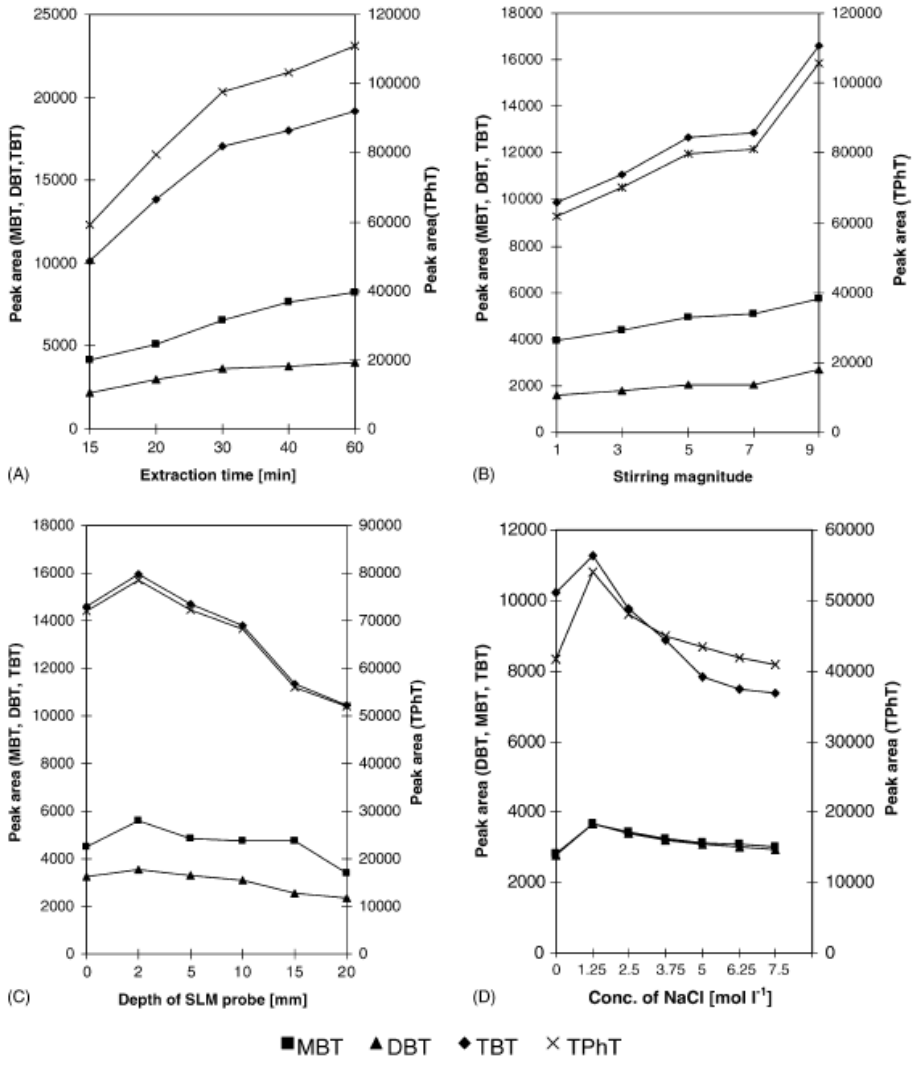


Fig. 2. Optimisation of OT extraction from aqueous samples to organic acceptor: (a) extraction time, (b) agitation intensity, (c) depth of the probe into the donor solution and (d) addition of sodium chloride.

3. Results and discussion

3.1. Optimisation of OT extraction from aqueous samples to organic acceptor

Four factors were optimized: extraction time, agitation intensity, depth of the probe into the donor solution and the addition of sodium chloride.

3.1.1. Effect of extraction time

The effect of extraction time on the membrane probe extraction of the OT compounds is shown in Fig. 2a. The experiment was conducted under the following conditions: constant stirring settings on a magnetic stirrer and a probe depth of 2 mm into a sample solution (no NaCl added). The amount of OT extracted increased with the extraction time. This trend can be explained by the fact that longer

Table 2
The linearity of extraction for the range of OT concentrations ($\mu g\,l^{-1}$) spiked

Compound	Concentrations spiked ($\mu g\,l^{-1}$)[a]					Calibration curves		
	10	15	20	25	35	*Y* intercept	Slope	*r* Value
MBT	1875.02	3344.01	4925.11	6650.46	8715.01	5.993	1698.6	0.9977
DBT	1654.05	2500.23	3438.36	4463.29	5124.33	905.33	2688.8	0.9954
TBT	2875.12	4944.42	7225.82	9760.99	12750.01	141.37	2456.6	0.9974
TPT	4063.33	5981.32	8581.19	11469.23	14763.28	764.97	890.36	0.9982

[a] Peak areas obtained × 100

Table 3
Recoveries of OT compounds from deionised water and sea water samples

OT compound	Concentration spiked ($\mu g\,l^{-1}$)	Average concentrations detected		Recoveries (%)	
		Deionised water	Sea water	Deionised water	Sea water
MBT	30	17.1	14.1	57 (9.8)	47 (10.1)
DBT	30	27.3	17.1	91 (8.1)	57 (8.9)
TBT	30	26.1	24.1	87 (9.5)	80 (8.7)
TPhT	30	28.8	27.2	96 (12.2)	91 (11.6)

R.S.D. (%) in parenthesis.

extraction time allows for the donor solution to be in contact with the acceptor solvent for longer periods, which enable greater degree of diffusion of the non-polar analyte from the donor to the acceptor phase through the hydrophobic membrane. However, this trend was more pronounced within the first 30 min of the extraction time and began to plateau as extraction time increased beyond 30 min.

3.1.2. Effect of agitation intensity

Effect of agitation intensity on membrane probe extraction of the OT compounds is shown in Fig. 2b. This parameter was investigated using a magnetic stirrer equipped with nine stirring settings. It was observed that generally, an increase in stirring intensity results in an increase in recovery of each OT compound. Although this trend is not very pronounced, it is definitely observed, and may be explained in terms of contact time between the analyte, the hydrophobic membrane, and the pressure exerted on the membrane. Increasing the rate of agitation enables more effective mixing of the donor solution. The result of the stirring is that fresh portions of donor solution are allowed to come into contact with the organic acceptor. This enhances the extent to which diffusion can occur, hence increases the concentrations of OTs in the acceptor phase. At a very high stirring rate the amount extracted does not depend on the transport to the membrane only on the kinetics of the extraction resulting in a plateau.

3.1.3. Effect of probe depth

The effect of probe depth on the extent of membrane probe extraction of OTs is shown in Fig. 2c. It shows the maximum in recovery of OTs when the membrane-bound probe is inserted 2 mm into the donor solution. Thereafter, the amount of extracted OT compounds decrease. This trend may be explained by the quantity of analytes in contact with the membrane probe. Because the working membrane area is only at the bottom of the probe, the deeper the probe is immersed into the donor solution, the smaller the volume of sample that comes into contact with the organic acceptor.

3.1.4. Effect of sodium chloride addition

Fig. 2d shows the effect of addition of sodium chloride on the extent of extraction. It was observed that the extraction of OT compounds reach the maximum at the concentration of 1.25 $g\,l^{-1}$ of NaCl. Sodium chloride is only in ionic form in aqueous solution and its role in the extraction is to increase

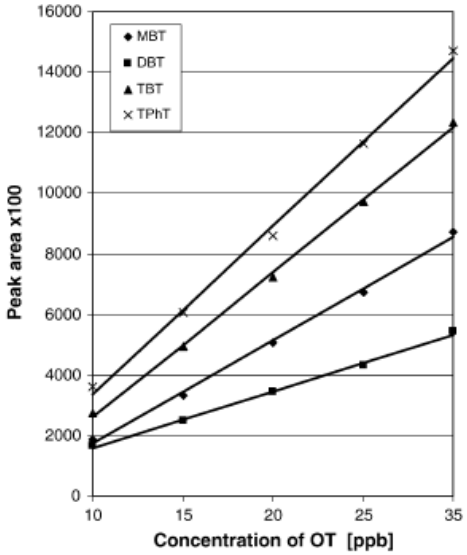


Fig. 3. The linearity of extraction efficiency for the range of OT concentrations spiked.

Table 4
Speciation of OT in varies environmental water samples

Sample id	pH	ORP vs. SHE (mV)	Conductivity ($\mu S\,cm^{-1}$)	Concentrations found ($\mu g\,l^{-1}$)				
				MBT	DBT	TBT	TPhT	Total Sn
Rustenberg water samples								
R1	10.20	321	1003	10.4 (5.7)	17.7 (6.2)	ND	ND	35
R2	8.13	433	1038	11.8 (6.5)	19.3 (6.8)	ND	ND	98
R3	7.80	440	672	12.3 (7.1)	23.0 (7.2)	ND	1.5 (9.1)	105
R4	8.40	442	510	10.9 (7.5)	18.6 (6.7)	ND	ND	43
R5	8.22	428	1008	11.2 (6.8)	18.9 (5.5)	ND	ND	82
G1	6.78	375	524	13.8 (8.4)	29.1 (7.1)	5.7 (6.3)	3.6 (4.9)	63
G2	6.37	312	722	14.3 (8.2)	33.9 (7.5)	6.8 (5.4)	4.5 (5.6)	70
G3	5.40	360	883	14.1 (7.9)	30.3 (6.8)	6.1 (5.7)	3.9 (4.3)	105
G4	2.90	691	1638	8.5 (6.9)	11.9 (7.0)	2.6 (5.1)	ND	125
G5	3.85	565	1418	13.9 (6.7)	29.5 (7.2)	5.9 (6.1)	3.8 (5.6)	117
Sea water								
S1	8.20	689	5468	2.3 (7.9)	4.6 (8.0)	10.4 (9.8)	6.3 (8.9)	113
S2	8.15	684	5519	2.4 (7.6)	4.5 (7.9)	10.9 (9.6)	6.2 (8.8)	120
S3	8.16	698	5575	2.8 (7.7)	4.8 (7.6)	11.5 (9.3)	6.9 (8.5)	134

R.S.D. (%) in parenthesis; ORP: oxidation/reduction potential.

the ionic strength of the donor solution, which results in increased partitioning of the analyte into the membrane. To high ionic strength course, the decrease of OT solubility leading to adsorption of compounds. This effect was also observed in other studies [43].

3.1.5. Extraction efficiency and linearity

The extraction efficiency was linear in the range 10–35 $\mu g\,l^{-1}$ investigated. Fig. 3 shows a GC result for OT peak areas after extraction of solutions spiked with the above concentrations. The results are average of three duplicate extractions. The extraction efficiency was calculated after extraction of deionised water and sea water spiked with 30 $\mu g\,l^{-1}$ of each OT compound. The results are shown in Table 2. The SLM probe allows the sample to come into contact with the membrane several times during extraction. This results in higher extraction efficiency compared to traditional set up where the sample is pumped over the membrane surface. Individual extraction efficiencies are influenced mostly by the polarity of the OT compounds, which in turn determines the partitioning into the membrane. MBT is the most polar and was, therefore, least extracted. TPhT is the most hydrophobic and gave highest extraction efficiency as it is partitioned more into the membrane. The extraction efficiencies were a little lower in sea water than in deionised water perhaps due to adsorption on various matrices found in sea water and high concentration of sodium chloride (Fig. 2d). The detection limit of the method was calculated from signal to noise ratio of 3:1 after extraction of 10 $\mu g\,l^{-1}$ spiked OT compounds. The detection limit ranged from 0.5 $\mu g\,l^{-1}$ for TPhT to 1.5 $\mu g\,l^{-1}$ for MBT.

3.1.6. Application to real water samples

Table 3 gives a summary of concentrations determined in water samples from various areas in South Africa. Generally, in each sample, there was at least one type of OT compound present. As shown by pH values, water samples from Rustenberg were alkaline while from Germiston were acidic. Overall, the concentrations of OT compounds detected in Rustenberg were higher compared to those from Germiston area. TBT was also absent in all Rustenberg samples while TPhT was only detected in one sample and at very low concentration. On the other hand, TBT was present in all the Germiston samples and TPhT was also present except in one sample. This observation suggests that the occurrence of most OT compounds particularly the trisubstituted compounds (TBT and TPhT) is favored by acidic conditions.

The oxidation reduction potentials measured in both the Rustenberg and Germiston samples were high (ranging between 312 and 691) indicating oxidizing conditions in both sample media. This observation supports the existence of tin as Sn(IV) ion as opposed to Sn(II) ion. Sn(IV) is the form in which OT compounds are stable. As seen from Table 4, the total concentration of Sn (determined by ICP–MS) is much larger than that of OT compounds. Total concentration of OT compounds also depended on the sample pH with more acidic samples containing high concentrations.

4. Conclusions

The developed SLM probe was previously used for the extraction and preconcentration of aqueous samples to aqueous acceptor solution. In this paper, we present the extraction of aqueous samples to organic acceptor solution. The SLM probe prooved to work successfully also in this system. In our future study, the SLM probe will be used for extraction from organic sample solutions into organic acceptor solution.

References

[1] I.K. Krull (Ed.), Trace Metal Analysis and Speciation, Elsevier, Amsterdam, 1991.
[2] A.M. Ure, C.M. Davidson, Chemical Speciation in the Environment, first ed., Chapman and Hall, New York, 1995.
[3] T.M. Florence, Talanta 29 (1982) 345.
[4] I.R. Pereiro, A. Wasik, R. Lobinski, J. Chromatogr. A 795 (1998) 359.
[5] S. White, T. Catterick, B. Fairman, K. Webb, J. Chromatog. A 794 (1998) 211.
[6] R.M. Harrison, S. Rapsomanikis (Eds.), Environmental Analysis Using Chromatography Interfaced with Atomic Spectroscopy, Ellis Horwood, Chichester, 1989.
[7] R. Reuther, L. Jaeger, B. Allard, Anal. Chim. Acta 394 (1999) 259.
[8] R. Lobinski, Metals biomolecules, Bioinorg. Anal. Chem., Analysis-Dossier 26 (1998) M17.
[9] A. Kot, J. Namesnik, Trends Anal. Chem. 19 (2000) 69.
[10] F. Adams, S. Slaets, Trends Anal. Chem. 19 (2000) 80.
[11] P. Quevauviller, R. Harrison, F. Adams, L. Ebdon, Anal. Chem. 19 (2000) 195.
[12] D. Amouroux, J.C. Wasserman, E. Tessier, O.F.X. Donard, Environ. Sci. Technol. 17 (1999) 3044.
[13] J. Szpunar, R. Lobinski, J. Anal. Chem. 363 (1999) 550.
[14] R.F. Bennet, Industrial Chem. Bull. 2 (1983) 171.
[15] K. Fent, Ecotoxicology of organotin compounds, Crit. Rev. Toxicol. 26 (1996) 1.
[16] B.S. Tselentis, M. Maroulakou, J.F. Lascoureges, J. Szpunar, V. Smith, O.F.X. Donard, Mar. Pollut. Bull. 38 (1999) 146.
[17] A.S. Stasinakis, N.S. Thomaidis, T.D. Lekkas, Ecotoxicol. Envir. Saf. 49 (2001) 275.
[18] R.D. Barnes, A.T. Bull, R.C. Poller, Pestic. Sci. 4 (1973) 305.
[19] A. Albalat, J. Potgrykus, J. Pempkowiak, C. Porte, Chemosphere 47 (2002) 165.
[20] G.E. Batley, Sci. Total Environ. 122 (1992) 301.
[21] A.C. Belfroid, M. Purperhart, F. Ariese, Mar. Pollut. Bull. 40 (2000) 226.
[22] www.ace.orst.edu.info/extoxnet/pips/tributiltin.htm.
[23] www.ortepa.org/about organotin.htm.
[24] www.fluoridealert.org/pesticides/triphenyltin.F1.1984.PubMe.htm.
[25] S. Chiron, S. Roy, R. Cottier, R. Jeannot, J. Chromatogr. A 879 (2000) 137.
[26] P. Quevauviller, Trends Anal. Chem. 19 (2000) 86.
[27] V. Camel, Trends Anal. Chem. 19 (2000) 229.
[28] J.L. Gomez-Ariza, E. Mortales, I. Graldez, D. Sanches-Rodas, A. Velasco, J. Chromatogr. A 938 (2001) 211.
[29] O.F.X. Donard, B. Lalere, F. Martin, R. Lobinski, Anal. Chem. 67 (1995) 4250.
[30] Y.K. Chau, F. Yang, M. Brown, Anal. Chim. Acta 304 (1995) 85.
[31] N.P. Vela, J.A. Caruso, J. Anal. Atom. Spectrom. 11 (1996) 1129.
[32] C.G. Arnold, M. Berg, S.R. Mueller, U. Dommann, R.P. Schwarzerbach, Anal. Chem. 70 (1998) 309.
[33] K.D. Buchholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160.
[34] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[35] S. Tutschku, S. Mothes, R. Wennerich, Fresenius J. Anal. Chem. 354 (1996) 587.
[36] J. Poerschmann, F.D. Kopinke, J. Pawliszyn, Environ. Sci. Technol. 31 (1997) 3629.
[37] G. Lespes, V. Desauziers, C. Montigny, M. Potin-Gautier, J. Chromatogr. A 826 (1998) 67.
[38] L. Soko, L. Chimuka, E. Cukrowska, S. Pole, Anal. Chim. Acta 485 (2003) 25.
[39] J.A. Jönsson (Ed.), J. Sep. Sci. (24) 7 (2001) 459.
[40] L. Chimuka, L. Mathisson, J.A. Jönsson, Anal. Chim. Acta 416 (2000) 77.
[41] L. Soko, E. Cukrowska, L. Chimuka, Anal. Chim. Acta 474 (2002) 59.
[42] L. Chimuka, E. Cukrowska, L. Soko, J. Sep. Sci. 26 (2003) 1–6.
[43] K. Ndungu, L. Mathiasson, Anal. Chim. Acta 404 (2000) 319.

Appendix 7: GC-ICP-MS chromatograms

This appendix gives the chromatograms obtained from the GC-ICP-MS analyses and the field measurement of Klip river samples.

GC – ICP - MS CHROMATOGRAM

Figures A7.1, A7.2 and A7.3 give the chromatograms obtained for a water sample spiked with inorganic mercury ^{199}Hg. The figure A7.4 compares all chromatograms obtained for different isotope. It shows the possibility of multiple ratio comparison.

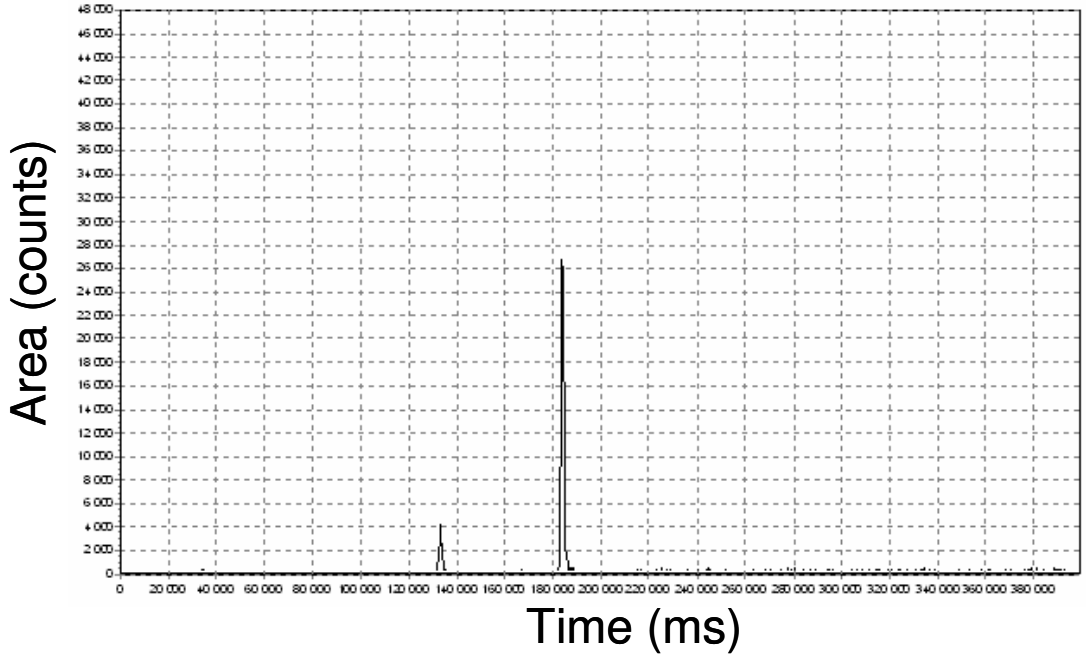


Figure A7. 1: Mercury species chromatogram for ^{199}Hg isotope

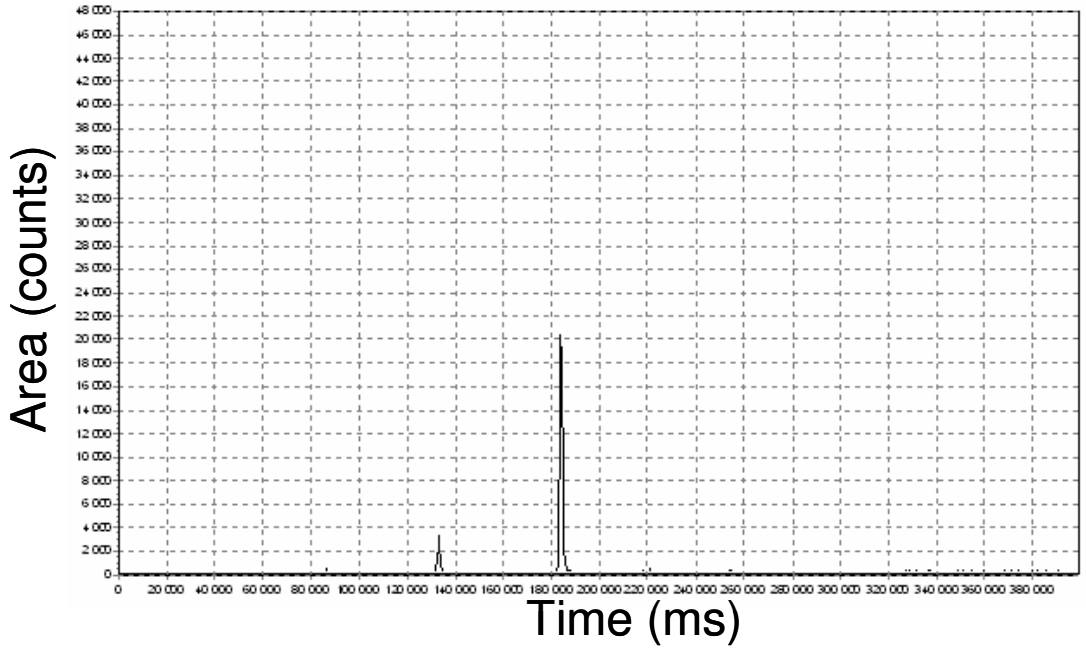


Figure A7. 2: Mercury species chromatogram for ^{201}Hg isotope

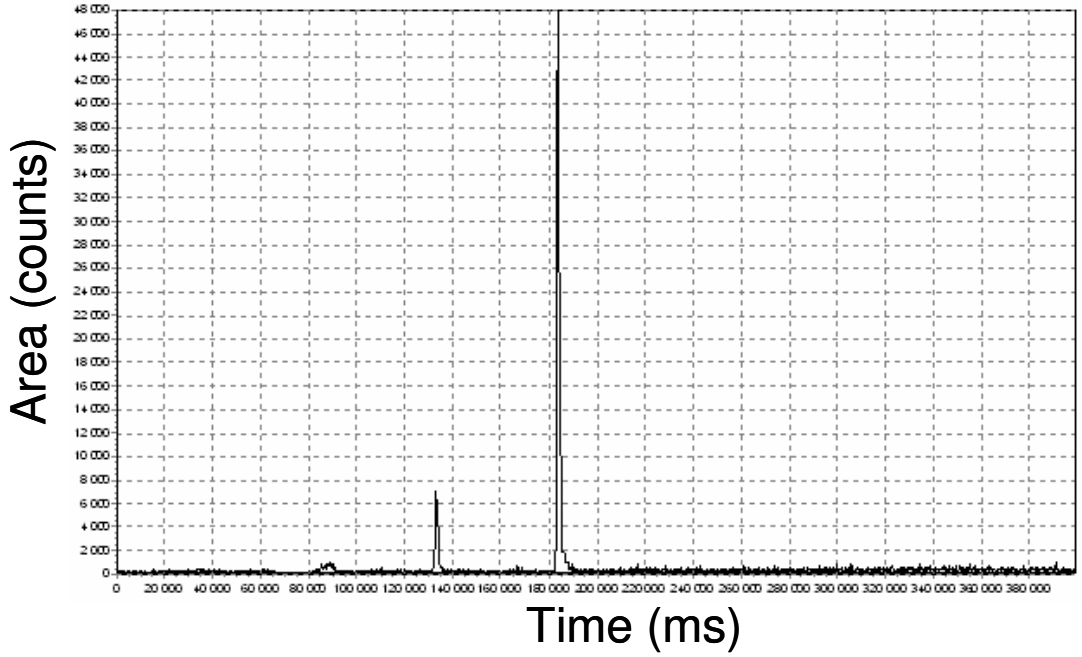


Figure A7. 3: Mercury species chromatogram for ^{202}Hg isotope

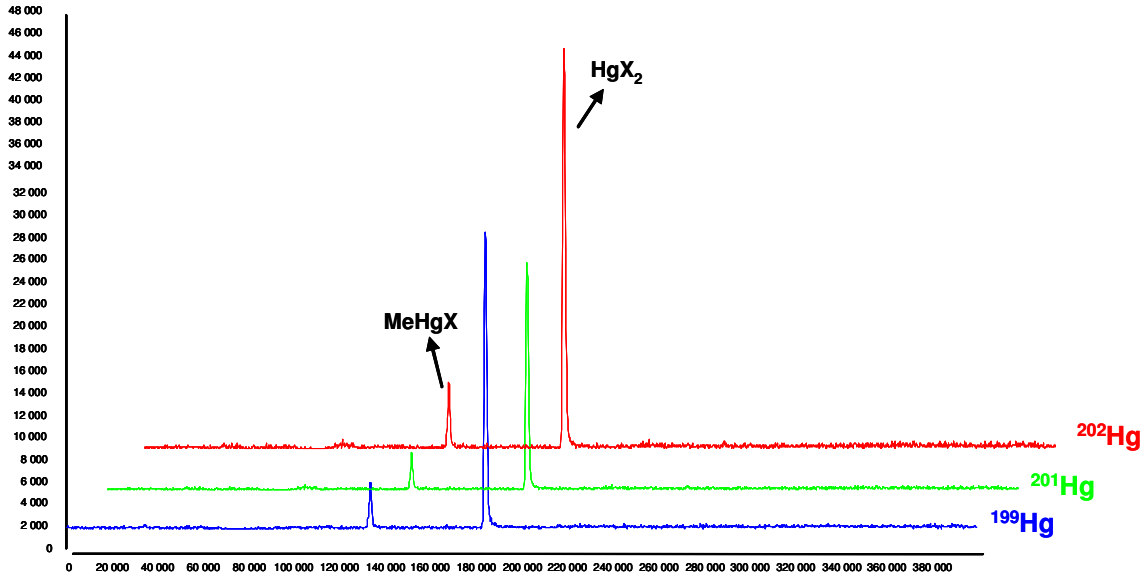


Figure A7. 4: Mercury species chromatogram comparison for different isotopes

Figures A7.5, A7.6 and A7.7 give the chromatograms obtained for a sediment sample without any isotope spike. The figure A7.8 compares all chromatograms obtained for different isotope. When we assume that there was no contamination of the sample, the ratio of those isotopes should relate to the natural abundances of ^{118}Sn, ^{119}Sn, ^{120}Sn isotopes.

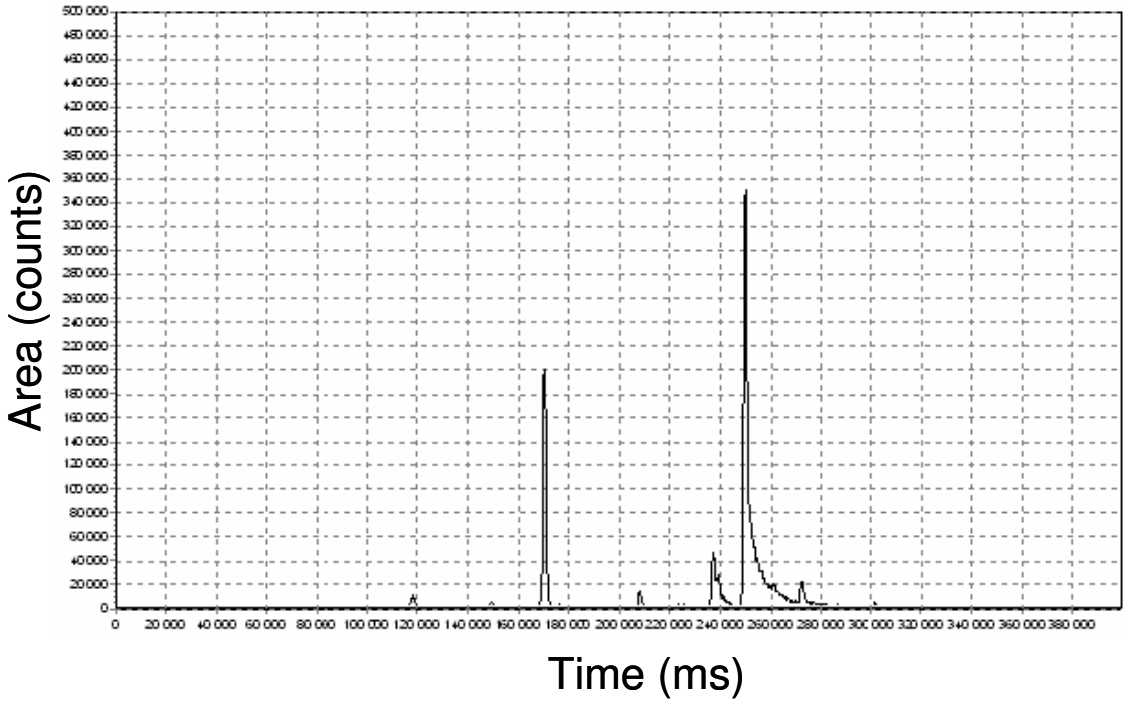


Figure A7. 5: Tin species chromatogram for ^{118}Sn isotope

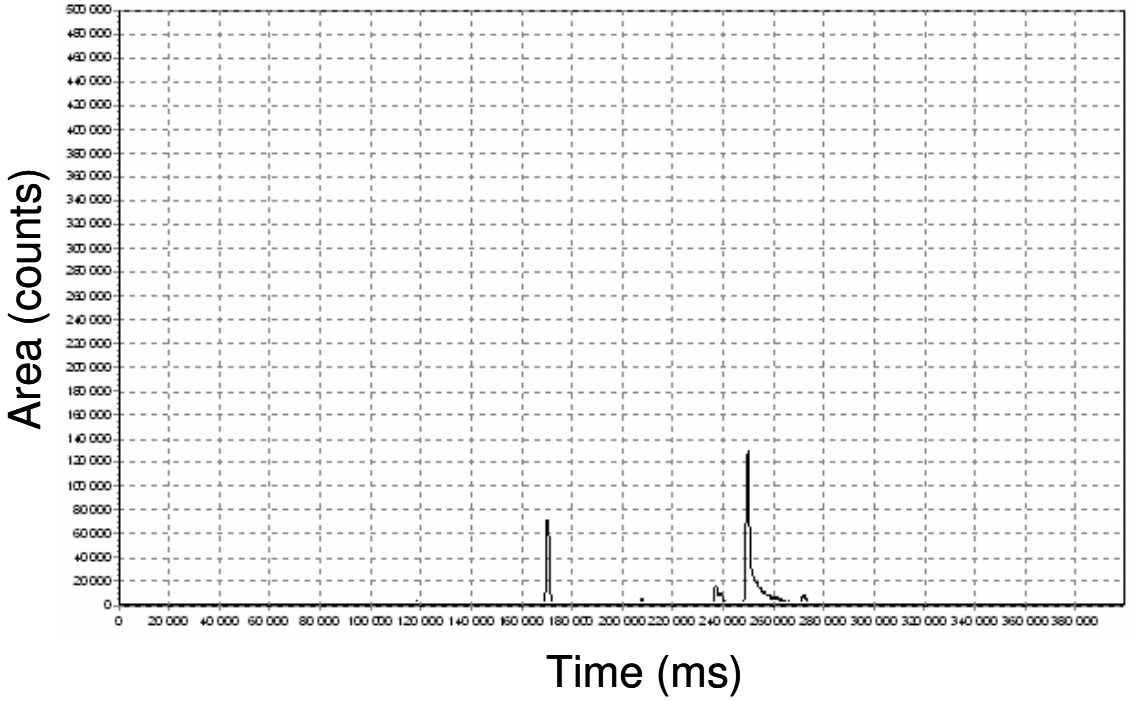


Figure A7. 6: Tin species chromatogram for ^{119}Sn isotope

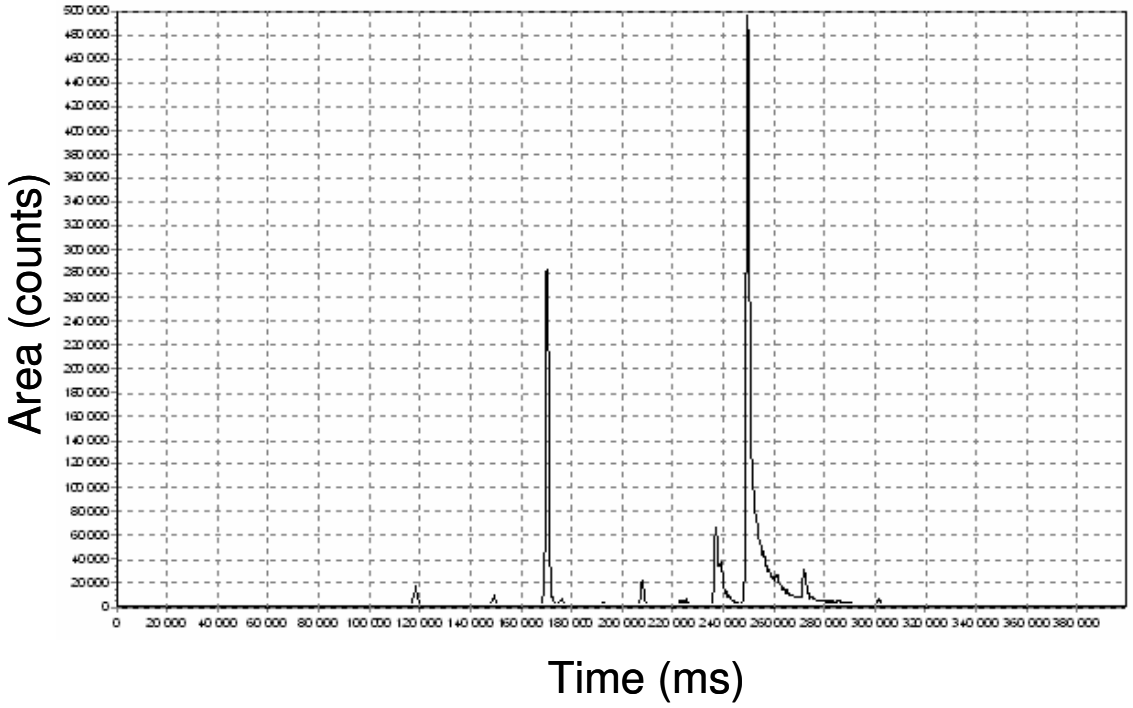


Figure A7. 7: Tin species chromatogram for ^{120}Sn isotope

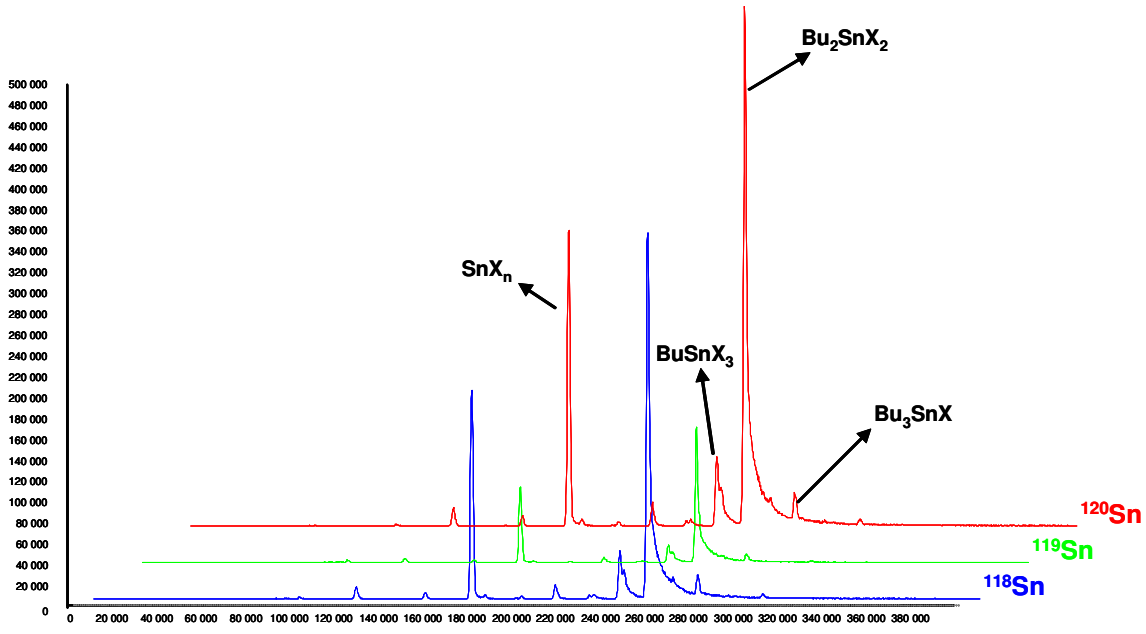


Figure A7. 8: Tin species chromatogram comparison for different isotope