2

Speciation Methods for the Determination of Organotins (OTs) and Heavy Metals (MHs) in the Freshwater and Marine Environments

Peter P. Ndibewu, Rob I. McCrindle and Ntebogeng S. Mokgalaka
Tshwane University of Technology,
South Africa

1. Introduction

Our primary goal for the development of analytical methods is their application in environmental monitoring to achieve good assessment of the contamination situation in freshwater and marine environments. As clearly stated in the endocrine disrupting contaminant (EDCs) program strategic plan for health related water issues (HRWI) of the Republic of South Africa (Version 1.2B, 7/02/2001), one of the objectives in the water research field is to protect aquatic ecosystems and human health based on sound science and defensible data through developing and validation of appropriate methods and by investigating the sources, persistence and effects of potential EDCs in water to support the risk assessment process and contribute towards a trustworthy environmental policy for endocrine disrupting contaminants.

Research goals around the globe in this area have focused on the development of speciation methods for the determination of organotins and heavy metal pollutants in both freshwater and marine environments. Research and development over the years has provided reliable and sensitive analytical techniques that can be used for water research analysis, monitoring and health risks assessment including sampling, testing and validation, although some challenges still exist in regard to the availability of efficient and cost-effective sampling techniques.

The procedures for method modification and development vary depending on the properties of the chemical, possible interferences, the desired sampling medium, the desired analytical technique, sensitivity required, and similar factors (Ombaba and Barry, 1992). The following are questions, which have to be considered and answered by any method modification or development:

- Can the analyte be collected by and removed from the sampling media?
- What are the collection and recovery factors and are they acceptable?
- Is the detection limit sufficiently low to provide meaningful data, especially when adjusted for collection and recovery factors?
- Will expected interferences produce false positive, false negative or biased results?
- If possible, can the results be verified by comparison with an accepted procedure?

This work is partitioned into two sub-sections covering the organotins (OTs) and the heavy metals which are toxic, (TMs) and in most cases are carcinogens. In the heavy metals group, only a few of them, known to cause serious health hazards are fully discussed. These are mercury (Hg), cadmium (Cd), arsenic (As), lead (Pb) and zinc (Zn), all known as endocrine disrupting contaminants (EDCs) (Fatoki and Ngassoum, 2000; HRWI, 2001; Ndibewu *et al*., 2002). Other toxic metals including chromium (Cr) and vanadium (V) will be briefly mentioned in our discussion.

The first part of this chapter discusses speciation analysis of organotins by liquid-liquid (Espadaler et *al*., 1997; Jiang et *al*., 2000; Mueller, 1984) and microsolid phase extraction methods (Mueller, 1987) followed by sodium tetrahydroborate (Jiang et *al*., 2000), sodium tetraethylborate (Cai and Bayona, 1995; Thomaidis et *al*., 2001; Ceulemans and Adams, 1995; Pereira et *al*., 1999) and the Grignard`s reagents (Chau et *al*., 1996; Ceulemans and Adams, 1995; Krull et *al*., 1985; Lucinda, 1983) derivatization. Separation and detection is usually accomplished using the GC-FPD/GC-AAS techniques (Fatoki et *al*., 2000). In the liquid-liquid extraction phase, solvents such as tropolone, hexane-soxhlet and/or diethyl ether have been used for water (Fatoki and Ngassoum, 2000; Mueller, 1987; Leal et *al*., 1995; Abalos et *al*., 1997), sediment samples (Fatoki et *al*., 2000; Abalos et *al*., 1997; Krull et *al*., 1985) and the biota (Kan-atireklap et *al*., 1998). In the derivatization step, two techniques have been used. Firstly, hybridization reactions using sodium tetrahydroborate as the reagent (Abalos et *al*., 1997) was used. Alternatively, derivatization technique based on alkylation reactions employ two reagents namely: the Grignard`s reagents (methylation or ethylation) and sodium tetraethylborate (Fatoki and Ngassoum, 2000; Cai and Bayona, 1995). While the GC-FPD (Fatoki *et al*., 2000; Richardson and Gangolli, 1994) and GC-AAS techniques (Fatoki *et al*., 2000) can be used for the speciation of various organotins compounds, elemental Tin (Sn) is analyzed using flame AAS (Quevauviller *et al*., 1989) in water and sediment samples and the biota.

For the determination of cadmium (Cd), mercury (Hg), arsenic (As), and zinc (Zn), while an ion chromatography-hydride generation-atomic absorption (HG-AAS) procedure (Wade *et al*., 1988) has been used for speciation of As, Cd and Zn are usually determined using flame AAS spectrometry (Lucinda *et al*., 1983; Maenpa *et al*., 2002), and Hg analyzed using the cold vapor technique (CVAAS) (Shrader *et al*., 1983; Willis, 1965). More recently, Fatoki *et al*. (2000) has used GC-FPD for the determination of tributyltin concentrations in the coastal water and freshwater sediments from both the Port Elizabeth and East London harbors in South Africa, which contributed to resources for building regulatory data in that part of the world.

2. Background

Aquatic pollution is a major cause in the decline of resources from water. It is, thus, important to monitor the condition of water. A major concern is the need to develop accurate, reliable and efficient speciation methods for the determination of the polluting compounds within ultra-low detectable ranges. Those known so far to be particularly toxic to the aquatic ecosystems are the organotins (Fent, 1996; Mueller, 1987) and the heavy metals (Cai and Bayona, 1995; Lucinda *et al*., 1983). The term "speciation" in analytical chemistry refers to the separation and quantification of the different oxidation states or chemical forms of a particular element (http://www.frontiergeosciences.com/ebru/).

Although the total concentration of an element is still useful to know, and sometimes essential, the determination of species is necessary to fully understand the biogeochemical and toxicological behavior of the metals. Pollution can influence aquatic life, either directly or indirectly in several ways. By pH changes (increase in acidity); decreasing dissolved oxygen (most common index for pollution); toxicity; mechanical injury to gills (for example from silt); thermal change of medium; killing food organisms through pH change or thermal changes; destruction of spawning grounds (FAO, 1978); shell malformation in oysters (Bayona and Cai, 1994), imposex in gastropods (Kuballa *et al*., 1995); mortality of the larvae of mussels (Jiang *et al*., 2000) and fish poisoning (Cai and Bayona, 1995; Stab *et al*., 1992; Shrader *et al*., 1983; Leal *et al*., 1995).

More specifically, maritime and coastal areas, as well as freshwater are definitely amongst today`s prominent endangered ecosystems. Industrialization and other human activities have caused major changes in these reservoirs` water quality, both inland and marine (Leal *et al*., 1995). Dumping at sea and maritime-based transport activities are mostly responsible for this problem. Polluting loads emptied into the aquatic environments are of various nature and types depending on the point or non-point source though some, like the heavy metals, occur naturally (Lucinda *et al*., 1983; Fatoki *et al*., 2000; Ndibewu *et al*., 2002). The point sources are essentially discharges of sewages and industrial effluents, and are easily identifiable and controllable (Maenpa *et al*., 2002; Lucinda *et al*., 1983). The non-point sources arise in part from natural phenomena, for example, soil erosion; irrigation return flows; outflow from fish farms, and are often diffuse, and so difficult to identify and to control (Leal *et al*., 1995).

3. Occurrence and ecotoxicity of heavy metals, TBT and other organotins

Unlike methyltin, which may be formed naturally in the environment, TBT is exclusively of anthropogenic origin (MCkie, 1987; Fent, 1996). This is why its occurrence in the aquatic environment has been directly attributed to its application as an antifouling agent. TBT residues in the sediments of harbors, marinas and shipping channels has been found to be considerably higher typically in the range of about 200 – 1000 μg kg^{-1} (Balls, 1987). Progressive introduction of organic groups at the tin atom produces increasing biological activity (Bayona and Cai, 1994). Organotin compounds with three alkyl groups attached to the tin atom, such as tributyltin (TBT), triphenyltin and tricyclohexyltin, have found wide applications as antifouling agents in marine paints formulations, bactericides in cooling water (MCkie, 1987; Fatoki, 2000), agricultural fungicides and acaricides (Leal *et al*., 1995). The most import of these is TBT, which is used in marine paints as an effective means of the growth of fouling organisms such as tubeworms, barnacles and mussels on seafaring vessels and marine structures (Brian, 1991).

Meech *et al.* (1998) has shown that TBT is acutely toxic to a variety of fresh water species at concentrations down to 0.1 μL^{-1}. TBT is particularly toxic (Fent, 1996; Reisch, 1996; Meech *et al*., 1998) to mollusks (oysters) and gastropods. The decline of dog whelk populations on various coasts of France and UK has been attributed to the occurrence of TBT in these waters (Fent, 1996). Chronic toxic effects on oysters in the form of shell deformation (Fent, 1996) and marine gastropods in the form of sterilization of females have been reported occurring at concentrations of a few ng L^{-1} (Fent, 1996; Fatoki *et al*., 2000).

Unlike TBT and organotins, metals are unique environmental and industrial pollutants in that they are found naturally distributed in all phases of the environment. The term "heavy metals" is generally interpreted to include those metals from periodic table groups IIA through VIA. The semi-metallic elements boron, arsenic, selenium, and tellurium are often included in this classification. At trace levels, many of these elements are necessary to support life. Heavy metals are elements having atomic weights between 63.546 and 200.590g (Kennish, 1992), and a specific gravity greater than 4.0 (Connell *et al*., 1984). Living organisms require trace amounts of some heavy metals, including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc (Nriagu, 1996). Excessive levels of essential metals, however, become toxic and may build up in biological systems, and become a significant health hazard (Brickman, 1978). Non-essential heavy metals of particular concern to surface water systems are cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), arsenic (As) and antimony (Sb) (Kennish, 1992).

During the last two decades, considerable attention has been given to problems concerning negative effects of heavy metals (HMs) on various ecosystems in different environmental media (Lucinda, 1983; Nriagu, 1996). The heavy metals rated among most of the environmental risk pollutants (Cai and Bayona, 1995) requires that, fast, accurate and reliable analytical techniques suitable for their assessment and for their determination in environmental samples at trace levels be developed. In the class of the heavy metal ecotoxicants, Hg, Cd, As and Zn are considered fairly hazardous because of their high toxicity (Schrader *et al*., 1983; Nriagu, 1996; Fent, 1996). These metal species actually occur in the environment at sub ultra-low trace concentrations level (Cai and Bayona, 1995; Lucinda *et al*., 1983). Therefore, accurate and sensitive determination techniques are of fundamental interest for the assessment of the effectiveness of regulatory control measures.

Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Therefore, they tend to accumulate in soils, seawater, freshwater, and sediments (Schrader *et al*., 1983; http://www.osha.gov/SLTC/cadmium/index.html). Excessive levels of metals in the marine environment can affect marine biota and pose risk to human consumers of seafood (http://www.msceast.org/hms/). Heavy metals are also known to have adverse effects on the environment and human health (Schrader *et al*., 1983). Numerous field observations also indicate a significant increase of HM concentrations in agricultural and forest soils as well as in marine and inland water sediments. This increase is frequently observed in remote areas thousands of kilometers away from major anthropogenic sources and can be explained by transboundary atmospheric long-range transport only (http://www.msceast.org/hms/). An assessment of the potential ecological and health risks associated with atmospheric fluxes of heavy metals requires an understanding of the relationships between sources of emission to the atmosphere and the levels of concentrations measured in ambient air and precipitation (Ikeda *et al*., 1996).

Since the industrial revolution, the production of heavy metals such as Pb, Cu, and Zn has increased exponentially (Lucinda, 1983; Maguire *et al*., 1982). Between 1850 and 1990, production of these three metals increased nearly 10-fold, with emissions rising in tandem (Maguire *et al*., 1982). The heavy metals have been used in a variety of ways for at least 2 millennia (Lu *et al*., 1996; Lucinda, 1983; Meech *et al*., 1998). For example, lead has been used in plumbing, and lead arsenate has been used to control insects in apple orchards. The Romans added lead to wine to improve its taste, and mercury was used as a salve to

alleviate teething pain in infants (Nriagu, 1996). Once emitted, metals can reside in the environment for hundreds of years or more (Nriagu, 1996), while causing immediate or long term damage depending on the concentration released. Evidence of human exploitation of heavy metals has been found in the ice cores in Greenland and seawater in the Antarctic (Nriagu, 1996). The lead contents of ice layers deposited annually in Greenland show a steady rise that parallels the mining renaissance in Europe, reaching values 100 times the natural background level in the mid-1990s (http://h2osparc.wq.ncsu.edu/info/hmetals.html). Mining itself, not only of heavy metals but also of coal and other minerals, is another major route of exposure. Despite some noted improvements in worker safety and cleaner production, mining remains one of the most hazardous and environmentally damaging industries (Nriagu, 1996; Maenpa *et al.*, 2002). In Bolivia, toxic sludge from a zinc mine in the Andes had killed aquatic life along a 300-kilometer stretch of river systems as of 1996 (http://h2osparc.wq.ncsu.edu/info/hmetals.html). It also threatened the livelihood and health of 50,000 of the region's subsistence farmers (Nriagu, 1996; Fent, 1996). Uncontrolled smelters have produced some of the world's only environmental "dead zones" where little or no vegetation survives. For instance, toxic emissions from the Sudbury, Ontario, and nickel smelter have devastated 10,400 hectares of forests downwind of the smelter (Nriagu, 1996). All heavy metals exist in surface waters in colloidal, particulate, and dissolved phases, although dissolved concentrations are generally low (Kennish, 1992). The colloidal and particulate metal may be found in (1) hydroxides, oxides, silicates, or sulfides; or (2) adsorbed to clay, silica, or organic matter. The soluble forms are generally ions or unionized organometallic chelates or complexes. The solubility of trace metals in surface waters is predominately controlled by the water pH, the type and concentration of ligands on which the metal could adsorb, and the oxidation state of the mineral components and the redox environment of the system (Connell *et al*., 1984).

Heavy metals in surface water systems can be from natural or anthropogenic sources. Currently, anthropogenic inputs of metals exceed natural inputs. Excess metal levels in surface water may pose a health risk to humans and to the environment (Nriagu, 1996). Considering that heavy metals are natural constituents of the Earth's crust, they are present in varying concentrations in all ecosystems and human activities have drastically changedthe biogeochemical cycles and balance of some of these heavy metals. The main anthropogenic sources of heavy metals are various industrial sources (Shrader *et al.*, 1983; Fatoki, 2000) including present and former mining activities (http://www.msceast.org/hms/), foundries and smelters (http://www.osha.gov/SLTC/cadmium/index.html), and diffuse sources such as piping (http://www.msceast.org/hms/), constituents of products, combustion by-products, traffic (Shrader *et al.*, 1983), etc. Relatively, volatile heavy metals and those that become attached to airborne particles can be widely dispersed on very large scales. Heavy metals conveyed in aqueous and sedimentary transport enter the normal coastal biogeochemical cycle and are largely retained within near-shore and shelf regions (http://www.msceast.org/hms/). The toxicity of these metals has also been documented throughout history: Greek and Roman physicians diagnosed symptoms of acute lead poisoning long before toxicology became a science (Nriagu, (1996). Today, much more is known about the health effects of heavy metals. Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage, and even death in some instances of exposure to very high concentrations. Exposure to high levels of mercury, gold,

and lead has also been associated with the development of autoimmunity, in which the immune system starts to attack its own cells, mistaking them for foreign invaders (Nriagu, 1996; http://www.mercurypolicy.org/). Autoimmunity can lead to the development of diseases of the joints and kidneys, such as rheumatoid arthritis, or diseases of the circulatory or central nervous systems. Despite abundant evidence of these deleterious health effects, exposure to heavy metals continues and may increase in the absence of concerted policy actions. Mercury is still extensively used in gold mining in many parts of Latin America. Arsenic, along with copper and chromium compounds, is a common ingredient in wood preservatives. Lead is still widely used as an additive in gasoline. Increased use of coal in the future will increase metal exposures because coal ash contains many toxic metals and can be breathed deeply into the lungs. For countries such as China, India and South Africa, which continue to rely on high-ash coal as a primary energy source, the health implications are ominous.

Mercury is a toxic metal that is liquid at room temperature (http://h2osparc.wq.ncsu.edu/info/hmetals.htm). Exposure to mercury is known to cause permanent damage to the brain, nervous system, and kidneys (http://www.mercurypolicy.org). Pregnant women are particularly vulnerable as mercury may damage the developing fetus. While mercury is released naturally from rocks, soil, and volcanoes, human activities have boosted atmospheric levels to some three times above pre-industrial levels, the experts say. Estimates vary, but the UNEP group of experts says some 5,000 to 10,000 metric tons of mercury are thought to enter the atmosphere every year and 50 to 75 percent of it from human activities (http://h2osparc.wq.ncsu.edu/info/hmetals.htm). The main human source of mercury emissions is coal combustion from electrical power plants and industrial, commercial and residential burners. Other sources include municipal solid waste incineration, mining of non-ferrous metals, and artisanal gold mining (http://www.mercurypolicy.org).

Interest in the biogeochemical cycle (Shrader *et al.*, 1983) of mercury in the environment has dramatically increased in recent years due to the observation that mercury accumulates in aquatic organisms. Moreover, methylmercury becomes magnified in the upper tropic levels as a result of bioaccumulation, from dietary intake of organisms containing methylmercury (http://www.frontiergeosciences.com/ebru/). It has been demonstrated that mercury can be methylated in the environment and bioconcentrated in the biota (Cai and Bayona, 1995). Ingestion of fish muscle is an important exposure pathway of mercury to humans (Cai and Bayona, 1995). Studying mercury in environmental systems requires a very sensitive method as typical mercury levels in aquatic environments range from 0.5 to 5.0 ng L^{-1} (http://www.frontiergeosciences.com/ebru/). Total mercury permissible in the environment is 0.005 mg L^{-1} (FAO, 1978). The high toxicity of methylmercury has been well recognized in fish (Cai and Bayona, 1995; Lucinda *et al.*, 1983; Wade *et al.*, 1988) and ingestion of fish muscle is an important exposure pathway of mercury for humans (Cai and Bayona, 1995).

Cadmium may interfere with the metallothionein's ability to regulate zinc and copper concentrations in the body. Metallothionein is a protein that binds to excess essential metals to render them unavailable when cadmium induces metallothionein's activity binding it to copper and zinc, disrupting the homeostasis levels (Kennish, 1992). Cadmium is used in industrial manufacturing processes and is a byproduct of the metallurgy of zinc. Acute

cadmium toxicity may result in brain damage. Metal fume fever may result from acute exposure with flu-like symptoms of weakness, fever, headache, chills, sweating and muscular pain. Acute pulmonary edema usually develops within 24 hours and reaches a maximum by three days. If death from asphyxia does not occur, symptoms may resolve within a week. Chronic cadmium poisoning can cause eventual death. The most serious consequence of chronic cadmium poisoning is cancer (lung and prostate). The first observed chronic effect is generally kidney damage, manifested by excretion of excessive (low molecular weight) protein in the urine. Cadmium also is believed to cause pulmonary emphysema and bone disease (osteomalcia and osteoporosis). The latter has been observed in Japan ("itai-itai" disease) where residents were exposed to cadmium in rice crops irrigated with cadmium-contaminated water. Cadmium may also cause anemia, teeth discoloration (Cd forms CdS) and loss of smell (anosmia) (http://www.osha.gov/SLTC/cadmium/index.html). Arsenic ingestion can cause severe toxicity through ingestion of contaminated food and water. Ingestion causes vomiting, diarrhea, and cardiac abnormalities (Viessman and Hammer, 1985).

The behavior of metals in natural waters is a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry (Nriagu, 1996). Sediment composed of fine sand and silt will generally have higher levels of adsorbed metal than will quartz, feldspar and carbonate-rich sediment. Metals also have a high affinity for humic acids, organo-clays, and oxides coated with organic matter (Connell *et al*., 1984). The water chemistry of the system controls the rate of adsorption and desorption of metals to and from sediment. Adsorption removes the metal from the water column and stores the metal in the substrate. Desorption returns the metal to the water column, where recirculation and bio-assimilation may take place. Metals may be desorbed from the sediment if the water experiences increase in salinity, decreases in redox potential, or decreases in pH controlled by the following mechanisms:

- Salinity increase: Elevated salt concentrations create increased competition between cations and metals for binding sites. Often, metals will be driven off into the overlying water. (Estuaries are prone to this phenomenon because of fluctuating river flow inputs).
- Redox potential decrease: A decreased redox potential, as is often seen under oxygen deficient conditions, will change the composition of metal complexes and release the metal ions into the overlying water.
- pH decrease: A lower pH increases the competition between metal and hydrogen ions for binding sites. A decrease in pH may also dissolve metal-carbonate complexes, releasing free metal ions into the water column (Connell *et al*., 1984).

3.1 Environmental effects

Aquatic organisms may be adversely affected by heavy metals in the environment. The toxicity is largely a function of the water chemistry and sediment composition in the surface water system, as clearly detailed under the section "Environmental fate/Mode of transport". Slightly elevated metal levels in natural waters may cause the following sublethal effects in aquatic organisms: (1) histological or morphological change in tissues; (2) changes in physiology, such as suppression of growth and development, poor swimming performance,

changes in circulation; (3) change in biochemistry, such as enzyme activity and blood chemistry; (4) change in behaviour; (5) and changes in reproduction (Connell *et al*., 1984). Many organisms are able to regulate the metal concentrations in their tissues. Fish and the crustacea can excrete essential metals, such as copper, zinc, and iron that are present in excess. Some can also excrete non-essential metals, such as mercury and cadmium, although this is usually met with less success (Connell *et al*., 1984).

Research has shown that aquatic plants and bivalves are not able to successfully regulate metal uptake (Connell *et al*., 1984). Thus, bivalves tend to suffer from metal accumulation in polluted environments. In estuarine systems, bivalves often serve as biomonitor organisms in areas of suspected pollution (Kennish, 1992). Shell fishing waters are closed if metal levels make shellfish unfit for human consumption. In comparison to freshwater fish and invertebrates, aquatic plants are equally or less sensitive to cadmium, copper, lead, mercury, nickel, and zinc. Thus, the water resource should be managed for the protection of fish and invertebrates, in order to ensure aquatic plant survival (USEPA, 1987). Metal uptake rates will vary according to the organism and the metal in question. Phytoplankton and zooplankton often assimilate available metals quickly because of their high surface area to volume ratio. The ability of fish and invertebrates to adsorb metals is largely dependent on the physical and chemical characteristics of the metal (Kennish, 1992). With the exception of mercury, little metal bioaccumulation has been observed in aquatic organisms (Kennish, 1992). Metals may enter the systems of aquatic organisms via three main pathways: (1) Free metal ions that are absorbed through respiratory surface (e.g. gills) are readily diffused into the blood stream, (2) Free metal ions that are adsorbed onto body surfaces are passively diffused into the blood stream, and (3) Metals that are sorbed onto food and particulates may be ingested, as well as free ions ingested with water (Connell *et al*., 1984).

3.2 Irrigation effects

Irrigation water may transport dissolved heavy metals to agricultural fields. Although most heavy metals do not pose a threat to humans through crop consumption, cadmium may be incorporated into plant tissue. Accumulation usually occurs in plant roots, but may also occur throughout the plant (De Voogt *et al*., 1980). Most irrigation systems are designed to allow for up to 30 percent of the water applied to not be absorbed and to leave the field as return flow. Return flow either joins the groundwater or runs off the field surface (tail water). Sometimes tail water must be rerouted into streams because of downstream water rights or a necessity to maintain stream flow. However, usually the tail water is collected and stored until it can be reused or delivered to another field (USEPA, 1993a). Tail water is often stored in small lakes or reservoirs, where heavy metals can accumulate as return flow is pumped in and out. These metals can adversely impact aquatic communities. An extreme example of this is the Kesterson reservoir in the San Joaquin Valley, California, which received subsurface agricultural drain water containing high levels of selenium and salts that, had been leached from the soil during irrigation. Studies in the Kesterson reservoir revealed elevated levels of selenium in water, sediments, terrestrial and aquatic vegetation, and aquatic insects. The elevated levels of selenium were cited as relating to the low reproductive success, high mortality, and developmental abnormalities in embryos and chicks of nesting aquatic birds (Schuler *et al*., 1990).

3.3 Health effects

Ingestion of metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), barium (Ba), and chromium (Cr), may pose great risks to human health. Trace metals such as lead and cadmium will interfere with essential nutrients of similar appearance, such as calcium (Ca^{2+}) and zinc (Zn^{2+}). Amongst the heavy metals pollution, mercury pollution has become a global problem (Schrader *et al.*, 1983) because of its occurrence from natural anthropogenic sources, and its biogeochemical processes (Cai and Bayona, 1995; Coello *et al.*, 1996). As public awareness regarding the toxicity and the environmental impact of mercury contamination increases, speciation analytical methods developed, are required to distinguish between organic and inorganic forms of mercury. The determination and monitoring of mercury and arsenic is a special concern in the field of mine works and food engineering respectfully (Leal *et al.*, 1995; Nriagu, 1996). It has been reported (Leal *et al.*, 1995; Fatoki, 2000) that mercury can be methylated in the environment and bioconcentrated in the biota. Mercury poses a great risk to humans, especially in the form of methylmercury. When mercury enters water, it is often transformed by microorganisms into the toxic methyl mercury form. Symptoms of acute poisoning are pharyngitis, gasteroenteritis, vomiting, nephritis, hepatitis, and circulatory collapse. Chronic poisoning is usually a result of industrial exposure or a diet consisting of contaminated fish (mercury is the only metal that will bioaccumulate). Chronic poisoning may cause liver damage, neural damage, and teratogenesis (USEPA, 1987).

The Global mercury assessment working group of the United Nations Environment Programme (UNEP) had in the past concluded a week long meeting in Geneva (2002) with the recommendation that governments negotiate a treaty to limit the amount of mercury traded worldwide. In the meantime, countries should reduce mercury risks by cutting or eliminating the production and consumption of the chemical by substituting other products and processes. Mercury has been widely used in consumer products because it is an excellent conductor of electricity and is highly malleable. Products containing mercury include thermometers, dental fillings, fluorescent lamps and other electrical equipment, and some batteries. Mercury is used in several types of instruments common to electric utilities, municipalities and households, such as switches, barometers, meters, temperature gauges, pressure gauges and sprinkler system contacts. It has been used as an ingredient in some pesticides and biocides, certain pharmaceuticals, and cosmetics such as skin lightening creams. In some countries, mercury has ritual religious uses. People are most likely to be exposed to mercury by eating fish or shellfish contaminated with methylmercury, and many jurisdictions have issued fish consumption warnings based on the presence of mercury in fish (Cai and Bayona, 1995; Abalos *et al.*, 1997; Fatoki *et al.*, 2000; Ndibewu *et al.*, 2002). People can be exposed when breathing vapours in air from spills, incinerators, and industries that burn fuels containing mercury (Nriagu, 1996). Mercury can be released from dental work or medical treatments and dental or health service workers can be exposed from breathing contaminated workplace air or skin contact during use in the workplace. When placed in landfills, mercury can slowly seep into groundwater or evaporate into the air. It can travel over long distances and persist in the environment for lengthy periods of time. Two studies released in March (2002) (http://h2osparc.wq.ncsu.edu/info/hmetals.html) show that mercury generated by fossil fuel burning power plants is falling from the sky in Antarctica and in the Arctic, and is entering the food chain.

Cadmium is an extremely toxic metal commonly found in industrial workplaces, particularly where any ore is being processed or smelted. Due to its low permissible exposure limit (PEL), over exposures may occur even in situations where cadmium is only in trace quantities in the parent ore or smelter dust. Cadmium is used extensively in electroplating, although the nature of the operation does not generally lead to overexposures. Several deaths from acute exposure have occurred among welders who have unsuspectingly welded on cadmium-containing alloys and among silver solders. Cadmium is also found in industrial paints and may represent a hazard when spray applied. Operations involving removal of cadmium paints by scraping or blasting may similarly pose a significant hazard. Cadmium emits a characteristic brown fume (CdO) upon heating, which is relatively non-irritating, and thus, does not alarm the exposed individual (Maenpa *et al.*, 2002; Meech *et al.*, 1998).

4. Pollution source – Points of TBT and organotins

Organotin compounds have found many important industrial and agricultural applications for more than three decades (Prudente *et al.*, 1999; Leal *et al.*, 1995). These include the use of mono-methyl tins, mono-butyltins and di-butyltins as stabilizers in polyvinyl chloride (PVC) and as catalysts in industrial processes. Organotin compounds with three alkyl groups attached to the tin atom, such as tributyltin (TBT), tri-phenyltin and tri-cyclohexyltin, have found wide applications as antifouling agents in marine paints formulations, bactericides in cooling water, agricultural fungicides and acaricides (Leal *et al.*, 1995), as previously mentioned. Most import of TBT is used in marine paints as an effective means of the growth of fouling organisms such as tubeworms, barnacles and mussels on seafaring vessels and marine structures (Abalos *et al.*, 1997; Fatoki, 2000).

5. Pollution source – Points of heavy metals (HMs)

Nonpoint sources of heavy metals pollution are mostly natural. Chemical and physical weathering of igneous and metamorphic rocks and soils often releases heavy metals into the sediment and into the air. Other contributions include the decomposition of plant and animal detritus, precipitation or atmospheric deposition of airborne particles from volcanic activity, wind erosion, forest fire smoke, plant exudates, and oceanic spray (Kennish, 1992). Anthropogenic sources are contributed by surface runoffs from mining operations usually has a low pH and contains high levels of metals such as iron, manganese, zinc, copper, nickel and cobalt. The combustion of fossil fuels pollutes the atmosphere with metal particulates that eventually settle to the land surface. Urban stormwater runoffs often contain metals from roadways and atmospheric fallout (Connell *et al.*, 1984). Currently, anthropogenic inputs of metals exceed natural inputs. Point sources include domestic wastewater effluents which contains metals from metabolic wastes, corrosion of water pipes, and consumer products. Industrial effluents and waste sludges may substantially contribute to metal loading (Connell *et al.*, 1984).

6. Mode of transport and environmental fate of HMs

Transport occurs mostly in water and air. Water can transport metals that are bound to sediment particles. The primary route for sediment-metal transport is overland flow. Water

also transports dissolved metals. Although dissolved metals are primarily transported in overland flow, some underground transport is possible (Nriagu, 1996). Metals that are introduced to the unsaturated zone and the saturated zone will most likely not be transported a long distance. Dissolved metals that are carried below the land surface will readily sorb to soil particles or lithic material in the unsaturated zone and the saturated zone (Nriagu, 1996). Metals introduced into the atmosphere may be carried to the land surface by precipitation and dry fallout. Additionally, because metals readily sorb to many sediment types, wind-borne sediment is a potential route for metal transport (Nriagu, 1996).

7. Regulatory measures applied to TBT and organotins

Zehra Aydin (2002) reported that by the early 1970s, there was clearly a need to promote better use and management of the seas and their resources which imposed a call on the international community to begin negotiating a comprehensive treaty on the law of the sea. What is remarkable is that, these laws had diversified in time to fit specific country`s standards and regulatory needs. For continual assessment, there had then been a growing need to develop suitable analytical tools to assess organotins and heavy metals. In response to this trend, countries with advanced economy began research in this area long ago. Today, a substantial body of knowledge on OTs and heavy metals in waters of the developed countries of Europe, America, Asia and Oceania has evolved. However, data are very scanty for developing nations' water environments (Bryan, 1991; ATRP Corp-U.S-EPA, 2000, 2001 and 2002).

Particularly, the ecotoxicological effects of TBT and other tri-organotin (Leal *et al.*, 1995) compounds in the aquatic environment have caused much concern in recent years leading to the control or banning of their use in a few developed countries (Jiang *et al.*, 2000). At present, it is doubtful if specific legislation exists controlling the use of TBT in many, if not all developing countries (Samson and Shenker, 2000). This is primarily due to the lack of supporting data on the occurrence and impact of TBT in these countries. Tributyltin has been described as "the most toxic substance ever deliberately introduced into the natural waters" (Jiang *et al.*, 2000; Leal *et al.*, 1995; Thomaidis *et al.*, 2001). Owing to its extremely toxic effects to aquatic life at low concentrations, TBT and other forms of organotin such as triphenyltin are legislatively banned to be used as antifouling paints from since the late 1980s in most European and North American countries (Jiang and Yang, 2000). The first regulatory and legislative control on the use of TBT was only adopted in France in 1982 followed by UK in 1985 (Meech *et al.*, 1998). Most of the control measures introduced since then involved banning the use of TBT in marine boats of less than 25 m length (Ceulemans and Adam, 1995). For marine water, the UK adopted an environmental quality target of 20 ng L^{-1} TBT in 1985 and environmental quality standard of 2 ng L^{-1} TBT was proposed in 1989 (Cai *et al.*, 1994). The US Environmental Protection Agency's proposed limits for TBT in fresh and marine waters were 26 ng L^{-1} (4-day average) and 10 ng L^{-1} (4-day average), respectively, (Dirkx *et al.*, 1994). The Canadian Council of the Ministers of Environment derived an Interim Water Quality Guideline of 8 ng L^{-1} TBT in estuarine or seawaters for the protection of aquatic life (Cai and Bayona, 1995).

Levels of TBT of the order of a few hundred ng L^{-1} have been reported in coastal waters with heavy marine traffic, such as ports, marinas and dockyards, as compared to open water

where TBT is found to be near or below 10 ng L^{-1} or less for Europe and North America and Hong Kong (Evans *et al.*, 2000; Forstner, 1983; Maguire *et al.*, 1982).

Fatoki, (2000) have reported a preliminary study of TBT in waters and sediments from some major ports in South Africa (Port Elizabeth and East London). This study indicates significant contamination of the East London and Port Elizabeth Harbors' aquatic environment with TBT (Fatoki *et al.*, 2000). This study also indicates contamination levels of 5.5 ng L^{-1} - 22.7 ng L l^{-1} (water samples) and 1.8 ng g^{-1} - 26.2 ng g^{-1} (sediments) for Port Elizabeth. The figures for East London are 3.3 – 49.9 ng L^{-1} and 3.5 – 1103.1 ng g^{-1} for water and sediments, respectively. As such, this should be viewed as danger to the biota in the aquatic system.

8. Regulatory measures applied to heavy metals in marine environments

In 1998, in Aarhus (Denmark), 36 Parties to the Convention on Long-Range Transboundary Air Pollution signed the Protocol on HMs. The Protocol was aimed at the elimination, restriction on use, and reduction of HM emissions to the environment. An integrated program for the inter-comparison study of mercury models was developed (http://www.msceast.org/hms/).

The US Food and Drug Administration (FDA) has set an action level of 1μg g^{-1} (wet mass) for fresh fish (Cai and Bayona, 1995; Lucinda *et al.*, 1983), Canada and several US states have set consumption limits for fish at 0.5μg g^{-1}. The European Union (UE) has set environmental quality objectives of 0.3μg g^{-1} (wet mass), 1μg L^{-1} for continental water, 0.5μg L^{-1} for estuarine water, and 0.3μg L^{-1} for coastal water as total mercury (Cai and Bayona, 1995). In addition to a legally binding mercury treaty, the Global Mercury Assessment Working Group (http://www.mercurypolicy.org) urges governments to establish a non-binding global program of action, and strengthen cooperation among countries on information sharing, risk communication, assessment and related activities. The Working Group recommended immediate action to enhance outreach to vulnerable groups, such as pregnant women and provide technical and financial support to developing countries and to countries with economies in transition. Increased research, monitoring, data collection on the health, environmental aspects of mercury and development of environmentally friendly alternative chemicals to this one are among the group's recommendations. Some countries have seriously taken action to deal with mercury pollution while others still take it slightly, especially the developing countries. The European Union had faced a bill of up to 330 million Euros (US$324 million) to dispose safely of excess mercury stocks from an obsolete method of chlorine production (http://www.mercurypolicy.org/). The U.S. Senate passed legislation in early 2002 (http://h2osparc.wq.ncsu.edu/info/hmetals.html) banning the sale of mercury fever thermometers anywhere in the United States. Similarly, in the same year, the U.S. Environmental Protection Agency proposed changing waste regulations for computers, televisions and mercury containing equipment to discourage the flow of these materials to municipal landfills and incinerators (http://www.mercurypolicy.org).

9. An overview of speciation methods and determination techniques of heavy metals, TBT and other organotins in seawaters

Organotins or butyl compounds (BCs) which are generally of interest for speciation include the tributyltins (TBTs), dibutyltins (DBTs) and monobutyltins (MBTs), as well as the

triphenyltins (TPTs), diphenyltins (DPTs) and monophenyltins (MPTs). Amongst them, TBT is acutely toxic to a variety of freshwater species at concentrations down to 0.1 μg L^{-1} (Prudente *et al.*, 1999; Chau *et al.*, 1996). Indeed, this toxicity limits level has been checked for only some marinas in South Africa (Fatoki *et al.*, 2000). Although, the lack of research in this area cannot be over stressed as an underlining factor in setting regulatory limits in developing countries, BT contamination should be regarded as a global pollution problem (Maguire *et al.*, 1982) particularly in countries where no regulation has been implemented such as South Africa.

We have mentioned that TBT and organotins once were the preferred universally available biocides for marine coatings (Mueller, 1987) and large amounts were used (Leal *et al.*, 1995; Kuballa *et al.*, 1995) for pleasure boats, large ship or vessels, docks and fish-nets, lumber preservatives and slimicides in cooling systems, as an effective antifouling agent in paints. Also, that dibutyltins (DBTs) and monobutyltins (MBTs) were mostly used as stabilizers in polyvinyl chlorides and as catalysts in the production of polyurethane foams, silicones, and in other industrial processes (Cai and Bayona, 1995; Fent, 1996). Finally, it suffices to recall that another well-known source (Leal *et al.*, 199) is their use in the manufacture of fungicides, acaricides and insecticides for use in agriculture. And after years of use, their effects on marine environments have brought about actions to limit the use of TBTs through laws and regulations (Kan-atireklap *et al.*, 1997; Jiang *et al.*, 2000; Fatoki *et al.*, 2000), especially in developed countries. With the growing concern regarding the impact on the environment with threats to aquatic life (Kuballa *et al.*, 1995; Kumar *et al.*, 1993) and human health (Fatoki, 2000), an international stakeholders' process (Murmansk-2000) considered a draft treaty to ban the use of TBTs on all hulls worldwide.

Actually, considerable work has been done in the area of techniques development for organotins speciation analysis elsewhere (Mueller, 1987; Prudente *et al.*, 1999; Cai and Bayona, 1995; Ikeda *et al.*, 1996; Jiang *et al.*, 2000; Meech *et al.*, 1998; Abalos *et al.*, 1997) while research efforts in most developing countries, including South Africa are still at their first endeavors at the moment (Fatoki *et al.*, 2000; Ndibewu *et al.*, 2002). Thus, lack of continual consistent research work in this field and lack of monitoring really present potential danger both to the aquatic biota and man. As reported for the case of man-generated heavy metals discharge into the environment (Lucinda *et al.*, 1983; http://www.mercurypolicy.org/), OTs, disperse on a global scale by long-range atmospheric transport and deposit into colder regions could cause an environmental disaster. If the lack of research interest in this area on a global scale stays as such, this will lead to no regular monitoring of water environments in order to avoid potential danger that can be caused by these endocrine-disrupting compounds to human and marine lives including the aquatic ecosystems.

Generally, any procedure for speciation analysis consists of five successive steps (Fatoki, 2000): (i) extraction of the analytes from the sample matrix, (ii) derivatization to form the volatile derivatives, (iii) pre-concentration (iv) clean-up and, (v) determination.

In the first step, extraction is critical, meaning that the choice of a particular extraction technique is also critical. Two extraction methods are popularly used (Abalos *et al.*, 1997), namely: the liquid-liquid (LLE) and the solid phase extraction (SPE) extraction techniques. Liquid-liquid extraction methods often require a large amount of hazardous solvents and tend to be replaced by the solid phase extraction (SPE) procedures (Fent, 1996). The

advantage of SPE include a higher pre-concentration factor and ease of application in the field and in on- line systems, while a drawback has been observed in that only filtered samples can be analyzed. More recently, the solid phase microextraction (SPME) technique has been applied to organotin speciation (Attar, 1996; Abalos *et al.*, 1997). SPME offers an attractive alternative method, which minimizes some problems associated with other methods (Fatoki *et al.*, 2000).

Using Liquid-liquid extraction methods, many non-polar and polar solvents have been used for extraction in water, sediment and biological samples. During early techniques applications for organotins determination in water samples, speciation analysis were based on acidification (hydrochloric acid-HCL, hydrobromic acid-HBr or acetic acid-HOAc), to release alkyl tin compounds from the sample matrix, then converting them to the halides or acetate forms (Forstner, 1983). Relatively high polarity solvents are now being used for extraction. These methods (Abalos *et al.*, 1997) succeeded for TBT, TPT and tricyclohexyl (TCyT) and failed for other species due to their high polarity.

For sediment analysis with regard to organotins speciation, acid leaching (Kan-atireklap *et al.*, 1997; Chau *et al.*, 1995; Tanabe *et al.*, 1998) to release organotin compounds from sediment was the basic approach in early use. Hydrochloric acids (HCl), Hdrobromic acid (HBr) and acetic acids (HOAc) are used. This is done in an aqueous or methanolic medium by sonification, stirring, shaking or Soxhlet extraction with an organic solvent (Dirkx *et al.*, 1994). As mentioned above, organotin compounds are not involved in biogeochemical process. They rather bind onto the surface of the sediment, hence, the complete dissolution of the later prior to the analysis is, therefore, not considered necessary. Extraction yield is increased by the addition of complexing agents such as tropolone or DDTC (Fatoki *et al.,* 2000). While the tri- and di-substituted compounds can be extracted quantitatively, only about 60 % or less of the mono-substituted compounds are recovered. Two approaches have been evaluated to improve the extraction efficiency of mono- and di-organotin species: (i) the addition of complexing agents (e.g. diethylammonium-diethylthiocarbamate, DEA-DDC or (DDC), and (ii) alkylation in a reaction cell with Grignard reagent prior to the extraction (Fatoki *et al.,* 2000; Abalos *et al.*, 1997). On one hand, recoveries obtained by the first approach are satisfactory for di- and tri-organotin species but a clean-up step is usually needed. On the other hand, the second method yields satisfactory recoveries only for TBT and TPT. Further developments are needed to bring these methods to routine analysis. Apparently, no reliable and efficient method for extracting all organotins from sediment has yet been developed (Abalos *et al.*, 1997).

Hexane, benzene, toluene or dichloromethane (DCM) are non-polar solvents used for the extraction of organotins with complexing agent (Cai and Bayona, 1995; Jiang *et al.*, 2000; Abalos *et al.*, 1997). The efficiency with which butyltins are extracted from spiked sediment with non-polar solvents in the presence of complexing agents is satisfactory. In contrast, very poor recovery is obtained with monobutyl tin and dibutyltin with DCM without a complexing agent (Abalos *et al.*, 1997). With volatile solvents such as hexane, hexane-acetone, dichloromethane (Abalos *et al.*, 1997; Tanabe *et al.*, 1998; Dirkx *et al.,* 1992), Soxhlet extraction is applied without complexing agent (Willis, 1965), since the more polar solvents are incompatible with the Grignard reagents used later for derivatization and favor co-extraction of organic interference compounds (Fatoki *et al.*, 2000). Therefore, the current recommended procedures (Wade *et al.*, 1988; Abalos *et al.,* 1997) are based on the extraction

of low polar organotin complexes with tropolone or diethyldithiocarbanate (DDTC). Tropolone is preferred to DDTC (Dirkx *et al.*, 1994), as under acidic condition, this undergoes decomposition, giving rise to extractable interference (http://h2osparc.wq.ncsu.edu/info/hmetals.html). Sample preparation procedures before analyses, such as liquid-liquid extraction of organotin chelates with fresh tropolone or diethyl dithiocarbamate (DDTC) (Fatoki et al, 2000) are also convenient.

Non-polar solvent plus acids are used in complex matrices' extraction (Tanabe *et al.*, 1998). For example, sediment sample is treated with hydrochloric acid with shaking or sonification, followed by sequential solvent extraction (Wade *et al.*, 1988). Hydrobromic acid or acetic acid or a mixture is also used (Tolosa *et al.*, 1992; Martin *et al.*, 1994). Sonification has become the most widely used stirring method for sediment matrix, whereas, energy-mixing methods are used for biotic materials. Mixtures of solvents have been used to increase the polarity of the medium, hexane-ethyl acetate, hexane -diethyl ether, and chloroform-ethyl acetate (Fatoki, 2000). The salting out effect or ion-pairing effect is used to increase the efficiency of extraction of organotins (OTs) from aqueous phase to the organic phase, when HCl is used (Dirk *et al.*, 1992; Tao *et al.*, 1999). Polar solvents have also been used to achieve extraction. The polar solvents which have been used are aqueous (i) HCl (Ceulemans and Adams, 1995); (ii) HCl or HOAc in polar organic solvents (MeOH, acetone) (Kuballa *et al.*, 1995; Cai *et al.*, 1993); (iii) acetic acid (Quevauviller, 1996); (iv) net polar organic solvents (MeOH, DCM-MeOH, butanol, MeOH-EtOAc (Han and Weber, 1988; Apte and Gardner, 1998); (v) polar organic solvents in basic conditions (Pawliszyn, 1997). In this case, sonification is used in most procedures. Very recently, a focused microwave field has been introduced to reduce extraction time from hours to several munites (Donard *et al.*, 1995). In some cases, after the acid or polar solvent extraction, a liquid-liquid extraction (LLE) with a non-miscible solvent (benzene, CH_3Cl-DCM, ETOAc-MeOH, DCM, hexane, cyclohexane, toluene, hexane-ETOAc) is used to recover OTs from the extract. Several authors (Abalos *et al.*, 1997) have used tropolone and salting out effect to increase the solubility of OTs in the organic solvent. Quite recently, as already mentioned, a more environmentally-friendly extraction technique developed is the supercritical fluid extraction (SFE). Advantages of these methods are shortened extraction time and limited amount of toxic solvents and acids used.

For biological samples analysis, tetramethyl ammonium (TMAH) hydrolysis is currently applied (Fatoki *et al.*, 2000), above room temperature (60^{o}C) for several hours, for example 1-2 h. (Leal *et al.*, 1995). The TMAH hydrolysis can be reduced from hours to minutes when the digestion is carried out under focused microwave irradiation. OTs are isolated from the hydrolyzed tissue by hexane (LLE) in the presence of tropolone. Alternatively, after a pH adjustment, simultaneous extraction derivatization with $NaBEt_4$ is used to reduce the numbers of LLE, compared to Grignard reagents. Also, ethanolic- KOH at 60^{o}C for 90 min or NaOH at 40^{o}C for 20 min followed by pH adjustment and LLE has also been applied to the determination of OTs from biotic matrices (Nagase *et al.*, 1998). The digestion time in basic extraction conditions is critical due to the lack of stability of mono- and di-organotin compounds. Basic and enzymatic hydrolysis methods, which are restricted to biotic samples, lead to tissue solubilization. This makes the embedded organotin more available to extracting agent (Tanabe *et al.*, 1998).

In the case of heavy metals speciation, many extraction techniques have been reported with differences in methods approach depending on the aqueous, solid or gaseous nature of the

species. Most of these techniques are in use today depending on individual situations and analytical goals. For the analysis of many metals in seawater or other matrices with strongly interfering elements, several different extraction techniques have been developed using co-precipitation with Co-APDC (ammonium pyrrolidine dithiocarbamate) or FeOH, or reductive precipitation using APDC, NaBH4, Fe, and Pd. These techniques allow quantitative extraction of metals from the interfering matrix. In addition, the extraction serves to pre-concentrate the metals, thus, improving detection limits.

After the above discussion, it is understood that, generally, testing and analysis of environmental pollutants demands the highest quality reagents for calibration and validation. Solvents used in the preparation of standard solutions must be validated free of interfering substances (Chemika-BioChemika Analytika–1995/96, 1905-1923). Quality parameters that need checking are: the physical characteristics and purity of the analytes, gravimetric data pertaining to solution preparation, actual concentration of analyte, chromatographic analysis of finished standard, and the expiration date or scheduled re-assay date. The second step involving derivatization is reviewed. For organotins speciation, GC methods require a derivatization reaction to produce volatile OT compounds for separation (Attar, 1996). The methods of conversion of ionic alkyl tins into gas chromatographable species include: (i) in situ hybridization using $NaBH_4$; (ii) ethylation with $NaBEt_4$; (iii) derivatization using Grignard reagents: methyl-, ethyl-, propyl-, pentyl-, hexyl-magnesium chlorides/bromides.

In in-situ process, Hydride generation with $NaBH_4$ has seldom been used in off-line methods, owing to the lack of hydride stability. However, this derivatization technique combined with CT-QFAAS allows for the determination of butyltins and highly volatile OTs (i.e methyltin), which cannot be determined by most off-line methods (Bayona, 1994). Furthermore; phenyltin cannot be analyzed by this method. The on-line HG-CT-QFAAS methodology allows for the reduction of the sample handling steps to a minimum, which makes this approach tone of the most rapid alternatives for the analysis of OTs (Quevauviller *et al.*, 1989). The amount of derivatization reagent needed to be optimized according to the matrix characteristics, since the matrix can inhibit the hybridization reaction. In this regard, the uncomplexed tropolone suppresses the hydride generation reaction. SPME technique has recently been used for speciation analysis of the hydride derivatives (Bayona and Cai, 1994). This method is also used in generating the hydride volatiles in the analysis of mercury using the cold vapor technique (CVAAS or CVAFS). Boron tetra-ethyl reagents have been developed (Schrader *et al.*, 1983; Leal *et al.*, 1995; Nagase *et al.*, 1995; Mueler, 1984) to minimize analyzing time. This allows carrying out the reaction in aqueous media under buffer conditions. In spiked river sediments, the derivatization yield of MBT using $NaBEt_4$ is lower than that given by hybridization methods, but matrix effects are reduced (Fatoki *et al.*, 2000; Cai *et al.*, 1993). The method is particularly successful for aqueous samples, but lower derivatization yields than those given by the Grignard reactions are observed in complexed matrix containing large amounts of co-extracted compounds. The $NaBEt_4$ procedure allows a simultaneous extraction-derivatization in the buffer medium. The ethylated derivatives are recovered with non-polar solvents (Tao *et al.*, 199). SPME technique has recently been used for speciation analysis of ethyl organotin derivatives (Millán and Pawliszyn, 2000). Alkylation with a variety of Grignard reagents (e.g. methylation, ethylation, propylation, pentylation and hexylation) is the most widely used derivatization technique for water, sediment and the biota (Tolosa et

al., 1996). However, the method is time consuming, and requires strict anhydrous conditions and non-protic solvents, which necessitate solvent exchange when polar solvents are used as extracting agents. Furthermore, the LLE step becomes necessary to isolate the derivatized OTs. Cai *et al.,* (1994; 1995) found the formation of dialkyl mono- and disulfide when the derivatization is performed in-situ on a sediment sample before the SFE, which necessitates large excess of Grignard reagents. Similar side reactions occur when the derivatization is performed on the extracts. A wide range of reaction times is reported (Ashby and Craig, 1989) but too long exposure of phenyl to Grignard reagent can lead to deproportionation reactions. Some workers have reported substantial losses of the most volatile tin species when the derivatization is performed with methyl and ethyl Grignard reagents. It is, thus, advisable to avoid evaporation to dryness of derivatized OTs. Another limitation of the methyl derivatives is that they do not allow for the determination of the naturally occurring methylbutyltins.

The next step, usually after the derivatization step is the clean-up phase. Most of the analytical procedures based on GC determination require a clean-up step or process, usually after the derivatization step as mentioned above. Silica is the adsorbent mostly used. Other adsorbent candidates in use are: florisil (Harino *et al*., 1992), alumina (Dirkx and Adams, 1992), alumina-silica (Willis, 1965), amino and C_{18} cartridges, florisil-alumina, and florisil-silica (Harino *et al*., 1992; Dirkx and Adams, 1992). In most of the methods applied to sediments that use GC-MS or GC-flame photometric detection (FPD), a desulfurization with activated copper following a clean-up is performed. However, alkylsulfides generated during the Grignard derivatization from elemental sulfur occurring in the sediment are not removed by this procedure. Alternatively, other desulfurization reagents such as tetrabutyl ammonium hydogensulfate and sodium sulfide have been successfully applied (Okamura *et al*., 2000). Florisil is a preferred adsorbent for biotic matrix with a high lipid content. Hexane or hexane-Et_2O mixtures are the most widely used eluents during the clean-up step because they allow GC determination without evaporation to dryness. More volatile solvent such as pentane is used to minimize the evaporation losses of the most volatile species. Other analytical procedures perform the clean-up before derivatization. Since underivatized OTs have a strong interaction in these adsorbents; polar eluents are needed to achieve quantitative recovery, which leads to poor clean-up efficiency. Tropolone in hexane has been used as an eluent in this case. Today, the most preferred approach, gradually gaining popularity, is the extraction of the analyte earlier derivatized in situ, preferably using sodium tetraethylborate ($NaBEt_4$) (Thompson *et al*., 1998) and sodium tetrahydroborate ($NaBH_4$) as a derivatization reagent (Balls, 1987). Hydride generation is more prone to interference and in the case of mono substituted organotin; it leads to very volatile derivatives, which can hardly be further pre-concentrated by evaporation of the extracting solvent. In addition, organotins are relatively reactive and decompose when subject to cleanup or harsh instrumentation conditions (Lespes *et al*., 1998).

The last step, which allows for the compound under investigation to be speciated, is detection. Many techniques have been developed although most of these methods are not commonly used due to their poor sensitivity or cost. From the detection point of view, GC is highly flexible (Fatoki *et al.,* 2000; Ndibewu *et al*., 2002]. In this respect, the following detector have been used for OTs speciation, GC (Fatoki *et al.,* 2000) coupled to flame ionization detection (FID), flame photometric (FPD) detection (Brickman, 1978); liquid chromatography (LC) (Fatoki *et al*., 2000), or supercritical fluid chromatography (SFC)

(Martin and Donard, 1994) with spectrometric (AAS) detection (DWAF, 1992; Prudente *et al*., 1999), atomic emission (AES) spectrometry (Fatoki *et al*., 2000; Ombaba and Barry, 1992), flame photometric (FPD) detection (Kumar *et al*., 1993; Fatoki and Ngassoum, 2000; Jiang *et al*., 2000), electron capture detection (ECD), mass (MS) spectrometry (Fatoki *et al*., 2000) or induced coupled-plasma (ICP-MS) spectrometry (Fatoki *et al*., 2000). Most of these techniques are based on an extraction (Ndibewu *et al*., 2002) step followed by derivatization using Grignard reagents (Fatoki *et al*., 2000, Abalos *et al*., 1997), sodium tetrahydroborate (Abalos *et al*., 1997) or sodium ethylborate (Abalos *et al*., 1997). However, some analytical techniques allow TBT determination by GFAAS after hybridization and selective extraction in water (Balls, 1987), sediments (Lespes *et al*., 1998) and biological samples (Prudente *et al*., 1992, Lespes *et al*., 1998). ECD and FID were used in the earlier speciation studies but seldom used during the last decade. The lack of selectivity and/or sensitivity of those detection systems for organotins led to their replacement by more sensitive low cost detector such as MS in the electron impact mode, FPD equipped with an interference filter at 610 nm or AAS. The low molecular masses of diagnostic ions in the electron impact or chemical ionization modes impair moderate selectivity in case of complex matrices (Morabito *et al*., 1995). Similarly, FPD suffers some interference associated with co-extracted sulfur species (Cai and Bayona, 1995). AED is one of the most sensitive and selective detection systems coupled to GC used in OT speciation. However the high cost and maintenance operation of the GC-microwave induced plasma (MIP)-AED system makes it unsuitable to monitoring studies involving a large number of samples.

Despite the more complex sample preparation procedure often required in GC because of insufficient volatility of the ionic organic compounds, GC is preferred (Sasaki *et al*., 1988; Arakawa *et al*., 1983) to the liquid chromatography-based technique (Fatoki *et al*., 2000) which suffers from poor resolution and usually a lack of sensitivity (Yang *et al*., 1995). Another advantage of GC over LC is the possibility of using several internal standards (IS) and surrogates, which allows the steps of analytical procedure to be traced. The main disadvantage of GC methods is that they usually require production of volatile OT derivatives to perform their separation. Packed columns are used exclusively in cold temperature (CT) when hydride derivatization is carried out. The hydrides are purged with a helium stream and trapped in a U-shaped packed column cooled by liquid N_2. The column is then heated rapidly until the purging step is complete. This method is only successful for the determination of methyl and butyl tins. Capillary column methods gained acceptance during the 1990's (Fatoki *et al*., 2000) and nowadays, they are commonly used rather than packed or megabore columns. Sample is usually introduced into the column by splitless injection because non-volatile co-injected compound is retained in the liner. Its limitation is the low sample capacity (up to 2 μL) and the discrimination of low volatile OTs against the high volatile tin species. Cold on-column and temperature programmable injectors avoid some of the limitations of the splitless mode and then allow up to 5 μL to be injected. In order to prevent column contamination, GC Tenax packing in the injection port or uncoated deactivated tubing has been used.

The high efficiency achieved by capillary GC (cGC) allows satisfactory resolution of OTs according to carbon number even with non-polar, non-selective stationary phases, such as dimethylpolysiloxane or 5 % diphenyldimethylpolysiloxane (DB-1, HP-1, SE-30). OTs with equal number of carbon co-elute (Mueller, 1987). The mid-polarity stationary-phases such as 50 % diphenyldimethylsiloxane (OV-17) or 14 % cyanopropylphenyl 86 % dimethyl

siloxanes (DB-1710) allow the resolution between specific OTs (phenyl and cyclohexyl) (Kuballa *et al*., 1995).

The use of liquid chromatographic separation is not very popular in speciation procedures. Most of the published works with LC have been done on standards (Fatoki *et al*., 2000), with few on environmental samples (Yang *et al*., 1995; Suyani *et al*., 1989). In spite of the advantage of avoiding derivatization step, LC has some limitation arising from the insufficient sensitivity of the most common detector for the levels found in environmental samples. Butyltin are the most species considered but in some cases phenyltin is considered.

Ion exchange chromatography is performed in the silica-based cation-exchange column and it has been the most applied (Rivaro *et al*., 1995; Leal *et al*., 1995). Mobile phases consist of mixtures of methanol or acetonitrile and water containing ammonium acetate or citrate. The separation of TBTs and DBTs amongst the other OTs is achieved at the same pH. In the separation of di- and triorganotin compounds based on normal phase mode, cyanopropyl have been used. The mobile phase consisted of high percentage of hexane together with polar solvent such as ethyl acetate, tetrahydrofuran (THF) and HOAc. A mobile phase consisting of tropolone in toluene has been used (Fatoki *et al.,* 2000). On one hand, reversed-phase with octadecyl silane stationary phase (C_{18}) has been used (Fatoki *et al.,* 2000) in the separation of butyltin compounds in sediments using a polar mobile phase containing complexing agent such as tropolone. On the other hand, reversed-phase ion pair approach has been used in the separation of tri-organotin compounds (Beyer *et al*., 1997). Polymeric based column (PRP-1) or octylsilane column was used, where pentane sulfonate or hexane sulfonate is used as an ion-pair (Kumar *et al*., 1993).

Several detectors or hyphenated techniques have been used in LC: AAS, ICP-MS (Beyer *et al*., 1997), fluorimetry, MS, laser-enhanced ionization (LEI) and ICP-AES. Among different AAS modes, flame AAS with pulse nebulization and off -line GFAAS were the earliest (Fatoki *et al.,* 2000). When ICP-MS is coupled to LC, pneumatic nebulizers and spray chambers are the common systems for sample introduction. ICP methods suffer incompatibility of most of the mobile phases. When fluorimetric detection is used, derivatization with fluoregenic reagent such as flavone derivatives is mandatory. The reaction is performed after chromatographic separation (McKie, 1987).

In any analytical method, a few important parameters are important to assure quality and reliability of the method involved. Some of these parameters are: detection limits, calibration, accuracy and precision. Bearing this in mind during methods development, any analytical methods developed for speciation should, therefore, provide sufficient sensitivity allowing for the determination of individual organotin compounds and elemental heavy metals below set limits. Selected absolute detection limits according to analytical techniques and analytes are reviewed below. Among the non-chromatographic techniques, ion spray mass spectrometry (ISMS-MS) is ca. 4-order of magnitude more sensitive than GFAAS (Fatoki, 2000). In the group of the GC detection techniques, AED, MS in the electron impact (selected ion monitoring) and FPD have the detection limit in the sub-to-low picogram range (Fatoki *et al., 2000;* Ndibewu *et al.,* 2002*).* The FPD configuration can lead to a remarkable difference in sensitivity. Filterless operation and quartz surface-induced luminescence are the most sensitive detection mode in FPD (Attar, 1996). Unfortunately, the dramatic deterioration of the selectivity due to sulfur emission at 390 nm was found in these operational modes. Also, oxidant flames can lead to poor selectivity since the luminescence

at 610 nm is attributed to tin hydride (Gomez *et al*., 1994; Martin *et al*., 1987). The sensitivity of the AES is strongly dependent on the plasma source. In this regard, alternating current plasma (ACP-AES) (Ombaba and Barry, 1992) has detection limit at least two orders of magnitude higher than MIP-AES.

The GC-QFAAS techniques have LODs ca. two orders of magnitude higher than the former detection systems (ECD, FPD, MS, AED) coupled to GC techniques. Nevertheless, the suitable design of the interface and GCs columns can improve the sensitivity of AAS by at least one order of magnitude (Kuballa *et al*., 1995). Among the LC methods, ICP-MS detection (Yang *et al*., 1995; Suyani *et al*., 1989), either with ultrasonic or pneumatic nebulization is the most sensitive for all the OTs, and is comparable to the most sensitive GC methods (Kumar *et al*., 1993). Concerning LC-MS interface, only thermospray has been applied to environmental studies; it has moderate sensitivity with a detection limit about 2- or 3-orders of magnitude higher than ICP-MS. The sensitivity attained with fluorimetric detection depend both on the species and the fluoregenic reagents used, and in some cases very low detection limits are achieved, only improved by LC-ICP-MS by one order of magnitude.

Calibration is another essential operation in the analytical method procedures. In some papers, especially in those devoted to environmental monitoring, little information, if any is provided on this aspect (Abalos *et al*., 1997) is very limited. In methods based on GC, calibration is generally carried out with an internal standard; however, external standards are almost extensively used. In contrast, the standard addition method is seldom used (Abalos *et al*., 1997). In those methods that involved cold trapping of volatile species, (hydride or ethyl derivatives) quantitation is usually performed by the method of standard addition or with matrix matched standards (Abalos *et al*., 1997, Fatoki *et al., 2000)*. When the technique applied is LC, calibration is usually performed with external standards, although the standard addition method is sometimes used (Abalos *et al*., 1997). When the external standards are used, the standard solution must be subjected to an entire extraction procedure (Tam and Wong, 1995; Sasaki *et al*., 1988). In other cases, in order to account for the matrix effects, matrix- matched standards are proposed (Han and Weber, 1988). However, suitable analyte-free matrices to match sample matrices may not be available. When using internal standard methods, several approaches are proposed. In the most common approach, the substance used as internal standard (IS) is added to the extracts before the derivatization step, usually as trialkyltin, or just before the injection to the chromatograph as the tetraalkyltin (Fatoki *et al., 2000*, Abalos *et al*., 1997). In the first case, the IS affords a compensation for the incompleteness of the derivatizatioin reaction, for the possible losses occurring in the operations subsequent to derivatization (extractions, evaporations, clean up) and for the instrumental variability. In the second case, it only compensates for the uncontrolled variations in the chromatographic measurements (Abalos *et al*., 1997). A second approach consists of the addition of IS (in this case also called surrogate) at the beginning of the extraction process, providing the compensation for the losses taking place in the whole process, including the variability in the determination step (Arakawa *et al*., 1983; Pereira *et al*., 1999). Some authors used both the surrogate and IS. This allows for the calculation of the recovery of the substance added as surrogate and, on this basis, correction of the amount of the analytes recovered (Willis, 1965). The substances most commonly used as IS or surrogates are tripropyltin (TPT), tetrabutyltin (TBT), tetraphenyltin

(TePeT), and triphenyltin (TPT). Generally, only IS and or surrogate is used but some alternative approaches have been proposed: (i) the used of different IS's such as monophenyltriethyltin (MPTT), diphenyldiethyltin (DPDT), triphenylethyltin (TPEtT) and tributylmethyltin (TBMEtT), depending on the nature of OTs being determined. This has been shown to be the more accurate way for correcting variations of the alkylation step (Stab *et al*., 1994), (ii) the use of several surrogates with different degrees of alkylation (Tripropyltin (TPrT), monophenyltin (MPT), diphenyltin (DPT) and triphenyltin (TPT)), in order to mach the behaviour of the different OTs in moieties in the extraction step (Fatoki *et al*., 2000; Garcia-Romero *et al*., 1993).

After considering both the detection limits and calibration, the accuracy of the analytical procedures is mostly evaluated through the analysis of either certified reference materials (CRMs) or spiked samples. In the field of OTs in sediments, nowadays, there are two CMRs available (Fatoki *et al*., 2000): the harbor sediments, PACS-1 with certified value of MBT (280 ± 170, ng g^{-1} as Sn), DBT (1160 ± 180 ng g^{-1} as Sn) and TBT (1270 ± 220 ng g^{-1} as Sn), and the coastal sediment CRM-462 with certified values for DBT (63 ± 8 ng g^{-1} as Sn) and TBT (24 ± 6 ng g^{-1} as Sn) (Fatoki et al., 2000; Abalos *et al*., 1997). There is also the reference material RM-424, with a reference value for TBT (8 ± 5 ng g^{-1} as Sn) and indicative value for DBT (27 ± 10 ng g^{-1} as Sn) and MBT (174 ± 36 ng g^{-1} as Sn) (Fatoki *et al*., 2000). The situation now is that, the CRM only allows for the assessment of the accuracy of butyltin compounds, and thus, the need for more CRMs with certified values for other OTs of environmental relevance, such as phenyl tin species is necessary.

Although the analysis of CRMs is preferable to that of spiked samples, only a few papers have reported the use of sediments certified reference materials, and in most cases PACS-1 is the CRM analyzed (Abalos *et al*., 1997). This is probably due to the fact that PACS-1 was the first CRM available for organotins, or because concentration levels of OTs are higher in PACS-1 than in CRM-462. In relation to the analysis of MBT in PACS-1, some problems have been reported though. None of the ten methods evaluated by Zhang *et al*. (1996) could recover MBT from PACS-1 satisfactorily. A higher scatter of results has also prevented the certification of MBT in CRM-462 (Martin *et al*., 1994).

In the field of biological samples, only one CRM for OTs has been available since 1991. This is a fish tissue (sea bass) from the National Institute for Environmental Studies (NIES) in Japan, with a certified value for TBT (475 ± 36 ng g^{-1} as Sn), and indicative value of TPHT (1942 ng g^{-1} as Sn (Abalos *et al*., 1997). Another method for the assessment of the accuracy of the analytical methods is based on the analyses of spiked samples, and the determination of the recoveries obtained for each analyte (Fatoki *et al*., 2000). The analyses of spiked samples are carried out in most of the papers reviewed for quality assurance. In this case the main problem lies in how the spiking has been performed, probably because; this is one of the most critical points. In any case, experiments should be performed with several kinds of matrices, and at several concentration levels, always in the range of concentrations usually found in environmental samples (Cai *et al*., 1994). Moreover, it should be taken into account that the availability of spiked analytes in the extraction step can be higher than that of the same substances incorporated into the matrices in the environment. So, using spiked samples can lead to an overestimation of the extraction efficiency, and, therefore, quantitative recoveries from spiked materials do not ensure that the same result will be achieved with natural samples (Abalos *et al*., 1997).

SAMPLE	SAMPLE TREATMENT STEPS	DETERMINATION TECHNIQUES	SPECIES
Water	1) 50 ml water + 50 acetate buffer pH 3.3 2) 1 mL $NaBH_4$ (3 %) in water) 3) SPME 15 min	GC/FPD	TBT, DBT, MBT.
Water	1) 1000 mL water 70 ^oC + 0.6 g $NaBH_4$ 2) air purged and trapping Porapak cartridge, 3) CH_2Cl_2 elution, evaporation to 0.1 mL	GC/FPD	TBT, DBT, MBT
Water	1) 250 mL water + adjust pH = 6 tris + AcOH + 1 mL isooctane 0.1 ml $NaBEt_4$ (2 % sol.) stirred 30 min, separation	GC/FPD	TBT, DBT, MBT.
Water	1) 25 ml water + standard 2) + Pd solution	GFAAS	Total tin (Sn)
Water	1) 500 mL H_2O + 5-20 mL HBr (48 %) + standards + 25 mL benzene tropolone, shaking for 15 min., 2) 3 mL MeMgBr (2.5 M sol. In diethylether), for 30 min., 3) Cooling + 25 mL 1 N H_2SO_4, shaking, separation, evaporation to 25 mL	GC/MS (if possible)	TBT, DBT, MBT.
Sediment (Also suitable for water)	1) 200 mL H_2O + 20 mL buffer pH 5 or 3 g wet sediment + 15 g Na_2SO_4, mixing, Soxhlet extraction 9 hrs (110 mL hexane-acetone 9:1, 2) acetate buffer 4 + 10 mL hexane + 4 mL $NaBTEt_4$ (2% sol.), reaction 30 min, separation, evaporation to 1 mL 3) clean up, 0.5 g silica gel 80-100 or alumina elution 5 mL hexane	GC/FPD	TBT, DBT, MBT,
Sediment (Also suitable for water)	1) 1000 mL H_2O + 5 mL conc. HCl 2) + 3* 10 mL pentane extraction by shaking, separation and evaporation to 5 mL 3) + 2 mL MeMgBr sol. reaction 10 min 4) + 5 mL H_2O + 0.5 conc. HCl, separation + $CaCl_2$ evaporation to 1 ml 5) clean up, 0.5 g silica gel 60 elution pentane 1') 20 g wet sediment + conc. HCl to pH 2 2') + 4 - 10 ml diethyl ether extraction by shaking separation and evaporation to 5 ml 3' – 6') following the same steps as for water sample	GC/FPD GC/MS	TBT

SAMPLE	SAMPLE TREATMENT STEPS	DETERMINATION TECHNIQUES	SPECIES
Biological material (biota) (Also suitable for water and sediment)	1) 500-1000l mL water or 1-5 g sediment or 1g fish tissue in 10 ml TMAH + 10 mL hexane or 10-500 l air trapping on carbosive, eluted with hexane 2) Acetate buffer 4 + 10 mL hexane + 4 ml $NaBEt_4$ (2% sol.), reaction 30 min, separation, evaporation to 1 mL 3) Clean up, 0.5 g silica gel 80-100 or alumina elution 5mL hexane	GC/FPD	TBT, DBT, MBT.

Table 1. Recommended analytical procedures for the speciation analysis of organotins

Precision is the hardcore of all analytical operations. The precision of analytical methods reviewed in this chapter corresponds to the whole analytical procedure, that is to say: extraction, the derivatization and the determination technique (Cai *et al.*, 1994). An attempt is made to point out some trends about the precision of the reviewed methods. Two groups including the method commonly applied have been considered: those based on GC-FPD as determination technique and those based on CT-QFAAS. Independent of the analytical method used, the analysis of OTs in biological material gives more precise results than in sediments (Fatoki, 2000; Abalos *et al.*, 1997). For instance, in the case of TBTs, relative standard deviations (R.S.Ds) calculated as the mean of the different methods and concentration, are 12% and 8.5% for sediments and biological materials, respectively. The precision of GC-FPD seems to be, somewhat, better than those using CT-QFAAS: 10.5 versus 13 for sediment and 7 versus10 for biological materials (Fatoki, 2000). This trend is also noticeable in the results of the certification campaign of coastal sediments (CRM-462) of the European Commission (Quevauviller, 1996). One of the reasons for the higher precision for GC-FPD may be the fact that ISs are used in the calibration step of this technique, whereas, in the case of CT-QFAAS, the standard addition method is usually applied. The MIP-AED was introduced in the '90s (nineties) in the field of GC. It has not been widely applied to the analysis of OTs. However, some trend in the precision of this technique can be pointed out. An inter-laboratory study carried out in the USA among ten laboratories (Sharron *et al.*, 1995) that analyzed fourteen OTs compounds in three pentylated extracts of soils and sediments gave inter-laboratory R.S.Ds between 2 and 4 % for most compounds. Some researchers have applied GC-MS to OTs analysis, the R.S.D given are higher than the techniques previously commented upon by Fatoki *et al.* (2000).

Various techniques (Unicam, 1992; Prudente *et al.*, 1999; Nriagu, 1996; Fatoki *et al.*, 2002),) have been developed and used for speciation analysis of heavy metals or analysis of total metals. The modification and development of these methods sometimes not only require acute analytical skills but do involve several steps. In order to reduce the number of steps and for optimization purposes], instrumentation design and set-ups nowadays use the combination of a number of the techniques. The validity of an analytical method is based, in part, on the procedures used for sample collection and analysis, and data interpretation. In many instances these procedures use approaches that have been refined over many years and are accepted by professionals as good practice. However, the multitude of variables within a specific workplace requires the professional to exercise judgment in the design of a particular assessment.

TECHNIQUES (Comparative methods and procedures)			Samples and species detected
Liquid-liquid (LLE)	Sodium tetrahydroborate ($NaBH_4$)	GC – AAS or GC – FPD	Tri-, Di- and Mo-clusters of the butyl compounds (TPh, DPhT, MPhT) can be monitored in water, sediment
Liquid-liquid (LLE)	Sodium tetraethylborate ($NaBEt_4$)	GC – AAS or GC – FPD	As above
Liquid-liquid (best solvent from above)	Best reagent out of three: $NaBH_4$, $NaBEt_4$ and EtMgBr	GC – AAS or GC – FPD	As above Water and sediment
Liquid-liquid (best solvent from above)	EtMgBr (Grignard reagent)	GC – MS (compared with GC-AAS/GC-FPD)	monitor both the butyltins and phenyltins (TPh, DPhT, MPh & TPh, DPhT, MPhT) ion species Water, sediment and biota
Liquid-liquid (best solvent)	Best reagent	Best detection technique	Monitoring of butyl and phenyl tins species in water, sediment and biota
Solid-phase Extraction (SPME)	Above protocol followed (slight modifications where necessary)	Slight modifications where necessary	Routine reason Sediment and biota

Table 2. Comparative extraction and detection techniques for speciation of organotins

For the analysis of total metals (including all metals, organically and inorganically bound, both dissolved and particulate) most samples will require digestion before analysis to reduce organic matter interference and to convert metal to a form that can be analyzed by atomic absorption spectroscopy or inductively coupled plasma spectrometry (APHA, 1992),

For speciation analysis, a few common direct determination methods have earlier been employed (Unicam, 1992). These are the dissolved metals by air/acetylene and direct determination with nitrous oxide/acetylene. In water samples analysis, elemental Cd, Zn and Sn have been determined directly by AAS after dissolving in air/acetylene (dissolved metals by air/acetylene). In another technique, Cd and Zn, if present in low levels is chelated, and aspired into the flame prior to detection by AAS (Mueler, 1984). This method consists of chelation with ammonium pyrrolidine dithiocarbamate (APDC) and extraction with methyl isobutyl ketone (MIBK), followed by aspiration into the flame (Liquid-Liquid Extraction Prior to Flame AAS) (Lucinda *et al.*, 1983). Results are achievable by adjusting the pH of the sample and the water blank, to the sample pH as the standard. While organic tin

is extracted with solvent, the inorganic tin is determined by AAS after digestion with nitric acid (Unicam, 1992).

For reliability, efficiency and sensitivity, most trace metals analyses currently involve inductively coupled plasma–mass spectrometry (ICP-MS). The standard ICP-MS technique works quite well for many matrices. But for some element/matrix combinations, it gives poor detection limits or accuracy because of elemental or molecular interferences. In choosing the most appropriate methods to analyze any element/matrix combination, ICP-MS has been coupled to dynamic reaction cell (IC-DRC-MS) (Han and Weber, 1988). This combined technique can detect trace levels in complex matrices, where the standard ICP-MS would be prone to interferences; the standard has also been coupled to a micro-mass platform ICP-MS with collision cell technology (IC-ICP-MS) (Han and Weber, 1988). Extremely low detection limits (especially for the higher mass elements) even in matrices traditionally considered difficult are achievable in an ultra-clean sample preparation and analysis environment.

An analytical technique utilizing hydride generation and atomic fluorescence spectrometry (HG-AFS) (Unicam, 1992) or atomic absorption (HG-AAS) has been developed for the analysis of arsenic, antimony, and selenium at either ultra-trace levels or in complex matrices. With HG-AFS, we can accurately measure total arsenic, antimony, and selenium in nearly all matrices at single-digit parts-per-trillion levels [http://www.frontiergeosciences.com/ebru]. Speciation information could be determined using modifications of this technique, including cryogenic trapping/GC and ion chromatographic separation (Wade *et al.,* 1988).

For mercury speciation, relatively poor sensitivity is provided by transitional flame absorption. Alternate atomization techniques for the AA determination of this element have been developed (Cai and Bayona, 1995; Shrader *et al.,* 1983; Maguire *et al.,* 1982; Wade *et al.*, 1988). Amongst them, the cold vapor atomic absorption technique has received the greatest attention (Schrader *et al.*, 1983, Wade *et al.,* 1988). Other techniques employ cold vapor atomic fluorescence spectrometers (CVAFS) (http://www.frontiergeosciences.com/ebru/), which give unparalleled sensitivity for the determination of low-level total mercury. Using this detector in combination with gold amalgamation or aqueous phase ethylation plus gas chromatographic separation allows determination of Hg speciation at the parts-per-quadrillion level (Wade *et al.*, 1988). Furnace methods for mercury are not recommended due to the extreme volatility of mercury, which has a significant vapor pressure even at room temperature (Stab *et al.,* 1992; Maenpa *et al.,* 2002). Although the first cold vapor principle was proposed by Poluekov and co-workers in 1963, the most popular method credited to Hatch and Ott was published in 1968 (Shrader *et al.,* 1983). In this method, an acidified solution containing mercury is reacted with stannous chloride in a vessel external to the AA instrument. Ground state mercury atoms are produced which subsequently are transported by an air or inert gas flow to an absorption cell installed in the AA instrument. This method provides sensitivities approximately four orders of magnitude better than flame AA (Schrader *et al.*, 1983; Wade *et al.*, 1988). It is critical to note that, unlike in the case of organotins speciation where a global approach is favorable, heavy metals speciation will very much require methods choice to consider the thermodynamics of the elemental compound (solid/liquid or gaseous state at room temperature). Therefore, methods for each metal speciation will be specific although the principle underlining the different steps are similar.

9.1 Methods and techniques for the determination of heavy metals in water, sediment and biota samples

Generally, for direct atomic absorption spectroscopy or inductively coupled plasma spectrometry, the sample must be colourless, transparent, odourless, single phase, and have a turbidity of < 1 Nephelometric Turbidity Unit. Otherwise, the sample must first be digested. The following digestion methods are generally used:

- Digestion methods (open beaker).
- Nitric acid digestion: digestion is complete when solution is clear or light-colored.
- Nitric acid-hydrochloric acid digestion: complete when digestate is light in color.
- Nitric acid-sulphuric acid digestion: digestion is complete when solution is clear.
- Nitric acid-perchloric acid digestion: digestion is complete when solution is clear and white $HClO_4$ fumes appear.
- Nitric acid-perchloric acid-hydrofluoric acid digestion: digestion is complete when solution is clear and white $HClO_4$ fumes appear.

For individual metals analysis, requirements vary with the metal and the concentration range to be determined (APHA, 1992) as follows:

Dithizone Method: Mercury ions react with dithizone solution to form an orange solution that is measured in the spectrophotometer. This method is most accurate for samples with [Hg] > 2μ L^{-1}. Known interferences are: Copper, gold, palladium, divalent platinum, and silver react with dithizone in acid solution.

Mercury: Cold vapor atomic adsorption method (CVAAS): detection Limits: Choice of method for all samples with [Hg] < 2μ L^{-1}. Here, there are no known interferences.

For the analysis of solid samples such as sediments and tissues, direct determination is not possible due to the very large matrix effects that are encountered. In order to provide accurate determination in these complex matrices, specialized digestion procedures is required that not only brings the analyte of interest into solution, but also diminishes the interfering compounds present in the matrix as much as possible. The relative extreme low detection limits that are achievable with cold vapor atomic absorption spectrometry (CVAAS) offer the option of dilution or smaller aliquot sizes to overcome sample matrix issues.

Due to the volatile nature of mercury compounds, wet digestion methods are preferred over other trace metal preparation techniques such as dry ashing. Tissue samples can be prepared for total mercury analysis using a heated mixture of nitric and sulfuric acids. After the tissues have been fully solubilized, the digestate can be further oxidized by the addition of BrCl, to bring all the mercury to the Hg^{2+} oxidation state. At the time of analysis, a sub-aliquot of the digested sample can be reacted with $SnCl_2$ to reduce the mercury to its elemental form, which can then be concentrated on a trap filled with gold-plated sand and introduced into the CVAFS instrument by thermal desorption with argon as a carrier gas. Tissues can be digested for methylmercury using a heated mixture of potassium hydroxide and methanol. A small aliquot of this digestate can be reacted with sodium tetraethyl borate to produce the volatile methyl-ethyl mercury species. All forms of mercury can be collected on activated carbon traps, and can be introduced into an AAS with an argon carrier gas. The detection limit is somewhat elevated due to the small analytical aliquot required to

overcome matrix interference. However, this procedure is preferred as it allows for complete digestion. This allows more accurate results than are possible for other methodologies such as distillations, and most tissues samples (especially from higher organisms) have sufficient methylmercury concentrations to allow the smaller aliquot size.

Sediment for methyl mercury analysis is prepared by extraction into methylene chloride from sulfuric acid/potassium bromide/copper sulfate slurry. After extraction, an aliquot of the methylene chloride layer is placed in reagent water and the solvent is purged completely from the solution with an N_2 gas stream, leaving the methylmercury in the relatively clean water matrix. A sub-aliquot of the water matrix is treated with sodium tetraethyl borate and is analyzed in the same manner as methylmercury in tissue (described above). This procedure is particularly useful as it isolates the methylmercury from the interfering matrix, and allows a large sample aliquot to be analyzed, which yields low detection limits. The extraction method was developed (Harino *et al.*, 1992) in order to overcome artifact formation that was present in the commonly used distillation procedure.

For water sample analysis, samples can be treated with 25 mL of 4% $KMnO_4$ to break up the organo-mercury compounds. Adding excess hydroxylamine sulphate and passing clean nitrogen through the sample removes free chlorine gas formed during the oxidation step. Reduction of mercury can be carried out in a similar manner to the other hydride forming elements. Samples can be placed in a reaction vessel (normally 20 mL). 1-2 mL of 20 % by weight $NaBH_4$ in concentrated HNO_3 can be placed in another vessel outside the cold vapor kit. The solutions can be conveyed into an enclosed system by a circulating peristaltic pump. The mercury vapor formed can then be flushed out of the system into a T piece aligned in the optical path of the AA instrument for recording.

For the analysis of cadmium (Cd) and lead (Pb) using the flame atomic absorption method, the sample is aspirated into a flame and atomized. The amount of light emitted is measured. Detection range may be extended (1) downward by scale expansion or by integrating the absorption signal over a long time and (2) upward by dilution of sample, using a less-sensitive wavelength, rotating the burner head, or by linearizing the calibration curve at high concentrations. Chemical interference occurs by a lack of absorption by atoms that are bound in molecular combination by the flame. By using electrothermal atomic absorption spectrometry, the high heat of a graphite furnace atomizes the element being determined and use of a larger sample volume or reduced flow rate of the purge gas increases sensitivity (detection limit: 0.1µg L^{-1}). Interferences by broadband molecular absorption and chemical (formation of refractory carbides) and matrix effects are common. The use of inductively coupled plasma (ICP) method with ionization of an argon gas stream by an oscillating radio frequency and high temperature dissociates molecules, creating ion emission spectra yielding detection limit > 4.0 1µg L^{-1}. Spectral interference from light emissions originating elsewhere (other than the source) and other physical interference from changes in sample viscosity and surface tension can affect sensitivity. In the determination of Cd, and Zn by liquid-liquid extraction prior to flame AAS, ammonium pyrrolidine dithiocarbamate (APDC) is used to chelate the compounds. Aspiration into the flame follows after extraction with methyl isobuthyl ketone (MIBK). To achieve results in normal conditions, the pH of the sample and the water blank are adjusted to the same pH as the standards. pH range for maximum extraction is 1-6 for Cd and 2-4 for Zn.

Samples are placed in a 200 or 250 mL separatory funnel fitted with a teflon stopcock and 4 ml of acetate buffer of pH 6.2 added. The mixture is well agitated. 5 mL of 1% w/v mixed solution of ammonium pyrrolidinedithiocarbamate and diethylammonium diethyldithiocarbamate in water (chelating agent) is added. The total mixture is briefly agitated and 10 – 20 mL of methyl isobutyl ketone (MIBK) is be added. The mixture is vigorously agitated for 60 seconds. The layers are allowed to separate. The lower aqueous layer is removed while the MIBK layer is retained in the tightly capped glass bottles until sample is ready for analysis. A standard of Cd and Zn stock solution are prepared so that 200 mL of water that is extracted would contain 1-20 µg Cd or Zn L^{-1}. In this way, a direct concentration relationship would exist with samples. A reagent blank is run and the sample is analyzed (AAS) under instruments recommended conditions (Van Loon, 1985).

For the determination of arsenic (As) by continuous flow hydride generation (CF-HG-AAS), suitable for volatile metals that produce a metal hydride, the sample is treated with sodium borohydride in the presence of HCL, and then detected by AAS. If recovery is poor, interring organics could be removed by passing the acidified sample through a resin.

TECHNIQUES (Methods and Procedures)		Observations
Extraction/Separation	Detection	
Microsolid phase extraction (MSE)	Flame photometry AAS	Water, sediment, biota
Liquid-Liquid Extraction (LLE): Open beaker digestion and extractive Conc. methods	Flame photometry AAS	Cd and Zn in water and sediment samples, Detection of Hg
Hydride Generation	CF-HG-AAS	As in water, sediment and Biota
Cold Vapor Technique	CV – AAS	Hg in water, sediment and biota

Table 3. Summary of techniques for speciation and determination of selected heavy metals

9.2 Sampling and sample location

In the investigation of the freshwater and marine waters environment, water, sediment and the biota, samples should be taken within the study program-site time schedule from selected locations that reflect different sea regions (for example, Atlantic and Indian oceans) and related shipping activities of that particular location. Sampling for heavy metals analysis, apart from the marine sites, should include other sites such as from rivers (sampling sites can be fixed). Locations such as upstream, midstream and downstreams the rivers should be targeted. Lakes or municipal stream water environments can also be considered. For biological materials, biota, more logically, sourcing should be matched as much as possible to the various sites chosen for freshwater and marine sampling.

About 2.5 L subsurface water samples should be collected at each sampling site. Before sampling, sample bottles should be cleaned by washing with detergent and then soaked in 50 % HCl for 24 h. Finally, bottles should be washed with water and then rinsed with doubly distilled or deionized water. Core sediment samples should be collected at the same site used for water samples by divers. Both sample types should be kept at about 4 ^{o}C until analyzed. The biota should be fresh and bought from catchmen direct from source.

9.3 Quality assurance planning

The accuracy of the method should be demonstrated by analyzing the samples and by performing spiking experiments with water samples and reference sediment materials as outlined in the methods above. In order to carry out a successful quality assurance program, the following plan is necessary and should be strictly implemented.

- Staff organization and responsibilities.
- Sample control and documentation procedures.
- Standard operating procedure for each analytical method and
- Analyst training requirements.
- Preventive maintenance procedure for equipment.
- Calibration procedures and corrective actions.
- Internal quality control activities and performance audit.
- Data assessment procedures for bias and precision, validation, and reporting.

10. Conclusion and recommendations

Both organotins (OTs) and Heavy metals (HMs) have been implicated in endocrine disrupting activities (Mueller, 1987; Fatoki *et al*., 2000; Ndibewu *et al*., 2002). Despite their potential danger to man and the ecosystem, the manufacture and uses of these compounds are not currently controlled in many developing countries. TBT-based antifouling paints are still currently being manufactured in some developing countries and there appears to be no legislation regulating use in the environment. Thus, there is the potential for significant contamination of marine water environments by TBT and heavy metals; hence, they need to be regularly monitored to prevent potential danger to man and the ecosystem due to their endocrine disrupting activities. Also, it is observed that there is a shortage of research capacity in this field, particularly in Africa, explaining why data are very scanty on the occurrence and levels of these toxic compounds. The toxicity of OTs and heavy metals and the ulta-trace levels at which they exist in the aquatic environment make it extremely important to have sensitive and reliable analytical methods available for their determination. Such techniques are not yet commonly available. The need to develop some of these techniques is of topical importance to analytical scientists.

11. Acknowledgements

Information presented in this chapter was partly researched through the endocrine disrupting contaminants (EDCs) global initiative funded by the National Research Foundation (NRF) of South Africa. The remainder of the chapter has been written based on the authors' own scientific endeavour.

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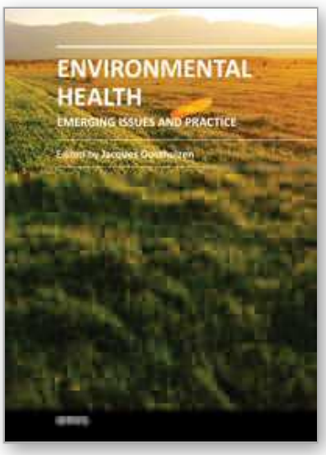

Environmental Health - Emerging Issues and Practice
Edited by Prof. Jacques Oosthuizen

ISBN 978-953-307-854-0
Hard cover, 324 pages
Publisher InTech
Published online 03, February, 2012
Published in print edition February, 2012

Environmental health practitioners worldwide are frequently presented with issues that require further investigating and acting upon so that exposed populations can be protected from ill-health consequences. These environmental factors can be broadly classified according to their relation to air, water or food contamination. However, there are also work-related, occupational health exposures that need to be considered as a subset of this dynamic academic field. This book presents a review of the current practice and emerging research in the three broadly defined domains, but also provides reference for new emerging technologies, health effects associated with particular exposures and environmental justice issues. The contributing authors themselves display a range of backgrounds and they present a developing as well as a developed world perspective. This book will assist environmental health professionals to develop best practice protocols for monitoring a range of environmental exposure scenarios.

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Peter P. Ndibewu, Rob I. McCrindle and Ntebogeng S. Mokgalaka (2012). Speciation Methods for the Determination of Organotins (OTs) and Heavy Metals (MHs) in the Freshwater and Marine Environments, Environmental Health - Emerging Issues and Practice, Prof. Jacques Oosthuizen (Ed.), ISBN: 978-953-307-854-0, InTech, Available from: http://www.intechopen.com/books/environmental-health-emerging-issues-and-practice/speciation-methods-for-the-determination-of-organotins-and-heavy-metals-in-the-freshwater-and-marine

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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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