SPECIATION OF MERCURY IN ENVIRONMENTAL SAMPLES FROM GOLD MINING COMMUNITIES IN SOUTHWESTERN GHANA

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Doctoral Dissertation
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MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
JOŽEF STEFAN INTERNATIONAL POSTGRADUATE SCHOOL

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SPECIACIJA ŽIVEGA SREBRA V OKOLJSKIH VZORCIH Z OBMOCIJ PRIDOBIVANJA ZLATA NA JUGOZAHODU GANE

Doktorska disertacija

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Ljubljana, Slovenia, October 2021

To . . .

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Abstract

Gold miners (Artisanal and Small-Scale [ASGM]) operating in southwestern Ghana use exclusive mercury-gold amalgamation, cyanidation of gold-rich mercury-contaminated tailings, or a combination of both techniques during gold extraction; exposing water bodies within the catchment to mercury (Hg) and cyanide (CN) from mining discharged effluents. Additionally, sporadic CN spillage/leakage from Large and Small-scale Gold mining which employs chiefly cyanidation also impacts water bodies. Formation of $Hg(CN)_2$ during cyanidation of Au-rich Hg-contaminated tailings, and interaction between Hg and CN in the aquatic environment increases the solubility of Hg, making it highly bioavailable for methylation. Studies on the quantitative influence of CN on Hg solubility and subsequent impact on methylation rates in the aquatic environment are rare. In Ghana, mercury contamination in the environment is mainly assessed in terms of total Hg concentrations, which lack explanation on its toxicity, mobility and bioavailability. This therefore makes an in-depth study on mercury speciation vital. Hg content in fish species depends on the age, feeding habit and location of the fish. The study investigated Hg (total) and MeHg levels in fish, water and sediment from two river systems within the Prestea-Huni Valley district, southwestern Ghana: a Hg-contaminated non-CN loaded (Ankobra River) and a Hg-contaminated CN-loaded (Aprepre River). The study also assessed Hg water-soluble fraction in sediments from both rivers, as well as identification of Hg compounds by thermal fractionation. Additionally, the depth distribution of total Hg (THg) and MeHg in soils from ASGM communities were also assessed. THg in sediments and fish was determined by Atomic Absorption Spectrophotometry (Cold Vapour technique [CVAAS]); in water by Atomic Fluorescence Spectrophotometry (Cold Vapour technique [CVAFS) after acid digestion. MeHg in sediment, fish and water was measured using aqueous-phase propylation/ethylation, preconcentration on Tenax, and gas chromatography coupled by CV-AFS. THg and MeHg levels in soils from the study sites decreased with depth, which is an indication of anthropogenic release and deposition of Hg. MeHg in sediments from the Aprepre River showed values in the range of 4.58-14.8 ng/g expressed as Hg on the dry weight (dw) basis, which represents 1.4-3.7% THg as MeHg; THg was found in the range between 241-415 ng/g, dw and 0.05–1.21 mgCN/kg, dw. For the Ankobra River, MeHg ranged 0.24-1.21 ng/g, dw (0.08-0.35% THg as MeHg) with THg in the range of 162-490 ng/g dw and CN <0.001 mg/kg. There was positive correlation (r^2=0.5974; $p<0.01$) between MeHg and CN in the Aprepre River. The water-soluble fraction of Hg in sediment from both rivers was <1% of THg. Hg in sediments from the Aprepre River was about four times more soluble than that from the Ankobra River; indicating that Hg in sediments from the Aprepre River was more bioavailable for methylation. Accordingly, the presence of CN in Hg-dominated river sediments quantitatively influences and enhances the solubility and mobility of Hg; resulting in high rates of Hg methylation. MeHg levels in fish species exceeded the USEPA MeHg guideline of 300 ng/g by 48% and 8% from the Aprepre and Ankobra River respectively. However, species from the Ankobra River were of larger sizes having an average weight of about four times bigger than that from the

Aprepre River. This is a result of the higher MeHg levels found in sediment and water samples from the cyanide loaded Aprepre River.

Povzetek

Pridobivanje zlata v jugozahodni Gani temelji na dveh postopkih. Prvi temelji na amalgamaciji z uporabo živega srebra (Hg) in je tipičen za majhne obrtniške dejavnosti (ASGM – Artisanal Small-Scale Mining); drugi pa temelji na cianidni metodi (CN) in se uporablja v večjih industrijskih obratih. Največkrat pa gre za kombinacijo obeh postopkov, kar pomeni veliko okoljsko obremenitev, zlasti vodnih teles. Prisotnost cianida v vodnem okolju povečuje topnost Hg, posledično se s tem poveča biorazpoložljivost Hg za biološko metilacijo Hg. Študije o vplivu cianidov na topnost, mobilnost in kasnejši vpliv na hitrost metilacije v vodnem okolju so redke. V Gani se onesnaževanje z živim srebrom v okolju ocenjuje predvsem glede na celokupne koncentracije Hg. Ta podatek pa žal ni dovolj, saj je znano, da so toksičnost, mobilnost in biološka razpoložljivost odvisne zlasti od kemijske oblike živega srebra. Zaradi tega je poglobljena študija o speciaciji Hg v okolju bistvenega pomena za razumevanje usode Hg. Vsebnost Hg v ribjih vrstah je odvisna od starosti, prehranskih navad in okolja v katerem živijo. V tej raziskavi je bila opravljena analiza celokupnega Hg in MeHg v sedimentih, vodi in ribah iz dveh rečnih sistemov na območju Prestea-Huni Valley, iugozahodna Gana. Območeje reke Ankobra je onesnaženo le s Hg, medtem ko je reka Aprepre onesnažena s Hg in CN. V študiji je bila opravljena tudi analiza vodotopne frakcije Hg v sedimentih ter termično desorpcijska analiza. Poleg tega je bila ocenjena tudi globinska porazdelitev celokupnega Hg in MeHg v tleh na območjih ASGM. THg smo določili z metodo atomske absorpcije hladnih par (CV-AFS) (usedlina in ribe) in atomske fluorescence hladnih par (CV-AFS) (voda) po predhodnem kislinskem razkroju. MeHg v usedlinah, ribah in vodi smo merili z uporabo propilacije / etilacije v vodni fazi, predkoncentriranjem na Tenax pasteh, plinsko kromatografijo ter detekcijo s CV AFS. Globinski profili koncentracij THg in MeHg v tleh kažejo trend zmanjšanja, kar je posledica antropogenega vnosa Hg v tla v novejšem času. Koncentracije MeHg v sedimentih reke Aprepre so bile v obsegu od 4,58 do 14,8 ng/g (izražene kot Hg na suho maso vzorca), kar predstavlja 1,4–3,7% THg. Koncetracije THg so bile med 241—415 ng/g in CN med 0,05—1,21 mg/kg. Koncetracija MeHg v sedimentu reke Ankobra je bila v obsegu med 0,24—1.21 ng/g, kar je predstavljalo le 0,08—0,35% THg. THg v obsegu med 162—490 ng/g in CN <0.001 mg/kg suha. MeHg in CN v reki Aprepre kažeta pozitivno korelacijo (r^2 = 0,5974; p <0,01). V vodi topna frakcija Hg v sedimentih iz obeh rek je bila pod 1% THg, vendar pa je bil Hg v sedimentih iz reke Aprepre približno štirikrat bolj topen kot v reki Ankobra. To potrjuje, da je Hg v sedimentih iz reke Aprepre, kjer sta prisotna Hg in CN, bolj topen in dostopen za metilacijo. Ravni MeHg v ribjih vrstah presegajo priporočila US EPA (300 ng/g) za 48% v reki Aprepre, v reki Ankobra pa le za 8%, kljub temu da so bile iste vrste iz reke Ankobra štirikrat večje kot v reki Aprepre. To je posledica višjih ravni MeHg v vzorcih sedimentov in vodah v reki Aprepre. V tej študiji smo tako potrdili, da prisotnost CN v rečnih sedimentih povečuje topnost in mobilnost Hg, kar posledično povečuje potencial metilacije in privzem MeHg v ribah.

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Abbreviations

AAS	. . . Atomic Absorption Spectrophotometry
AES	. . . Atomic Emission Spectrophotometry
AFS	. . . Atomic Fluorescence Spectrophotometry
ASGM	. . . Artisanal and Small-Scale Gold Mining
CIFA	. . . Committee for Inland Fisheries of Africa
CRM	. . . Certified Reference Material
CN	. . . Cyanide
CV	. . . Cold Vapour
DDW	. . . Deionised Distilled Water
DMA	. . . Direct Mercury Analyzer
DOC	. . . Dissolved Organic Carbon
DOM	. . . Dissolved Organic material
dw	. . . Dry weight
GEPA	. . . Ghana Environmental Protection Agency
GC	. . . Gas Chromatography
GEM	. . . Gaseous Elemental Mercury
Hg	. . . Mercury
$Hg(CN)_2$	. . . Mercury (II) cyanide
IAEA	. . . International Atomic Energy Agency
ICP-MS	. . . Inductively Coupled Plasma- Mass Spectrometry
INAA	. . . Instrumental Neutron Activation Analysis
LSGM	. . . Large-Scale Gold Mining
LOD	. . . Limit Of Detection
LOQ	. . . Limit Of Quantification
MeHg	. . . Methyl Mercury
mg/kg	. . . Milligram per kilogram
mg/L	. . . Milligram per litre
mL	. . . Millilitre
ng/g	. . . Nanogram per gram
ng/L	. . . Nanogram per Liter
NAA	. . . Neutron Activation Analysis
OC	. . . Organic Carbon
PEC	. . . Probable Effect Concentration
PHg	. . . Particulate Mercury
PTWI	. . . Provisional Tolerable Weekly Intake
ppm	. . . Part per million
ppt	. . . Quality Assurance Quality Control
QA/QC	. . . Reactive Gaseous Mercury
RGM	. . . Reactive Gaseous Mercury

SRB ... Sulphate Reducing Bacterial
SSL ... Soil Screening Level
TGM ... Total Gaseous Mercury
TDC ... Thermo Desorption Curve
THg ... Total mercury
UNEP ... United Nations Environmental Programme
UNIDO ... United Nations Industrial Development Organization
USEPA ... United States Environmental Protection Agency
UV ... Ultra Violet
WHO ... World Health Organization
ww ... Wet weight
µg/g ... Microgram per gram
µg/L ... Microgram per litre

Chapter 1

Introduction

1.1 Background of the Study

Mercury (Hg), a toxic pollutant present in the environment exists in different oxidation states [elemental Hg (Hg^0), mercurous (Hg_2^{2+}) and mercuric (Hg^{2+})]. The various oxidation states give Hg wide properties, and this influences its toxic nature, bioavailability and dispersion. There are a suite of organic forms; monomethylmercury [MeHg or CH_3Hg+], dimethylmercury [DMeHg or CH_3HgCH_3], ethylmercury [EtHg], and phenylmercury acetate [$C_8H_8HgO_2$] (Alpers et al., 2005; USEPA, 1997a; Leermakers et al., 2005). Organic Hg, particularly MeHg, is the most toxic to humans because of its neurotoxic nature. It readily incorporates into biological tissues, accumulates in the tissue leading to its biomagnification in the aquatic food web. Poisoning from MeHg causes neurological damage in both adults, children, babies, and developing foetus (Zahir et al., 2005; Karagas et al., 2012).

MeHg's toxicity emanates from the strong attraction of Hg in alkylated Hg compounds for sulphur and the thiol group (-SH) in the cysteine units present in proteins of enzymes (Baird, 1998). MeHg interferes with the fundamental cell metabolism as it enters the cell and causes it to malfunction leading to irreversible damage to metabolic processes (Barkay et al., 2003; Baird, 1998). Acute mercury exposure results in lasting injury to the brain, liver, kidneys, lungs, and the human nervous system [paresthesia, ataxia, sensory disturbances, tremors, distorted sight, inaudible speech, complications in hearing, loss of sight, and even death] (Liu et al., 2012, USEPA, 1997a). Although the inorganic forms are less potent, they can enter the organisms in enormous quantities.

Presently, human health and environmental effect of Hg exposure is an intense global concern. Following the 1950's Minamata-Japan widely-known incidence of Hg poisoning, where a large number of the natives were exposed to elevated levels of MeHg through consumption of Hg-contaminated seafood; with MeHg produced from $HgSO_4$ used as a catalyst by native/local acetaldehyde factory, and subsequently released into near-by water bodies (Akagi et al., 1998; Fujiki and Tajima, 1992). The second widely reported incident is the chlor-alkali factory accident which occurred near Ontario, Canada around 1970. This incident was a result of Hg contamination of a river system after eight years of effluent discharges by the chlor-alkali factory. Inorganic Hg was subsequently methylated in the environment. The indigenous population suffered mostly from this incident (McKeown-Eyssen and Ruedy, 1983).

Another severe tragedy occurred in Iraq around the year 1972. According to reports, a large amount of methylmercury was exposed to the natives through eating of bread prepared from grain that has been treated with a fungicide made from alkylmercury

(Mergler et al., 2007; Amin-zaki et al., 1974). The incident recorded about four hundred and sixty (460) deaths.

However, there have been no reported cases of high Hg exposure leading to incidences of severe Hg poisoning in recent times. The human health risk posed are chiefly from consumption of contaminated fish and other aquatic organisms (Mergler et al., 2007; USEPA, 1997a; Karagas et al., 2012).

Environmental contamination of Hg from natural releases as well as widespread anthropogenic use of Hg has for decades been a global concern (Liu et al., 2012). Anthropogenic source of Hg constitute about two-thirds of Hg existing in the environment (Snider, 2011). Besides coal burning, ASGM (artisanal and small-scale gold mining) has in recent years been identified as the major contributor to worldwide atmospheric Hg from anthropogenic source (Rajaee et al., 2015a). ASGM operators adopt the use of Hg to extract gold through amalgamation [Hg-Au]; interestingly, the miners generally discard the Hg used freely into the natural environment. Significant quantities of airborne emissions and liquid effluents released enters the aquatic environment and may be transformed into methylmercury (the highly toxic Hg form). Methylmercury in the aquatic environment progressively bioaccumulate in fish and later in the foodweb, posing a danger to humans (Hilson et al., 2006; Liu et al., 2012; UNEP, 2019).

Gold miners in Africa, South America, Asia has persistently been using Hg. Most emissions occur in developing nations (Donkor et al., 2006). Ghana, a country in sub-Saharan Africa has a long history of intense and active gold mining (artisanal and small-scale). The ASGM activities have resulted in extensive application of Hg by the miners; resulting in the release of a significant amount of Hg into the environment. This has led to research into Hg contamination of environmental meadia by Ghanaian scientists, civil society organizations in Ghana and international bodies like UNIDO.

Most of the environmental Hg monitoring programs have focused on total mercury determination (Enti-Brown et al., 2016; Akabzaa and Yidana, 2011; Donkor et al., 2006; Adimado and Baah 2002; UNIDO, 2001). This however, does not give an idea about the environmental fate of Hg, and its attendant consequences. Speciation of Hg is vital in appreciating Hg cycling in the environment and its toxicity. Speciation of Hg has become progressively more significant as available health records have indicated toxicological differences between the organo-Hg and metal cation forms (Shahid et al., 2020; WHO, 2013; Bernhoft, 2012).

1.2 Physical and Chemical Properties of Hg Compounds

Mercury is an element that is naturally present in the lithosphere (earth crust) and occurs chiefly as cinnabar, HgS and the ore. It exists in diverse forms: Hg^0, Hg_2^{2+} and Hg^{2+}, in the 0, +1 and +2 oxidation states respectively (Ryaboshapkoa et al., 2002). The chemical behaviour of Hg is dependent on the oxidation state.

Elemental Hg (Hg^0) is a metal with a silvery-white colour, a liquid at 25 ˚C, [it is a sole metal which occurs as liquid at room temperature] (Lambertsson, 2005). It belongs to Subgroup IIB of the Periodic Table with an atomic number of 80; and a mass number of 200.59, density 13.55 gcm^{-3} (at 25 °C) with an extremely low melting point of -38.83 °C as well as a boiling temperature of 356.73 °C. Hg^0 is transformed effortlessly into the vapour phase and emitted into the atmosphere due to elevated vapour pressure of 0.3 Pa at a temperature of 25 °C. Hg^0 is comparatively insoluble in H_2O [56 µg/L at 25 °C] but soluble in lipids, as well as in C_5H_{12} (2.7 mg/L). Hg^0 is insoluble in HCl, but on boiling, it is soluble in H_2SO_4 (WHO, 2003a).

Hg_2^{2+} and Hg^{2+} forms a number of inorganic and organic compounds. Nonetheless, under normal environmental conditions, Hg_2^{2+} is seldom stable. Hg^{2+} may possibly through covalent bonding be converted from mono or di-substituted organometallic compounds to short-chained alkyl or phenyl substituents. Under normal environmental conditions, the most probable compounds are the salts: $HgCI_2$, $Hg(OH)_2$ and HgS; the CH_3Hg compounds, CH_3HgCl and CH_3HgOH, as well as in less fractions of organomercurials [$(CH_3)_2Hg$, $C_2H_5Hg^+$, $C_6H_5Hg^+$] (USEPA, 1997a).

Environmental mercurial species are divided into volatile [Hg^0, $(CH_3)_2Hg$] water-soluble mercurials (Hg^{2+}, $Hg(CN)_2$, $HgCl_2$); and, scarcely soluble complexes (CH_3Hg^+, CH_3HgS). Most of the organomercuric are insoluble and unreactive with weak acids/bases due to mercury's low affinity for C-O bonds. Notwithstanding, the strong hydrogen bonding capability of the hydroxide group makes CH_3HgOH highly soluble. The inorganic mercuric salts exhibit broad variability in solubility. $HgCI_2$ is highly soluble in H_2O, while HgS is unreactive as an organomercuric due to the immense affinity between Hg and S (USEPA, 1997a).

1.2.1 Solubility of mercuric compounds

Mercury is usually soluble in water when it is a complex with carbonate, hydroxide, chloride, cyanide etc. Mercuric sulphide complexes are the most insoluble. The sulphides usually forms stable compounds with Hg under anoxic conditions, (Hutchison and Atwood, 2003). Notwithstanding, in the presence of mercury cyanide complexes, stable mercury precipitates are not obtained (Tassel et al., 1997). Soluble mercury-cyano complexes [$Hg(CN)_2$ and $Hg(CN)_4$] increases mercury mobilization. Table 1.1 shows the solubility of mercuric compounds in water.

Table 1.1: Solubility of mercuric compounds.

Mercuric Compounds	Chemical formula	Solubility in water (g/100 mL)
Mercury (II) Chloride	$HgCl_2$	3.6 (0 °C) 7.4 (20 °C) 48 (100 °C)
Mercury (II) Cyanide	$Hg(CN)_2$	9.3 (14 °C) 53.9 (100 °C)
Mercury (II) oxide	HgO	0.0053 (25 °C) 0.0395 (100 °C)
Mercury (II) sulfate	$HgSO_4$	Decomposes in H_2O to yellow mercuric subsulfate and sulfuric acid
Mercury (II) sulfide	HgS	Insoluble

1.3 Toxicity of mercury compounds

The toxicity of Hg depends on the chemical form, or species in which it is present. The three main forms (elemental, inorganic and organic mercury) of mercury develop different symptoms on exposure. However, the organic and divalent inorganic mercury are known

toxicants, the former, more specifically the methylated form of mercury (CH_3Hg^+), is of most concern to public health (WHO, 1989; USEPA 1997a). Mercury toxicity to man is by virtue of the fact that they interfere with / inhibit the enzyme system in the human body (Walbolt, 1982; Ajsuvakova et al., 2020).
Four unique properties make mercury a potent environmental toxicant (Barkay, 1992; Ajsuvakova et al., 2020):

- Hg(II) and organomercurial compounds have a strong affinity for thiol groups (SH).
- Strong likeness to maximize bonding to two (2) ligands (Linear Stereochemistry)
- Stability (high) of Hg-C bond resulting from low likeness for O_2
- Inclination to covalent bonds formation with organic molecules.

1.3.1 Mercury exposure due to ASGM

The population living in the catchment areas of ASGM activities in Ghana are potentially exposed to fish containing MeHg or elemental Hg vapour as a result of Hg-Au amalgam roasting (UNEP, 2012).

Vapours of elemental Hg in the atmosphere close to amalgam roasting sites may be disturbingly high; and above the WHO recommended limit of 1.0 µg/m^3 for public exposure. The released Hg vapours from amalgam roasting affects the miners and the inhabitants of nearby settlements (WHO, 2013). A large amount of the vaporized Hg finally settles locally on soil surfaces and the sediment of lakes, rivers, bays, which can be methylated. MeHg bioaccumulate in fish thereby contaminating the food chain (UNEP, 2013; USEPA, 1997a).

Released of Hg during amalgamation and amalgam burning at ASGM sites result in high surface soil contamination which is subsequently washed by rain into river bodies in the mining area. Soil ingestion in ASGM communities in Ghana may not be the main route to Hg exposure; however, it is of utmost concern if high Hg concentrations and significant quantities are ingested by the inhabitants. Inhabitants near and around ASGM communities may be exposed to inorganic mercury through consumption of edible plants cultivated on contaminated soil (Rajaee et al., 2015a). Fish mercury concentrations depict current exposure and can represent both MeHg and inorganic Hg exposure while sediment and water reflect predominantly inorganic Hg, and MeHg.

The majority of the available work done concerning Hg exposure in ASGM communities, as well as communities far away but affected by ASGM activities examines levels of mercury in biological and geological samples (Kumah et al., 2015; Armah et al. 2014; Nartey et al., 2006; Nyarko et al., 2004). Several Hg monitoring methods have been used to assess levels of Hg in ASGM communities in southwestern Ghana. In some cases one monitoring methods is used, in other cases, a combination of the monitoring technique is used. The monitoring techniques so far used in Ghana include:

a) Demographic Survey
b) Epidermiological Survey
c) Biomonitoring
d) Biological Tissue Analysis (Biota)
e) Food Quality Monitoring
f) Lake and River Sediment Monitoring
g) Soil Quality Monitoring

1.3.2 Exposure and effect of MeHg

The major source of exposure to MeHg by human is through the consumption of fish and other seafood. MeHg is taken up through the gastrointestinal tract absorption (WHO, 1990; Chamaly, 2002). The Hg-C bond in MeHg is stable in biological media, and due to its high lipid solubility, enable efficient penetration of the blood brain barrier as well as the placental barrier (USEPA, 1997a; ATSDR, 2005; Clarkson and Magos, 2006). MeHg is not degraded nor excreted from the body at significant rates. As a result, it accumulate in the brain causing lysis of cells of the central nervous system resulting in accumulations and irreversible damage of the central nervous system (Rabenstein, 1978; ATSDR, 2005). The half-time of methylmercury for humans is 70 days, causing it to concentrate to very high levels (D'Itri, 1991). The risk of MeHg is especially severe for young children and foetus whose nervous systems are in a highly active developmental damage. MeHg denatures DNA and causes chromosomal damage (WHO, 1989).

1.3.3 Exposure and effect of elemental mercury

Exposure to elemental mercury (Hg^0) is of most concern in industries such as mining and smelting, where volatile Hg^0 is emitted in closed spaces [concentrations > 0.05 mg/m^3 are considered hazardous] (WHO, 1980). Inhalation is the main route of exposure, and 80% of inhaled mercury is retained. Absorption through the gastro-intestinal and pulmonary walls is very low [less than 0.01%] (WHO, 2003a), Nevertheless, if the vapours are inhaled 100% efficiency of absorption occurs. Hg^0 is lipid soluble, and once absorbed it can easily pass through biological membrane including the blood-brain barrier. Hg^0 can be oxidized to Hg^{2+} by hydrogen peroxide-catalase pathway. Long-term exposure to elemental mercury vapour causes neurological disorders with symptoms such as tremors, neuromuscular changes, memory loss, emotional lability, insomnia, headaches, polyneuropathy, motor difficulties and proteinuria and ultimately-death has been reported (WHO, 2003a). The ingestion of Hg^0 from amalgams in dental fillings constitutes a significant source of exposure to elemental mercury. The main route of excretion of Hg^0 is through the urine. The USEPA has developed a Reference Concentration (RFC) for Hg^0 of 0.3 µg/m^3 body weight/day (UNEP, 2008).

1.3.4 Exposure and effect of inorganic mercury

Ingestion or dermal application of inorganic mercury-containing medicinal products, such as teething powders, ointments, and laxatives, and ingestion of contaminated food are the main exposure pathway. Hg^{2+} compounds may be absorbed through the skin, and only about 10% are being absorbed (WHO, 2003a). Hg^{2+} is excreted from the body within 24 hours of exposure, but the primary effect of chronic exposure may cause renal damage (WHO, 1980; WHO, 2003a). Severe exposure affect organs like the kidney and intestines. Reference doses (RfDs) for mercuric compounds are 0.3 µg/kg body weight/day (UNEP, 2008). At exposures increasingly greater than the RfD, the potential for adverse health effect increases.

1.4 Fish Advisory Related to Hg

The degree of exposure to MeHg depends on the amount and the type of fish species eaten. Because fish are an essential part of a healthy diet, fish consumption standard

guideline are issued for safe eating. This enables people to enjoy the benefit of eating fish while reducing exposure to MeHg. These guidelines control/regulate consumption of fish with typical high levels of Hg; thereby encouraging intake of fish with low Hg.
There are differences in the action levels for advisories from continents, countries and states (Table 1.2). Some advisories may apply to fish from specific water bodies or focus on groups of particularly sensitive people (pregnant women, nursing mothers, children, women of childbearing age etc.). The Joint Food and Agriculture Organisation, and World Health Organisation (FAO/WHO) Expert Committee on Food Additives have established regulatory guidelines on dietary Hg intake. They recommend a Provisional Tolerable Weekly Intake (PTWI) of 300 µg of THg per 60 kg body weight, of which not more than 100 µg should be present as MeHg. This translates to 5 µg/kg body weight of THg, and 1.6 µg/kg body wt of methylmercury (WHO, 2003b).

The recommended limit by the FAO/WHO have been adopted by many countries including Ghana (CIFA, 1992).
The United States of America (USA) have standard guidelines of 0.5 mg/kg and 1.0 mg/kg set for fish consumption by their Environmental Protection Agency and Food and Drug Administration (USFDA), respectively (USEPA, 1999). The USFDA, in coordination with USEPA, issued a final advice concerning fish consumption on January 18, 2017. This includes a recommendation for vulnerable groups and fish consumption advisory for MeHg (USFDA, 2017; Cunningham, 2017). Currently, Dietary Guidelines of the duration 2020-2025 have been released for Americans. These include dietary recommendations for children under two (2) years of age.

The European Commission Decision 93/351 of 19 May 1993 (Official Journal of the European Communities, 1994) set the maximum limits for Hg in all species of fish at 0.5 mg/kg for THg. This limit is, however, 1 mg/kg for larger predatory fish species (tuna, sharks, pike, swordfish, halibut, marlin etc.). This decision has been transferred and updated to include recommendation for specific groups of people (women of child bearing age, pregnant women, nursing mothers, children etc.) at risk to European Commission Regulation 466/2001, and subsequently amended in regulation 221/2002.

In 2004, the European Commission requested the European Food Safety Authority (EFSA) to assess if the Provisional Tolerable Weekly Intakes (PTWI) established by the FAO/WHO were considered suitable. Based on recommendations by EFSA, the Commission established a Tolerable Weekly Intakes (TWI) of 1.3 µg/kg b.w for MeHg expressed as Hg; and, 4 µg/kg b.w for inorganic mercury expressed as Hg (EFSA Panel on Contaminants in the Food Chain, 2012). The European Union (EU) member states differ largely in their guidelines on fish consumption. Luxembourg and Hungary have no specific guideline for vulnerable groups whiles other countries have stricter recommendations than those by EFSA (Mercury - Health and Environmental Alliance, 2006).

Table 1.2: Standard mercury guidelines for safe consumption of fish

Organization/Country	Standard guidelines (µg/kg bw/week)			Reference
	$PTWI_{THg}$	$PTWI_{MeHg}$	$PTWI_{Hg^{2+}}$	
JECFA	5	1.6	-	WHO, 2003b
EFSA	-	1.3	4 µg/kg	EFSA, 2012
United States[a]		0.7		USEPA,1997b; UNEP, 2008
Canada[c]		1.4[d]		UNEP, 2008
Japan[e]		2.0		UNEP, 2008
Netherlands[f]		0.7		UNEP, 2008

PTWI: Provisional Tolerable Weekly Intake EFSA: European Food Safety Authority bw: body weight
JECFA: Joint FAO/WHO Expert Committee on Food Additives
a US Environmental Protection Agency
c Bureau of Chemical Safety
d For pregnant women, women of childbearing age and young children. The reference level for the general population of 3.3 µg MeHg/kg bw was established in 1972
e Food Safety Commission
f National Institute for Public Health and the Environment

1.5 Mercury in the Environment

1.5.1 Mercury sources and sinks

Hg emissions into the environment are mainly of natural and anthropogenic sources. Nevertheless, the third source of Hg release which is considered neither natural nor anthropogenic is the re-emission from old deposited Hg from natural and man-made sources. The primary pathway through which Hg enters the environment is atmospheric emission.

1.5.1.1 Natural sources

A number of natural processes leads to the emission of Hg into the atmosphere. The major natural sources are: degassing of the earth crust by incessant and universal natural weathering of Hg-containing rocks through geothermal activities or volcanic eruption (UNEP, 2013; Boening, 2000). Volatilization from marine and aquatic environments such as the earth crust is another important source of Hg in natural water. In addition, re-emission of atmospheric Hg earlier deposited by wet and dry processes from natural and man-made sources (Gustin et al., 2008). According to Global Mercury Assessment (GMA) 2018, Natural Hg emission to the atmosphere is about 10% of the total annual estimate (UNEP, 2019). Gaseous Elemental Mercury (GEM) is the main form (>99%) of mercury from natural releases.

1.5.1.2 Anthropogenic sources

Hg emissions into the atmosphere due to human actions are from intentional (use) and unintentional release. Artisanal and small-scale gold mining, followed by stationary combustion of coal, are the principal sources of man-made Hg emission. Other

anthropogenic sources (intentional and unintentional) are releases from industry (involving mercury-related processes). The production of iron, non-ferrous metals, chor-alkali, cement and smelting activities also results in the release of Hg. Waste from consumer products (including incineration of municipal and medical waste), combustion of other fuels including biomass, amalgam dental filling, (UNEP, 2019; UNEP, 2013; Boening, 2000).

Worldwide, aquatic and terrestrial ecosystems are contaminated with Hg due to emissions from human activities. Global anthropogenic Hg emissions between 1990 and 2010 decreased by 20% according to various studies (UNEP, 2019). However, Street et al., 2019 estimated that worldwide Hg emissions between 2010 and 2015 have increased at a rate of 1.8% per year. Globally, annual Hg emission to the atmosphere from anthropogenic sources in 2015 is 2220 tonnes (which has an approximate range of 2000-2820 tonnes) This account for about 30% of annual Hg emission to the atmosphere, with 60% coming from natural processes that result in re-emission of Hg previously deposited to soils and water (which is much derived from earlier anthropogenic emission). Annual emission in 2015 is estimated to be approximately 20% higher than it was in 2010 (UNEP, 2019). Total atmospheric Hg concentrations have increased by about 450% above natural levels by human activities. Most of anthropogenic emissions occurred in Asia, South America, and Sub-Saharan Africa with emission rate of 49%, 18% and 16%, respectively (UNEP, 2019). No study has generated annual emission trends for recent years. Gaseous Elemental Mercury (GEM), Reactive Gaseous Mercury (RGM), in addition to Particulate Mercury (PHg) are discharged through activities of humans; with a distribution of about 50-60% GEM, 30% RGM, and 10% PHg.

1.5.2 Mercury cycling in the environment

1.5.2.1 Mercury cycling in the atmosphere

Mercury is released into the atmosphere from both natural and man-made sources. Worldwide, the main anthropogenic sources of mercury emissions are the burning of fossil fuels, primarily coal, and artisanal and small-scale gold mining. Elemental mercury (Hg^0) is the main form of mercury released into the air as vapour. The main mercury species in the atmosphere are elemental mercury (Hg^0; ~ 95%), divalent reactive gaseous mercury (Hg^{2+}), and particle bound mercury (Hg^p) (Pietilä, 2014). Although the existence of methylmercury has also been reported (<3%) of the total gaseous Hg except at near emissions sources (Lin and Pehkonen, 1999).

Hg^{2+} constitutes about 3% of the total gaseous mercury. Gaseous mercury is vertically well mixed in the troposphere with background concentration of ~1-4 ng/m^3. Background concentration of particulate mercury has been shown to be a minor constituent [0.3-0.9% of total gaseous mercury (TGM)], (Lin and Pehkonen, 1999) except in industrialized region (up to 40% of TGM).

The speciation and chemical transformations of mercury in the atmosphere strongly influence its deposition mechanism and global cycling. Hg^0 is not significantly removed from the atmosphere by wet and dry deposition due to relatively low deposition velocity and water solubility. It therefore remains in the atmosphere long enough (atmospheric lifetime of ~ 1 to 2 years) to travel far from the source. The oxidation of Hg^0 in the atmosphere is an important mechanism involved in the deposition of mercury on land and water. Oxidation reactions convert Hg^0 into reactive gaseous mercury that represents a mixture of gaseous divalent mercuric compounds (reactive gaseous mercury, RGM) and particulate mercury (mercury associated with atmospheric particulate matter, PHg) (Liu et al., 2012). Fig. 1.1, shows the chemical processes of mercury in the atmosphere.

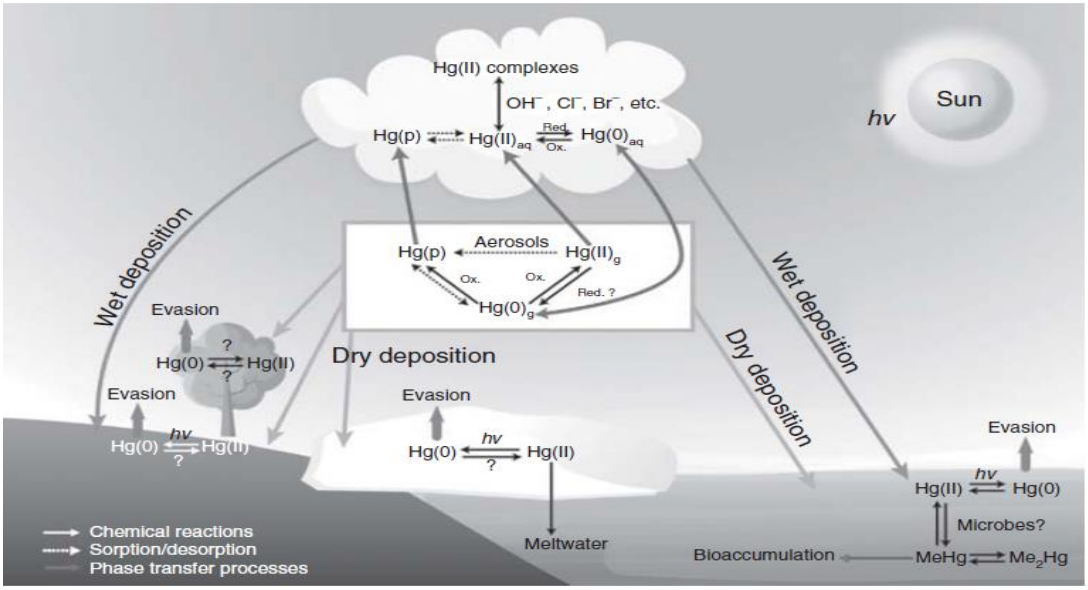

Figure 1.1: Atmospheric chemical processes of Hg [(Red) Reduction; (ox) Oxidation] (Liu et al., 2012).

The most significant oxidants are ozone, hydroxyl radical, hydrogen peroxide, nitrate radicals and reactive species of halogen (such as the atomic, molecular; and chlorine, bromine and iodine radicals). The oxidized Hg^{2+} compounds are removed or deposited more readily because of their higher water solubility and high reactivity with surfaces. Deposited mercury is easily accumulated in soils; it has been estimated that over 90% of annual mercury deposition is retained within soils. In freshwaters, mercury can originate from direct atmospheric deposition and run off from soils. Mercury can be re-emitted from land and sea back to the atmosphere, and then re-deposited.

The chemistry of mercury in the atmosphere

The atmospheric oxidation of Hg^0 to Hg^{2+} is very important in the cycling of mercury from the atmosphere to other environmental compartments. It is also critical in the estimation of the atmospheric lifetimes, as well as atmospheric transport distances of mercury (Gaffney and Marley, 2014). The oxidation of Hg^0 to Hg^{2+} increases mercury deposition rates; while reduction of Hg^{2+} to Hg^0 increases its atmospheric lifetime. Understanding the oxidation-reduction chemistry of atmospheric mercury is critical in the understanding of mercury cycling through the environment.

In the atmosphere, Hg partitions among gas, aqueous (e.g., cloud, fog, and rain waters), and solid (e.g. particulate matters) phases. In the gas phase, the chemical processes are dominated by oxidation reactions that convert Hg^0 into RGM and PHg. Reduction reactions, though yet to be verified experimentally, have been proposed (Schroeder et al., 1991). In the aqueous phase, both oxidation and reduction reactions occur (Gaffney and Marley, 2014). The predominant oxidants are ozone (O_3), hydroxyl radicals (OH), reactive halogen species (atomic and various molecular and radical forms of chlorine, bromine and iodine), hydrogen peroxide (H_2O_2), and nitrate radicals [NO_3.] (Liu et al., 2012).

Formerly, the major oxidation pathways for Hg^0 were believed to be the gas phase and aqueous phase reaction with O_3. However, the gas reaction of elemental mercury with ozone has been brought into question as there is now evidence suggesting that the primary reaction product is HgO_3; which can then decompose to the observed products HgO and O_2. The decomposition of HgO_3 is expected to occur on wet aerosol surfaces to form $Hg(OH)_2$ (Gaffney and Marley, 2014). Mercury oxidation processes with ozone increases with relative humidity (Vehedpour et al., 2011). Hence, the reaction of elemental mercury with ozone in the aqueous phase is much faster than in the gas phase. Oxidation reaction of GEM with ozone is now found to be slow. Gas phase reaction of elemental mercury with ozone has an atmospheric lifetime of mercury for approximately 1.4 years.

Presently, it is known that the oxidation of GEM by Br and BrO radicals are involved in the rapid removal process of atmospheric Hg. Consequently, Br atoms are considered as the main oxidant for GEM (Obrist et al., 2011; Lyman and Jaffe, 2012). Tables 1.3 & Fig. 1.2 show the important reactions of mercury relevant to the atmosphere with overall rate constants and atmospheric mercury lifetimes estimated from reaction kinetics in gas and aqueous phase, respectively.

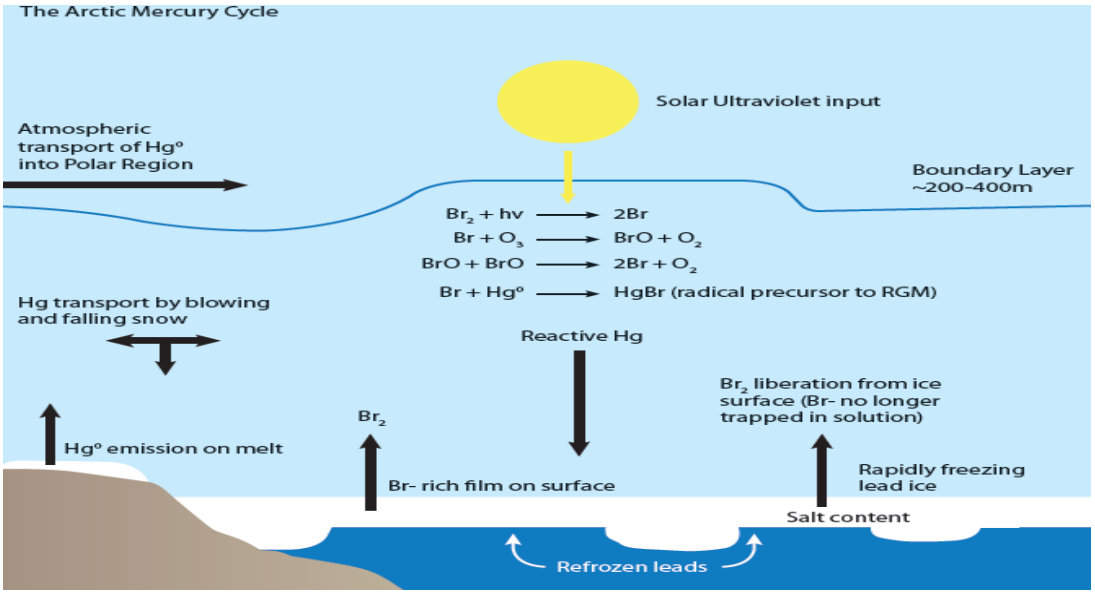

Figure 1.2: Atmospheric bromine production and the possible fate of mercury after its chemical reactions with bromine-containing compounds and its deposition (AMAP/UNEP, 2013).

Table 1.3: Reactions of mercury relevant to the atmosphere (Gaffney and Marley, 2014).

Gaseous phase			Aqueous phase	
Reactions	Rate constants ($cm^3\,molec^{-1}sec^{-1}$)	Lifetimes	Reactions	Rate constants ($M^{-1}\,sec^{-1}$)
1. $Hg^0 + O_3 \rightarrow HgO + O_2$	$3x\ 10^{-20}$	1.4 years	9. $Hg^0 + O_3 \rightarrow Hg^{2+} + OH^- + O_2$	$5x10^7$
2. $Hg^0 + OH \rightarrow HgOH$	$6x10^{-19}$	25 days	10. $Hg^0 + OH \rightarrow Hg^{2+}$	2x10
$HgOH + O_2 \rightarrow HgO + OH$	$9x10^{-14}$	8 month	11. $Hg^0 + HOCl \rightarrow Hg^{2+} + Cl + OH$	$2x10^6$
3. $Hg^0 + H_2O_2 \rightarrow Hg^{2+}$	$<8x10^{-19}$	>15 years	12. $Hg^0 + OCl^- \rightarrow Hg^{2+} + Cl + OH^-$	$2x10^6$
4. $Hg^0 + Cl_2 \rightarrow HgCl_2$	$2x10^{-10}$	50 years	13. $Hg^0 + Br_2 \rightarrow Hg^{2+} + 2Br^-$	$2x10^{-1}$
5. $Hg^0 + Br_2 \rightarrow HgBr_2$	$<9x10^{-17}$	>5 days	14. $Hg^0 + HOBr \rightarrow Hg^{2+} + Br + OH^-$	$3x10^{-1}$
6. $Hg^0 + Cl \rightarrow HgCl$	$1x10^{-11}$	3-4 months	15. $Hg^0 + OBr^- \rightarrow Hg^{2+} + Br + OH^-$	$3x10^{-1}$
7. $Hg^0 + Br \rightarrow HgBr$	$3x10^{-12}$	9 hours	16. $Hg^{2+} + HO_2 \rightarrow Hg^0$	$2x10^4$
$HgBr \rightarrow Hg + Br$			17. $Hg(OH)_2 + hv \rightarrow Hg^0$	$3x10^{-7}$
$Hg^0Br + Br \rightarrow HgBr_2$	$8x10^{-3}$		18. $HgSO_3 \rightarrow Hg^0 + S(IV)$	$< 1x10^{-4}$
$HgBr + OH \rightarrow HgBr(0H)$	$2x10^{-10}$		19. $Hg(SO_3)_2^{2-} \rightarrow Hg^o + S(IV)$	$5x10^3$
	$2x10^{-1}$			
8. $Hg^0 + BrO \rightarrow HgBrO$	$1x10^{-14}$	12 hours	20. $Hg(COO)_2R \rightarrow Hg^o + RCOO^{-2}$	$1x10^4$

1.5.2.2 Mercury cycling in soil and sediment

On deposition, 60% of the atmospheric mercury is deposited to land and 40% to water (Morel et al., 1998). The greater proportion of Hg deposition on land presumably reflects the proximity of its sources since water precipitation is three times lower on land than on the oceans. Deposited mercury is easily accumulated in soils; it has been estimated that over 90% of annual mercury deposition is retained within soils. Transformations of Hg^0 take place as soon as this element is deposited on the soil. Approximately, 25% of the deposited Hg remained in the Hg^0 form, and 75% turned into the active forms.

Deposited Hg^{2+} species are subject to a wide array of chemical and biological reactions. Soil conditions (e.g., pH, temperature and soil humic content) are typically favourable for the formation of inorganic Hg^{2+} compounds such as $HgCl_2$, $Hg(OH)_2$, $Hg(CN)_2$ and complexes with soil particles of sulphur content (USEPA, 1997a). Though inorganic Hg(II) compounds are quite soluble (and, thus, mobile), they form complexes with soil organic matter (fulvic and humic acid) and mineral colloids; the former is the dominating process (USEPA, 1997a). These chemical bonds and complexing behaviour limit mercury's mobility in soil and its availability for uptake by living organisms (USEPA, 1997a). The adsorption properties of soil or sediment are the main factors that control the transformation of species in soil. The extent of adsorption depends on the organic matter content and cation exchange capacity of soil or sediment (Hermogene, 2007).

In soil and sediments, Hg associates with organic matter and iron oxides under oxidizing conditions; and under reducing conditions, with organic matter and sulphides (Krabbenhoft et al., 2005). Bacteria and organic substances can reduce Hg^{2+} to Hg^0 or they can methylate and subsequently demethylate mercury (USEPA, 1997a). The reduction is mediated by humic substances and by light and according to several authors, the production of methylmercury is mediated by sulfate-reducing bacteria (Bouffard and Amyot, 2009). About 1-3% of THg in surface soil is MeHg, and the remaining 97-99% can be considered largely as Hg^{2+} complexes; though a small fraction of Hg in typical soil will be Hg^0 (USEPA, 1997a).

It is assumed that a natural concentration of total mercury in bottom sediments varies from 10 to 200 ng/g dry mass (Boszke et al., 2002). Terrestrial ecosystems are an

important link between atmospheric deposition and aquatic ecosystems. Table 1.4 shows the main species of Hg in the geochemical cycle (Issaro et al., 2009).

Table 1.4: The main species of Hg in the geochemical cycle (Issaro et al., 2009).

Properties	**Chemical Species**
Volatile Compound	Hg^0, $(CH_3)_2Hg$
Reactive species	Hg^{2+}, HgX_2, HgX_3^- and HgX_4^{2-} with X- OH^-, Cl^- and Br^-; HgO on aerosol particles; Hg^{2+} complexes with organic acids, methylmercury (CH_3Hg^+, CH_3HgCl, CH_3HgOH) and other organomercuric compounds.
Non-reactive species	$Hg(CN)_2$; HgS; Hg^{2+} bound to S atoms in humic matter

1.5.2.3 Mercury cycling in water and biota

Hg^{2+} and MeHg from atmospheric deposition (wet and dry) can enter water bodies directly. Hg^{2+} and MeHg can also be transported to water bodies in runoffs (bound to suspended soil/humus or attached to dissolved organic carbon); or enter water bodies from groundwater flow in the upper soil layers through the process of leaching (USEPA, 1997a). Aquatic systems can also be affected by point sources of mercury owing to local contamination (Selin, 2009). Once in the aquatic system, it may complex with anions like chloride and hydroxide or to thiol sites on dissolved or particulate organic material. The various inorganic mercury species subsequently settle at bottom sediments where they are either immobilised by formation of cinnabar (HgS) or undergo biological transformations into methyl, dimethyl or reduction to elemental mercury (Lambertsson, 2005).

Mercury species formed in sediments may thereafter transfer to the water column where MeHg is available for further biomagnification in fish through the aquatic food webs. Nearly 100% of the mercury found in fish muscle tissue is methylated (Bloom et al., 1991). Bioaccumulation of MeHg in fish occurs in water bodies that are remote from emission sources and seemingly pristine, as well as in water bodies that are less isolated (USEPA, 1997a). Not all mercury compounds entering an aquatic ecosystem undergo methylation, and demethylation reactions (USEPA, 1997a) as well as volatilization of dimethylmercury would decrease the amount of MeHg available in aquatic environment. The majority of the dimethylmercury and elemental mercury ultimately are emitted to the atmosphere.

Mercury levels in uncontaminated freshwaters are at ultra-trace (ng/L) level and the proportion of MeHg is typically less than 10% of the total Hg concentration (Pietilä, 2014). However, due to bioaccumulation, mercury concentrations in fish can be up to a million times higher than in ambient water; and over 95% of accumulated mercury can be in the form of MeHg. It has been shown that MeHg concentrations in fish respond rapidly to the Hg^{2+} added directly to the surface waters. Thus, a fall in mercury emissions and subsequent decrease in mercury depositions may have a significant effect on mercury contamination in fish (Pietilä, 2014).

1.6 Mercury in Gold Mining

ASGM produces 20-30% of global gold supply (about 500-800 tonnes per annum). It involves an estimate of about 40 million miners, including women and children. ASGM workforce in Africa includes about 40 to 50% of woman. According to the World Gold Council (2017) about 150 million people depend on ASGM for their livelihood across 80 countries in the world. ASGM takes place mostly in the global south, sub-Saharan Africa, Asia, Oceania, Central and South America.

Mercury amalgamation is the main gold extraction technique used in ASGM. Though the optimum mercury to gold ratio (Hg: Au) is about 1 [v/v] (Babut et al., 2003; Rajaee et al., 2015b), most miners add excess Hg so that all available gold will be amalgamated (Babut et al., 2003; Valdivia et al., 2011). Approximately 1 to 3 grams of Hg is lost to the environment for every gram of gold produced by ASGM. This is of great concern due to global environmental impact of mercury. About 650 to 1000 tonnes of Hg is discharged annually. This is equivalent to one-third of global anthropogenic mercury releases into the environment.

According to the Global Mercury Assessment Inventory for 2010 and 2015, the use of Hg in ASGM is the main source of Hg emissions to the atmosphere at the global level. The mean global annual Hg emission to air by ASGM in 2015 was 838 tonnes (estimated to be 675-1000 tonnes per year). This account for almost 37.7% of the global annual anthropogenic release (UNEP, 2019). South America and Sub-Saharan Africa are the main contributors to emissions accounting for about 70-75%. Hg emission from ASGM rises from 177 tonnes in 2010 to 340 tonnes in 2015 for South America; and about 230 to 250 tonnes for Sub-Saharan Africa. The annual Hg released for 2015 was higher than in 2010 by 158 tonnes (AMAP/UNEP, 2019).

Annual global Hg released into terrestrial and freshwater environment in 2015 was assessed to be 1220 tonnes. The demand of Hg in ASGM continues to upsurge with the increasing demand for gold. China consumes the highest levels of Hg, and releases about 200 to 250 tonnes; followed by Indonesia (100 to 150 tonnes). Brazil, Bolivia, Colombia, Ecuador, Ghana, Peru, Philippines, Venezuela, Tanzania and Zimbabwe releases between 10 and 30 tonnes each (UNEP, 2019).

1.6.1 Gold mining in Ghana

Ghana has vast gold deposits and have resulted in Artisanal and Small-Scale Gold Mining (ASGM), in addition to Large-Scale Gold Mining (LSGM); with southwestern Ghana being the hub of gold mining activities. Besides there is gold mining in the mid-belt (Kenyase in the Ahafo region to be precise) and northeastern Ghana (that is Talensi district in Upper East region). Gold has been one of the mainstays of Ghana's socio-economic development, contributing about 6% of Ghana's Gross Domestic Product (GDP). The gold mining sector is a major economic driving force and the biggest single contributor to government revenues and a leading source of export earnings. Globally, Ghana is among the top ten gold producing nations. On the African continent, Ghana and South Africa are the top two gold producing nations (Ghana Chamber of Mines, 2019). For over 1000 years, gold has been mined in the territory of present-day Ghana (Hilson, 2002). Ghana's gold output of 4.8 million ounces in 2018 surpassed South Africa's 4.2 million ounce total for the first time (Theafricareport, 2019). Ghana's mining industry contributes around 37% to the country's total exports, 38.3% of Ghana's total corporate tax earnings, and 27.6% of government revenue in recent years. Gold exports from Ghana makes up close to 90% of overall mineral exports (Ghana Chamber of Mines, 2019).

Gold exploration and large-scale mining activities are mostly carried out by foreign companies. The number of multilateral large-scale gold mining companies in Ghana presently (as at 2020) amounts to about fourteen (14). This makes mining companies engaged in gold exploration activities one of the busiest industries in Ghana. The sector employs 28,000 people in the large scale mining (LSGM) sector whilst over one million people are engaged in artisanal and small-scale gold mining (ASGM). ASGM activities have increased tremendously in recent years with chronic unemployment, increasing poverty and deregulation of gold mining (Ghana Chamber of Mines, 2011; Hilson, 2010; Hilson and Potter, 2005). ASGM plays a significant economic role and offers livelihood for rural and poverty-driven population in gold mining communities. The economic gains are however achieved at a great threat to the environment and human health, due to the discharge of Hg as a result of the application of Hg amalgamation as the predominant and preferred gold extraction technique used by the miners. The miners are also covertly engaged in cyanidation of gold-rich Hg-contaminated tailings.

Reports on extensive damage of farm-lands (cocoa, food crops and rubber plantations) result in low food production, with its adverse effect on food security. Additionally, the destruction of cocoa farms affects Ghana's total foreign earning from its main export crop. Gold production also has an adverse effect on human health; and has reportedly led to upsurge in diseases like malaria resulting from abandoned water ponds at ASGM sites. Furthermore, pollution of water bodies by the activities of ASGM has affected availability of portable water due to high cost of water treatment (Mensah et al., 2015). The gold processing methods used resulted in Hg and CN contamination of humans, soil, and water bodies (Adjei et al., 2012; Obiri et al., 2006; Hilson and Pardie, 2006).

1.6.2 Structure of gold mining in Ghana

Gold mining in Ghana is undertaken under two (2) broad categories: the large-scale gold mining (LSGM), and artisanal and small-scale gold mining (ASGM). The latter sector has two classes: artisanal gold mining and small-scale gold mining.

1.6.2.1 Artisanal and Small Scale Gold Mining (ASGM)

ASGM, popularly called 'Galamsey' (meaning "gather and sell") in Ghana, has in many centuries been a vibrant indigenous industry in Ghana. Gold production from ASGM activities has increased tenfold over the past decades. ASGM accounted for 35% of total national gold production in 2019 (Ghana Chamber of Mines, 2019). ASGM is undertaken by individuals, group of individuals, workers laid-off by LSGM companies, or co-operatives with weak monetary base with little or no mining expertise. Small-scale gold mining refers to the winning of gold dust by people whose activities are known to and overseen by the government, whereas artisanal gold mining refers to the winning of gold dust by people who do not have the sanction of government.

Small-scale gold mining (SSGM) was legalised in 1989, with the enactment of the Small-Scale Gold Mining Law (PNDC Law 218 of 1989), the Mercury Law (PNDC Law 217 of 1989), and the Mineral Marketing Corporation Law (PNDC Law 219). These were adopted to regularise and streamline small-scale gold mining by granting the relevant license to prospective miners, regulate the use of mercury by small-scale miners to ensure that they do not pose excessive danger to human life and the environment, and in addition provide official marketing channels for gold produced by small-scale miners (Minerals and Mining Policy of Ghana, 2014). These actions led to significant investment and a substantial rise in the production of gold in the country. The total gold production by artisanal and small-scale gold mining increased from 2.2% in 1989 to 35% of total gold

produced in 2019 (Ghana Chamber of Mines, 2019). Under the Mercury Law (PNDC law 217 of 1989), registered ASGM operators and licensed traders can purchase and trade Hg legally through authorized dealers, such as the Precious Minerals Marketing Company (PMMC) Limited (previously Precious Minerals Marketing Corporation). Hg use, however, appears to be greater than what is officially available, suggesting a significant "black market" for mercury. Mercury can be purchased at local stores or sourced from gold dealers. In some mining areas, gold buyers provide mercury to miners as an incentive to buy their gold (Nyame, 2010). Government bodies or institutions regulating mining in Ghana include:

a) Minerals Commission of Ghana, under the Ministry of Lands and Natural Resource
b) Ghana Chamber of Mines
c) Precious Minerals Marketing Company Limited
d) Precious Minerals Marketing Company Limited.
e) Environmental Protection Agency

In 2006, all the laws governing mining in Ghana were combined and replaced with the Minerals and Mining Law 2006 (Act 703), with the aim of providing a more comprehensive legal framework to guide mining operations. This law was amended in 2015 (Minerals and Mining Act, 900); and more recently (The Minerals and Mining Act, 995 in December 2019) the law was again amended after the government of Ghana imposed a ban on ASGM activities effective from January, 2017. The ban was however lifted in 2018 (Mining Law, 2021). The ban was implemented with the aim of formalizing and cleaning the artisanal mining sector (and the small-scale gold mining sector to a lesser extent) before a new roadmap could be deployed to manage activities due to concerns over environmental degradation, loss of biodiversity, the employment of child labour and chemical contamination (Ghana Web, 2018; Mining Review Africa, 2019). However, the ban has been criticized for being a heavy-handed and ineffective strategy over the long term because it does not address the root causes of illegality and informality. Hence, galamsey remains a prevalent practice in Ghana despite constant government effort to regulate it (BBC, 2017).

1.6.2.2 Large-Scale Gold Mining (LSGM)

LSGM has been a prominent industry in Ghana for decades. Apart from South Africa's Anlogold Ashanti (formerly Ashanti Gold) there has been an emergence of a number of gold mining companies from USA, Canada, and Australia engaged in large-scale gold mining in Ghana. The government of Ghana owns stakes in some of the companies. These companies have invested hugely in large-scale mining. As a corporate social responsibility, communities within their catchment area have benefitted from health facilities, school building, scholarships, employment as well as other amenities. The LSGM sector produced 2.807 million ounces of gold in 2019, which accounted for 65% of the total national output (Ghana Chamber of Mine, 2019).

1.6.3 Mercury gold mining in Ghana

1.6.3.1 Gold extraction by ASGM

Exclusive Hg amalgamation

In Ghanaian ASGM, gold ore is excavated from surface and shallow underground mining, and panning in streams (Rajaee et al., 2015a). The process of gold extraction from gold-bearing rocks, alluvial gold deposits, and gold-bearing river sediments is presented in Fig. 1.3. The gold-bearing ore is generally milled in a grinding machine and screened manually. The fine fraction is mixed with water and the mixture is allowed to gently flow down an inclined wooden sluice board covered with hemp tissue or jute sack. As the mixture flows down the board, gold particles are trapped on the sack due to gravity concentration. The concentrate is removed from the sack by washing with water, followed by separation from small amount of sand particles trapped together on the jute sack. The concentrate (impure gold dust) is panned with Hg in a rubber pan to form a mercury-gold amalgam. The amalgam is heated (roasted) with a blowtorch or on an open fire to volatize the Hg, leaving the raw gold (gold dore). During this process liquid and gaseous elemental Hg are released into the environment. The essential processes involved in gold extraction at the small-scale and artisanal gold mining sites are depicted in Fig 1.4 [photographs were taken during sample collection].

Processing of alluvial gold deposits, and gold-bearing river sediments to obtain gold commences by mixing the alluvial gold or river sediments with water. From this stage, the mixture is allowed to flow on a sluice board covered with jute sack (same as described for gold-bearing rock). The remaining extraction processes are the same as outlined/described for gold-bearing rocks.

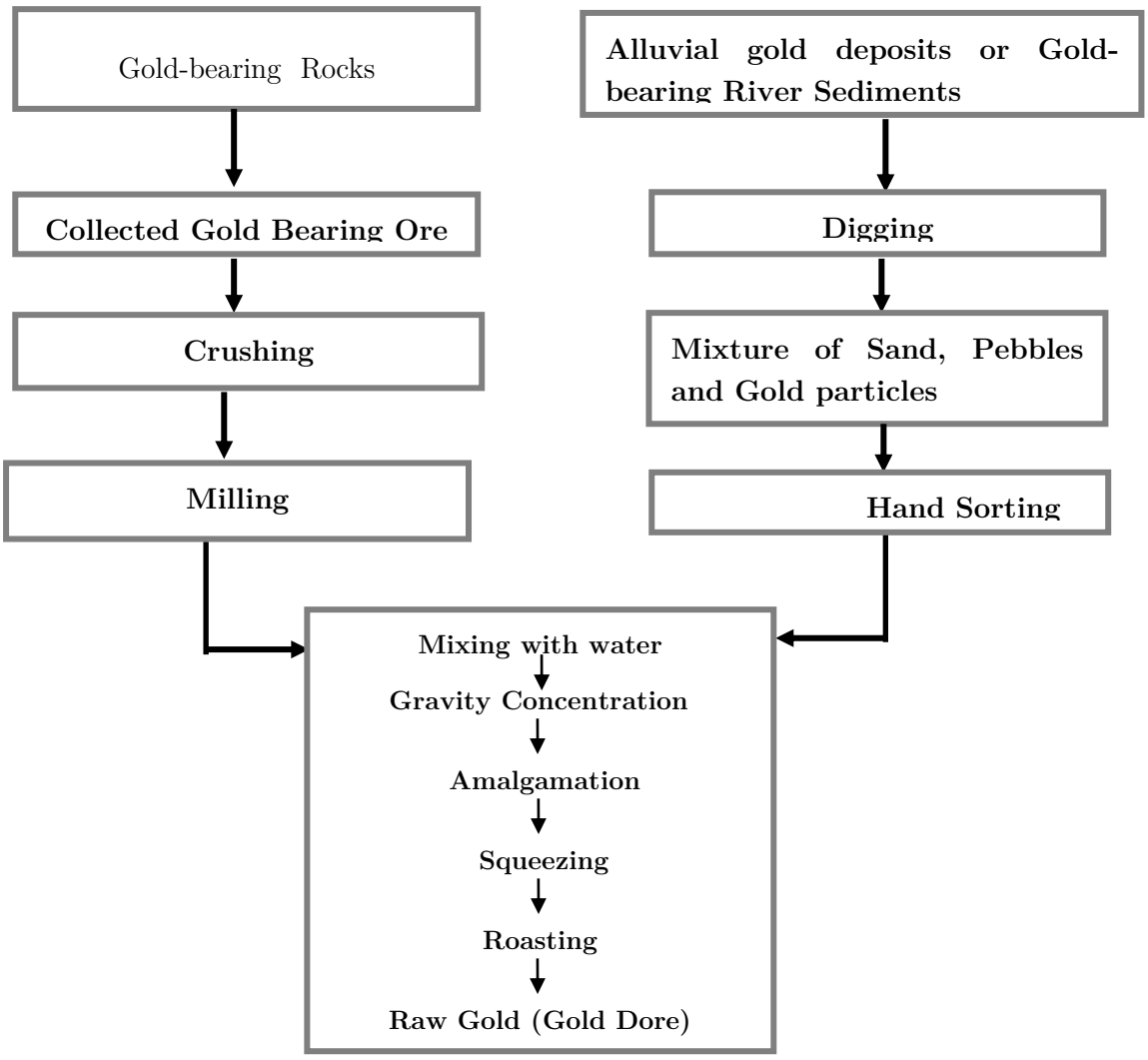

Figure 1.3: Schematic flow diagram of the essential processes of gold extraction from gold bearing rock, alluvial gold deposits, and gold-bearing river sediments.

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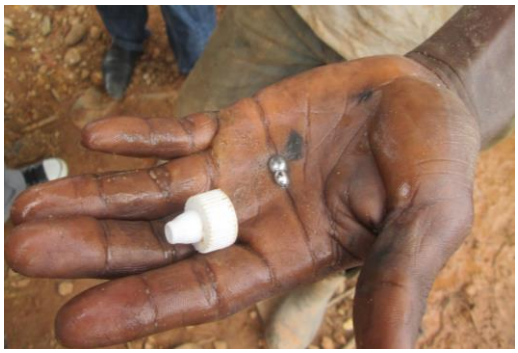

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h

Figure 1.4: The essential processes involved in gold extraction at the small-scale and artisanal gold mining sites

ASGM miners have no system for recovering the mercury used; therefore large amounts of Hg vapour settles in the surrounding environment or are deposited in areas far from the operation site. Also, the deposited Hg can be re-emitted from water and soil surfaces (Liu et al., 2012). Surface soils, water bodies, and sediment are the major biospheric sinks for Hg (UNEP, 2002). About 20% to 30% of the Hg introduced is lost to the tailings, streams and river sediments, and water bodies close to the processing sites. Approximately, between 70 to 80% of the mercury is lost to the atmosphere during processing (Straaten, 2000).

The miners have refused to use simple mercury distillation retorts introduced by the Minerals Commission of Ghana (in collaboration with UNIDO) to recycle mercury safely by distilling the amalgam. The reasons vary from suspicion that some gold is lost in the distilled mercury, to resistance of gold buyers to purchase retorted amalgam (which tends to be darker than that heated in open air), to tradition and indifference to mercury cast (Veiga, 1997). The problem of mercury poisoning due to the activities of small-scale and artisanal gold miners has been a topmost agenda for UNIDO. In 2000, small-scale and artisanal gold mining, and mercury pollution were among the priority programmes of UNIDO.

There are existing regulatory procedures, which the miners are required to comply with. The provisions of the mercury and small-scale gold mining laws of 1989 basically required that good mining practices be observed and due regard paid to environmental protection. However, this has not been adhered to by the miners. In recent years, extensive environmental contamination has occurred in gold mining areas as a result of gold recovery process.

Cyanidation of Au-rich Hg-contaminated tailings

Previously regarded as a gold extraction technique solely used in large-scale gold mining, cyanidation has been introduced into ASGM operations as a supplement to mercury amalgamation. Although the miners recognize the beneficial, simple and less tedious nature of Hg-Au amalgamation, its gold recovery efficiency of about 30% is considerably lower compared to the alternate process like cyanidation which has about 90% efficiency (Veiga et al., 2009). Although current regulations do not permit the use of cyanide or other leaching techniques for small-scale mining, the miners are covertly using cyanidation to extract gold in some mining areas of the Eastern, Northern and Upper regions of Ghana. Some miners in southwestern Ghana are also clandestinely engaged in the use of the cyanidation technique.

The artisanal and small-scale miners use exclusive amalgamation, or a combination of amalgamation and cyanidation (Velasquez-Lopez et al., 2011; Veiga et al., 2014; tulasi et al., 2021). The miners first employ amalgamation, and subsequently the Au-rich Hg-contaminated tailings are subjected to cyanidation to extract residual gold. The use of cyanide mostly occurs at gold processing centers and larger AGSM operators within gold mining communities (Velasquez-Lopez et al., 2011; Veiga et al., 2014). Central Processing Centres in AGSM communities are facilities where miners send their gold-bearing ores or tailings to be extracted by dedicated operators for a fee. Accordingly, the miners do not invest in costly equipment to crush, grind, concentrate and amalgamate the gold (Veiga et al., 2014). The processing centres purchase Au-rich Hg-contaminated tailings from the miners; and subsequently use cyanide to extract residual gold.

A significant number of miners in southwestern Ghana sell the Au-rich Hg-contaminated tailings to processing centres or larger ASGM operators within the community and other parts of Ghana; for reprocessing with cyanide (Tulasi et al., 2021).

The miners in addition sell their mercury-laced tailings to ASGM agents from bordering West African nations like Cote d'Ivoire, Burkina Faso and Mali (Veiga et al., 2014; Macdonald et al., 2014).

Cyanide processing is not preferred among smaller miners due to the large capital investment required for gold extraction (Sousa et al., 2010; Sulaiman et al., 2007).

1.6.3.2 Mercury and cyanide interaction in gold mining

During cyanidation of Au-rich Hg-contaminated tailings, Hg^0 present in the tailings reacts with cyanide or in the environment due to inappropriate waste disposal, to form water-soluble $Hg(CN)_2$ complexes. The Hg released during Au-Hg amalgam formation, Hg-CN complexes released with cyanidation tailings along with Hg in solution are discharged into nearby rivers (Castilhos et al., 2006; Marshall et al., 2018; Seney et al., 2020). In addition, cyanide in the aquatic environment can be a result of the activities of LSGM which solely employs cyanidation in the gold extraction process. Cyanide released in this case into the aquatic systems happens mainly due to escapes from tailing dumps or inadvertent releases. This may occasionally contribute to CN in the aquatic environment. There is therefore the likelihood of mercury also interacting with cyanide in the aquatic environment due to discharged effluent containing Hg and CN from both types of mining (ASGM and LSGM).

The presence of soluble $Hg(CN)_2$ in aquatic media results in substantial upsurge in Hg solubility and mobility; thereby making Hg further bioavailable for formation of organic Hg or direct bioaccumulation (Veiga et al., 2006; Coles and Cochrane, 2006; Velasquez-Lopez et al., 2011; Marshall et al., 2020). Fig. 1.5 shows a schematic diagram of the fate of mercury and cyanide released into the environment during gold mining.

The continuous release of Hg and CN into the aquatic environment due to gold mining activities poses a health risk to the inhabitants within the catchment area of the ASGM. This is because there is the potential of inorganic mercury in aquatic environment to be methylated into the more toxic organometallic (MeHg) form through microbial action (Guevara, 2002). The degree to which microbial mercury methylation occurs in aquatic systems depends on factors that can affect the bioavailability of Hg to sulphate reducing bacteria, and the activity and structure of the microbial community (Ullrich et al., 2001).

Aquatic organisms such as fish which feed on sediment are more likely to ingest methylmercury into their system. The majority of the inhabitants within the catchment area are consequently exposed to MeHg through consumption of fish from the water bodies.

Contamination of the aquatic environments with Hg in southwestern Ghana is mainly assessed in terms of total Hg concentrations. It is an established fact that levels of total mercury alone are insufficient for enhanced understanding, prediction and clarification of their behavior [mobility, bioavailability, and toxicity] (Issaro et al., 2009). This therefore, makes an in-depth study on mercury speciation vital. Literature data on Hg speciation in aquatic sediments exposed to both CN and Hg are rare. The rate of Hg methylation increases resulting in a corresponding increase in MeHg levels. Due to the amalgamation and cyanidation techniques adopted by ASGM in southwestern Ghana, it has become imperative to systematically assess the levels of MeHg in Hg-contaminated cyanide-loaded aquatic environments.

The present study therefore, seeks to investigate total and MeHg in sediments, fish and water from Hg-contaminated cyanide-loaded (Aprepre River) and Hg-contaminated non-cyanide (Ankobra River) aquatic environments in the Prestea Huni-valley district of southwestern Ghana.

Additionally, the study will evaluate the temperature fractionation of Hg in solid samples (sediment and soils) aimed at understanding the temperature stability of Hg compounds; and also identify the mercuric compounds.

Data generated from the study will be useful in the assessment of environmental risk; besides aiding in the development of remediation and control strategies. Furthermore, measurement of Hg water-soluble fraction in sediment will serve as an important tool for the assessment of the potential biological uptake and potential risk for Hg methylation (Wahle and Kordel, 1997; Reis et al., 2014).

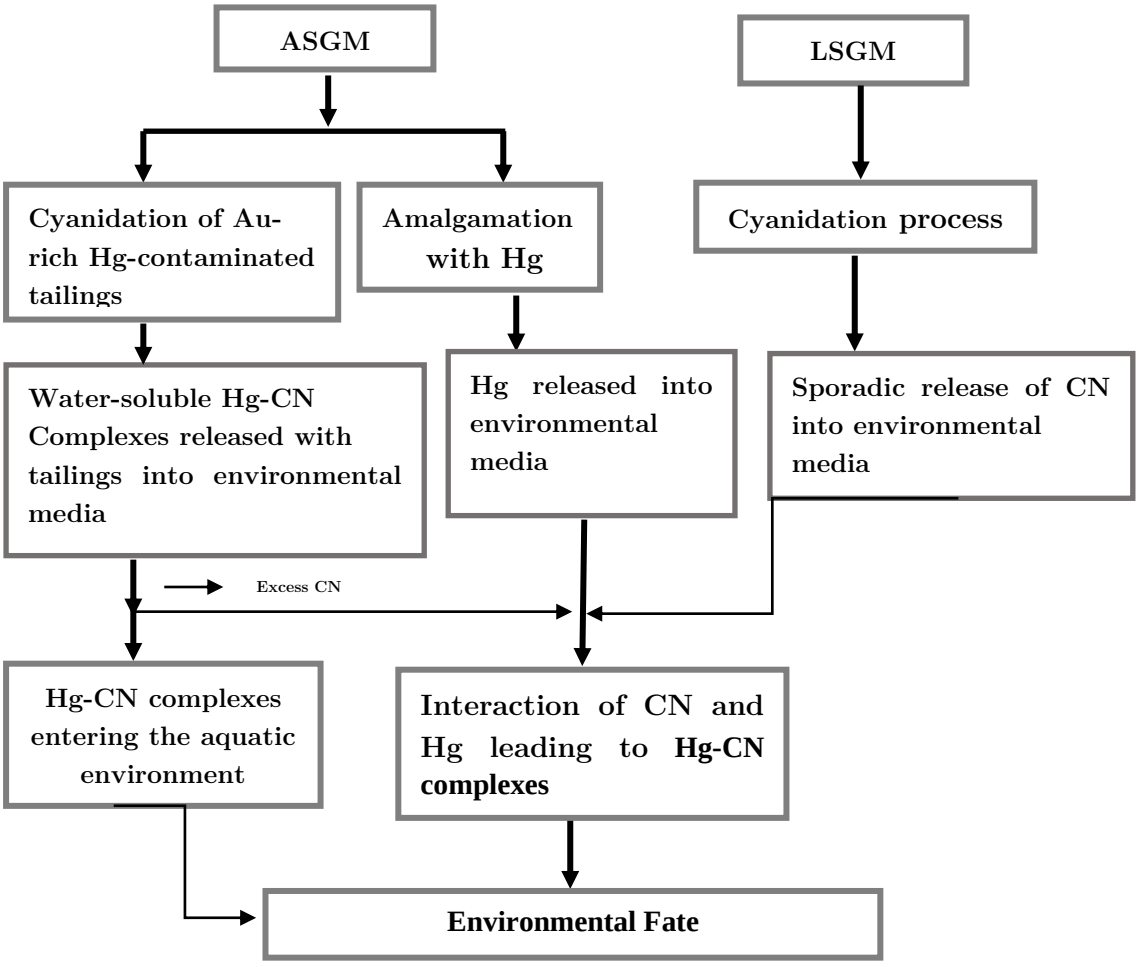


Figure 1.5**:** Schematic diagram of the fate of mercury and cyanide released into the environment during gold mining.

1.6.4 Previous Work Done on Mercury Pollution in Ghana

UNIDO, (2001), conducted a study to assess and reduce mercury pollution emanating from artisanal gold mining in Ghana. The study area was Dumasi. Blood, urine, hair and finger nail samples were collected from people in full-time "Galamsey" work, part-time "Galamsey" and people who have nothing to do with "Galamsey". Samples were taken from people permanently residing in Dumasi. The analytical procedures used in the analysis involved aqua regia digestion, centrifugation and dilution. Cold Vapour Atomic Fluorescence Spectrometry (CVAFS) was used to detect mercury. Hg Vapour was purged

from solution by Argon gas carrier stream. Results obtained led to the following intermediate conclusions:

1. "Galamseys" workers are more exposed to Hg than "non-galamseys" workers.
2. There is a strong evidence of Hg exposure among the Dumasi population.
3. Young illiterate "galamsey" workers shows the strongest exposure. People inhabiting the village for a long time are also among the most exposed people.
4. Many "non-galamsey" workers, even less exposed than "galamsey" workers, show obviously higher Hg blood levels than reference values, meaning that there is an exposure through the environment (food).

In the 2nd part of the study, water samples from water bodies in the village (Aprepre and Rora Rivers), sediments, fishes, vegetables and chicken were analysed for Hg. Detection of Hg in the various samples was by CVAFS after acid digestion, $KMnO_4$ oxidation and $SnCl_2$ reduction. The average concentrations of Hg obtained are stated below:

a) The average concentration of Hg in water samples from boreholes is 0.165 µg L^{-1} (±0.05). This is far beyond the limit for drinking water.
b) The average concentration of Hg in sediment samples is 5.091 µg g^{-1} (dry weight).
c) For surface water, the average concentration of Hg in fishes is 3.371 µg g^{-1} (dry weight) and 0.744 µg g^{-1} (dry weight).
d) For chicken, the average concentration (µg g^{-1} wet wt and dry wt) is 0.045 and 0.176 µg g^{-1}, respectively.
e) Average Hg concentration (µg g^{-1} wet wt) of 0.085 in vegetable samples

The findings of the work are summarised below:

(i) Sediments are significantly contaminated, even though less than heavily contaminated areas in other parts of the world.
(ii) Fishes are also significantly contaminated; the concentration range is comparable to those observed in other gold mining areas.
(iii) Vegetables show generally low concentrations of Hg, except cocoyam; the weekly Hg intake exceeds the acceptable level set by WHO/FAO committee for several vegetables.

Adimado and Baah (2002) determined mercury in human blood, hair, nail and fish from the Ankobra and Tano River basins in southwestern Ghana. In their work, Hg in all the samples was oxidized by acid digestion, then reduced to elemental mercury vapour prior to instrumental analysis. Cold Vapour Atomic Absorption Spectrometry (CVAAS) was used to determine the total mercury concentrations in the samples. Summary of the results is presented in Table 1.5.

Table 1.5**:** Summary of mercury levels in human blood, urine, hair, nail and fish tissues from different locations in the Ankobra and Tano River basins in southwestern Ghana (Adimado and Baah, 2002).

River Basin	Location	Blood µgg^{-1} Mean± SD (Range)	Urine µgg^{-1} Mean±SD (Range)	Hair µgg^{-1} Mean± SD (Range)	Nail µgg^{-1} Mean ± SD (Range)	n	*Fish tissues µgg^{-1} Mean ± SD (Range)	n
Ankobra	Anwiaso	102.0±55.8 (30.2-218)	34.2±36.0 (1.0-183)	1.61±1.33 (0.15-5.86)	2.65 ±2.0 (0.57-10.0)	50	0.18±0.1 (0.03-0.29)	7
	Sahuma	13.4±15.3 (2.1-68.1)	2.6±1.9 (0.13-6.96)	0.62± 0.41 (0.32±2.19	0.73±0.91 (0.18-5.40)	50	0.32±0.6 (0.01-2.40)	2 1
Tano	Tanoso	16.5±10.7 (2.1-57.2)	6.4±2.7 (2.0-14.3)	4.27±6.26 (0.06-28.3)	3.45±4.16 (0.13-22.9)	51	0.20±0.1 (0.09-0.39)	1 1
	Elubo	39.5±16.2 (1.8±70.4)	7.3±6.9 (0.02-42.5)	1.21±0.65 (0.07-3.19)	1.05±1.30 (0.22-9.68)	66	0.32±0.6 (0.05-2.50)	1 5

n represents the number of independent determination

* wet weight

Donkor et al. (2006) examined THg levels in water, soil and sediment from gold mining-impacted Pra River basin in southwestern Ghana. The results are shown in Table 1.6. THg levels in the water samples collected from all the mining-impacted sites along the Offin River, Lower and Upper Pra basin range from 24-420 ng/L. These levels are below the WHO (1996) and USEPA (2009) safe water limit of 1.0 and 2.0 µg/L. The sediment and soil samples are below the USEPA (1985) limit of 200 ng/g and the 1.06 µg g^{-1} Probable Effect Concentration (PEC). Only the maximum THg level of 2.15 µg/g in soil from Offin exceeds the Probable Effect Concentration.

Table 1.6: Hg concentrations found in River Pra basin, Ghana (Donkor et al., 2006).

		Lower Pra River basin	Offin River basin	Upper Pra River basin	Non-impacted site
Hg in River H_2O (ng/L)	Range	28.70-403.00	41.60- 420.00	24.00-294.00	148.92
	Mean	144.10	205.36	150.68	148.92
Hg in sediment (ng/g)	Range	6.52-57.32	2.73-49.86	13.07-23.22	11.21
	Mean	25.89	23.00	18.09	11.21
Hg in soil (ng/g)	Range	3.40-202.32	1.56-2146.96	12.09-34.26	4.00
	Mean	75.61	263.79	24.61	4.00

Akabzaa and Yidana (2011) reported the spatial distribution of mercury in the Ankobra River basin in southwestern Ghana. THg was analysed for water and streambed sediments from active and historic artisanal mining, as well as historic large-scale mining using CV-AAS. THg concentration from the streambed sediment ranged from 63 to 270 ng/g and from <1 to 8 ng/L in the water samples. The maximum THg levels in the sediment exceeded the USEPA (1985) THg sediment standard limit of 200 ng/g. THg levels in the water samples were low, with the highest level found in streams close to small-scale mining activities. They reported that all samples from streams with artisanal mining activities gave THg concentrations of at least 1 ng/L. THg levels in water from most of the streams located in areas with intense small-scale mining activity exceeded the WHO maximum limit of 1.0 ng/L.

Chapter 2

Aims and Hypothesis

Research on the quantitative influence of CN on Hg solubility and subsequent impact on methylation rates in the environment (soil, freshwater fish, river sediment and water) is rare. In Ghana, mercury contamination of the environment is mostly assessed in terms of total Hg concentration; this however, does not give information on the transport, fate and speciation of Hg on entering environmental systems. This study is, therefore, designed to fill gaps and challenges in current knowledge on the fate of Hg on entering CN-influenced environmental media in ASGM/LSGM communities in southwestern Ghana.

2.1 Research Aims and Goals

2.1.1 Overall aim and goal

The study assessed the distribution of THg and MeHg in sediment, soil, water and fish from Hg-contaminated CN-loaded and Hg-contaminated non-cyanide aquatic environments of ASGM and LSGM communities of southwestern Ghana.

2.1.2 Specific aims and goals

(a) Application of a well-validated analytical method for total and methylmercury in soil, sediment, water and fish;

(b) To ascertain the levels of THg and MeHg in water using CV-AFS and GC-CV-AFS, respectively, and also to evaluate the respective levels of THg and MeHg in soil, sediment and fish using CV-AAS and GC-CV-AFS;

(c) To appraise the impact of the presence of CN on Hg methylation through the estimation of the levels of MeHg in sediment, fish and water samples from the Hg-contaminated cyanide-loaded and Hg-contaminated non-cyanide aquatic system;

(d) To assess the water-soluble Hg fraction in Hg-contaminated sediment;

(e) To identify the easily temperature-released mercuric compounds in sediment and soil samples by thermal fractionation;

(f) To use Kayzero Instrumental Neutron activation (k_0-INAA) analytical technique to assess the levels of THg in soil and sediment, and compare with THg levels obtained using CV-AAS;

(g) To estimate the weekly dietary intake of THg and MeHg by adults through fish consumption.

2.2 Hypotheses

- Factors such as organic carbon and cyanide can influence the methylation of mercury.
- Hg water-soluble fraction can be used to assess the potential biological uptake and potential risk for Hg methylation.
- Speciation of mercury in soil depends on depth and location.
- Speciation of mercury in fish depends on location, feeding habit and weight.

Chapter 3

Materials and Methods

3.1 The Study Area

3.1.1 Geographical location of study area

The study was conducted in the Prestea Huni Valley District of the southwestern part of Ghana with Bogoso as its district capital. The Prestea Huni Valley Municipality (5° 58´ 0´´ North, 1° 55´ 0´´ West) shares boundaries to the north west with Wassa Amenfi East District, to the west with Axim Municipality, to the south with Tarkwa Nsuaem Municipality and to the north with Wassa Amenfi West District. According to 2010 Population and Housing Census, the district has an estimated population of 159,304 people. The district is predominantly rural and has its rural population of 62.9% exceeding the regional average of 57.6%. The majority of its inhabitants are into peasant farming and gold mining. The Prestea Huni Valley Municipality is a major gold mining hub in Ghana and one of the administrative districts in the Western Region of Ghana (southwestern Ghana to be precise). Lying about 300 km west of Accra (capital city of Ghana), the municipality is rich in gold and cocoa, timber and magnesium, and covers a land area of about 1376 sq. km. It has four major towns, namely Prestea, Huni Valley, Aboso and Bogoso (district capital) with about 29 villages and small-towns; notable among the small-towns are the popular gold mining towns, Damang and Dumasi. Gold mining takes place in all four major towns of the municipality.

3.1.2 Climate

The district is located in the rain forest zone of Ghana which is fast depleting by human activity, including mining and farming. The climate is of the wet equatorial type. Mean annual rain fall ranges from 1449 mm to 2608 mm, with an annual temperature range from 25 to 29 °C. The annual mean humidity is 86% and ranges from 70 to 90% (Akabzaa and Yidana, 2011). It is highest in August to September and lowest in January to February. The topography is undulating.

3.1.3 Geology

The geology of the study area is dominated by Birimian and Tarkwaian formations (Kesse, 1985). Gold deposits from the Birimian metavolcanic and metasedimentary rocks contain more quartz lodes that consist of quartz vein type and disseminated sulphide type. The gold occurs in lenses of sulphide-bearing quartz veins, mainly in carbonaceous phylillite as disseminated sulphides in the metavolcanics, or as oxidised derivatives of the two types in the Birimian rocks and in conglomeritic horizons as free milling gold in the

Tarkwaian (Akabzaa and Yidana, 2011). Gold deposits of the Tarkwaian are in most cases considered as paleo-placers, which is the source of "alluvial gold" in Ghana (Milesi et al., 1991).

3.1.4 Gold mining in Prestea Huni-Valley district

The district is a major mining hub in Ghana, defined by several major mining towns and villages such as Bogoso, Dumasi, Prestea and Damang. Out of the ten (10) multilateral large scale gold mining companies (LSGM) actively operating in the Ghana's Western region, five (5) are located in the Prestea Huni-Valley District (Owusu Nimo, et al., 2018). They include: Golden Star Bogoso/Prestea Limited (GSBPL), Abosso Goldfield, Goldfields Damang, Golden Star and Prestea Sankofa Gold. The district has about 156 galamsey (illegal artisanal mining) sites with over 1130 individual operations (Owusu Nimo, et al., 2018). Also, the district has 89 registered and actively operating small-scale gold mining companies. (Ghana Statistical Services, 2014). The artisanal and small-scale gold miners in the district mine gold from three main sources: gold-bearing rock ore, tailings from large-scale gold mining, and gold-rich sediment of water bodies (alluvial mining).

The techniques used in processing gold in LSGM companies operating in southwestern Ghana include: Carbon in Leach (CIL), Biological Oxidation (Biox), and Heap Leach Carbon in Column (CIC). These processing techniques employ the use of cyanide. A significant number of Large-Scale Gold Mining (LSGM) Companies uses the cyanidation technique to extract gold from its ore. The effluent from the extraction which contains residual cyanide is discharged into tailings dams and sumps. There have been reports of occasional unintentional and sporadic cyanide spillage/leakage from the tailing damps of LSGM companies into water bodies (especially the Aprepre River) within the catchment (Singh et al., 2007; Obiri et al., 2006; The Denver Post, 2006, July 27). The Aprepre River is the main source of drinking water and fish for the Dumasi community and surrounding satellite settlements.

Artisanal and small-scale gold miners in the Prestea Huni Valley district apply the following procedures in mining gold: **[i]** excavation/digging of earth surface/sediments; **[ii]** crushing and grinding (for gold bearing rocks); **[iii]** chuting/shanking (shifting); **[iv]** washing/sluicing (based on gravity concentration); **[iv]** amalgamation (Hg-Au amalgam formation); **[vi]** amalgam roasting (burning); **[vii]** amalgam squeezing for raw gold acquisition (removal of residual Hg).

Hg is released into the water bodies/environment during amalgamation, amalgam roasting and amalgam squeezing.

The post-amalgamation tailings are Au-rich and Hg-contaminated. This Au-rich Hg-contaminated tailings are subjected to cyanidation by some ASGM to extract additional gold; resulting in tailings contaminated with both Hg and CN [as $Hg(CN)_2$]. $Hg(CN)_2$ is the soluble mercury cyanide complex routinely discharged untreated into local water bodies with the tailings; resulting in Hg-contaminated CN-loaded sediments.

Interestingly, the majority of the artisanal minors working along the Aprepre River send the Hg-contaminated tailings to nearby processing centres in Dumasi (close to the Aprepre River) for cyanidation of the tailings to extract Au. Only a small percentage of the miners working along the Aprepre River sell their Hg-contaminated tailings to tailings-buyers.

After amalgamation of the tailings, miners along the Ankobra River sell the Hg-contaminated tailings to buyers who convey them to processing centers several kilometres away from the Ankobra River for cyanidation.

3.1.5 Study towns and draining rivers

Samples were collected from the Bogoso/Prestea environs. This area is drained by a number of rivers and streams. Two major rivers (Aprepre and Ankobra) within the district were used for the study. The Aprepre River is located at Dumasi (a village about 3 km from Bogoso; on the Bogoso-Prestea main road). Dumasi is located close to the processing facilities of a renowned large-scale industrial gold mining company operating in the district (5° 32' 27'' N, 2° 03'48'' W). The LSGM company is one of the largest formal gold mining companies in the district that adopt cyanidation in gold recovery. Historically, Dumasi village has been known to be an active artisanal and small-scale gold mining (ASGM) community. The Aprepre River which drains Dumasi flows from the eastern to the western part of the village. Dumasi also houses the tailing dam and sump of a vibrant large-scale gold mining company within the Prestea Huni Valley district. The tailing dam and sump are situated upstream of the Aprepre River. Mine effluents from the large-scale gold mining company end up in the Aprepre River during run-offs, in addition to unintentional spillage of CN. Effluents from artisanal/small scale gold mining activities (both amalgamation and cyanidation of Hg-contaminated tailings) are discharged directly into the Aprepre River. Thus the Aprepre River receives effluents containing CN and Hg [largely as $Hg(CN)_2$] from ASGM, and sporadically from LSGM. For that reason, the Aprepre River is likely to be Hg-contaminated CN-loaded aquatic environment. Apart from $Hg(CN)_2$ formed during cyanidation of Hg-contaminated tailings, there are probable interactions between CN and Hg in the aquatic environment, which imparts on Hg methylation.

The Ankobra River (Fig. 3.1) which flows through Ankobra Town-Prestea is about 20 km from Dumasi township and 3.8 km from the centre of the Prestea township. The Ankobra River receives mine-impacted water (Hg-contaminated) predominantly from artisanal and small-scale miners (alluvial and gold-bearing mineral ore mining) operating along the banks of the river and within the catchment area. A survey contacted during the study revealed that no LSGM company discharges its residual CN containing effluent upstream or mid-stream the Ankobra River, and also no processing centre engaged in cyanidation of Hg-contaminated tailings operates close to the Ankobra River within the catchment area of the Ankobra town (samples from the Ankobra River were collected from this town). This made the Ankobra River flowing through the Ankobra Township a probable Hg-contaminated non-cyanide aquatic ecosystem. This accounted for the selection of the Ankobra River along the Ankobra Township stretch. A map of the study area showing the sampling locations is presented in Fig 3.1.

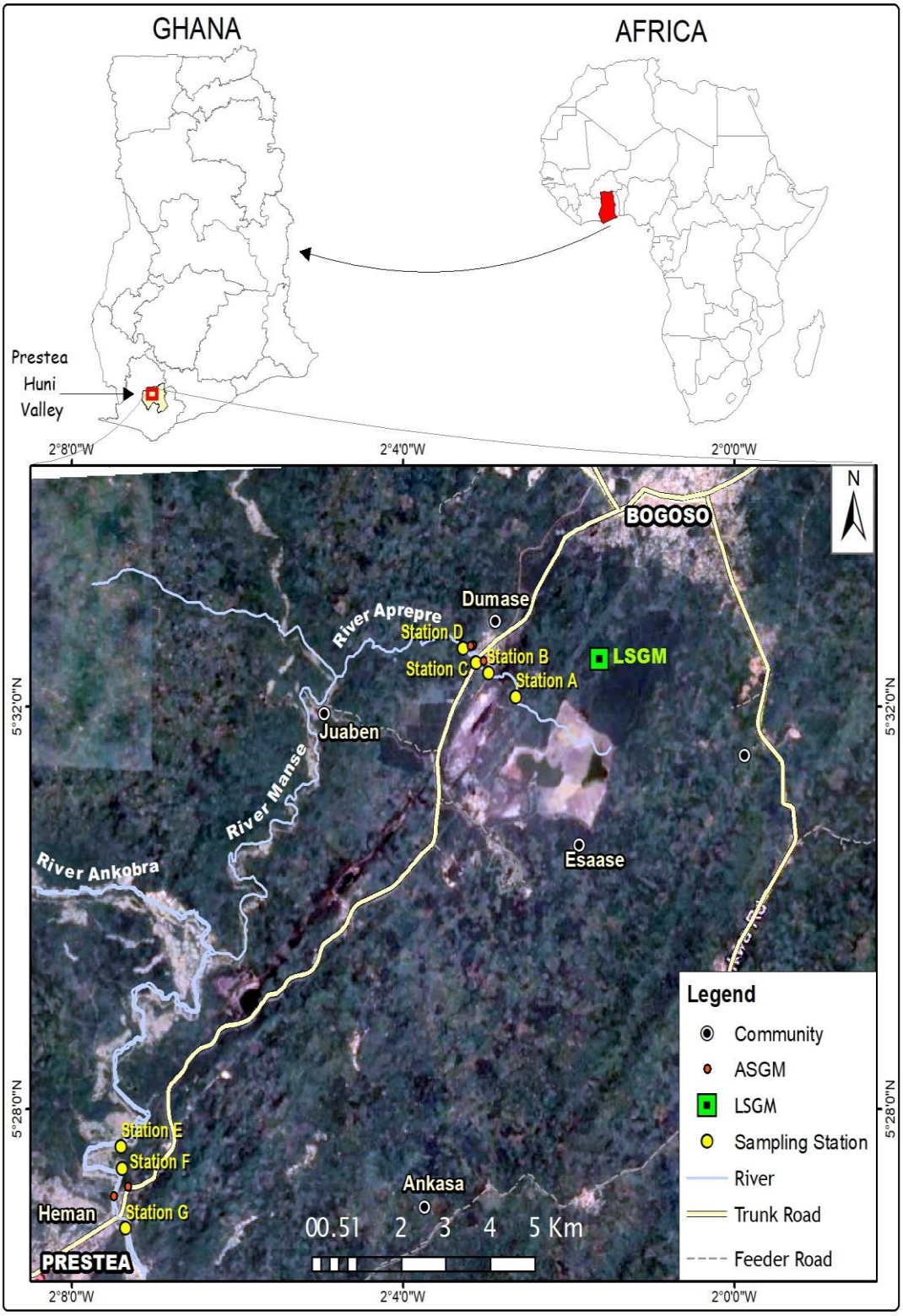


Figure 3.1: Map of the study area showing sampling locations

3.2 Pre-Sampling Activities

Reconnaissance study

Two reconnaissance surveys were conducted in July 2016 in order to locate ASGM sites and LSGM companies in southwestern Ghana. Secondly, it was to identify and select water bodies within the catchment area of gold mining activities to be used for the study. Also the survey was to gather initial locational information regarding land use within the selected gold mining sites. Lastly it was to ascertain equipment/implements and other logistics that would be required for sampling.

Permission to conduct research

Permission was sought from the Prestea Huni valley district, Mineral Commission, Environmental Protection Agency, Chiefs and Opinion Leaders in the various communities used for the study. The study commenced after the permission was granted in June 2016.

3.3 Sampling

3.3.1 Cleaning of sampling containers

Teflons and high purity polyethylene containers were used for sampling water and sediment respectively. Sampling bottles were handled with extreme cautions to avoid contamination or losses of Hg. Teflons were soaked in 50% hot HNO_3 for 48 h after being thoroughly rinsed with distilled deionized H_2O (Milli pore). The bottles were filled with 10% HCl for 24 hr. Finally, they were rinsed and filled with 1% HCl that was emptied just before sampling (Horvat et al., 1993b). The polyethylene containers were soaked in 10% HNO_3 for seven days and then rinsed thoroughly with double distilled water. They were subsequently soaked in 10% HCl before use (Mahapatra et al., 2001; Bank 2012).

3.3.2 Description of sampling locations for water, sediments, fish and soil samples

Samples investigated for the study were: water, sediment, soil and fish.

Water sampling

Water samples were collected from the Aprepre River and the Ankobra River. The water bodies were divided into three main zones (up-stream, mid-stream and down-stream) within the study community where it drains. Seven (7) sampling stations were located: four stations (Station A to Station D) from the Aprepre River and three stations (Station E to Station G) from the Ankobra River. The choice of the sampling stations was due to the bulk of human activities (mainly gold mining) happening at the banks and/or within these water bodies and their effect on the aquatic environment. At each station, a minimum of three (3) samples were taken at random at the sediment-water interface into a 500 mL Teflon [polytetrafluoroethylene (PTFE)] bottles. However, more samples were collected in suspected hot spot. A total of 28 water samples were collected. Detailed

descriptions of the sampling locations are presented in Table 3.1. Sediments were sampled at the same sampling stations where water samples were collected. About 57 sediment samples were collected in all, with a minimum of four samples taken at each sampling station.

Fish sampling

Fishes were sampled from two locations: Station D from the Aprepre River and Station G from the Ankobra River at Prestea. Thirty-three (33) fishes were caught, of which twenty-one (21) were from the Aprepre River and twelve (12) from the Ankobra River.

Soil sampling

Soils samples were collected from various parts of the study area. Fourteen (14) sampling sites were located. Eleven (11) sites were within Bogoso and Prestea environs. The three (3) remaining sites were located in gardens in Takoradi, Cape Coast and Accra. Soil samples were collected from various active ASGM operation sites, amalgamation and amalgamated Hg roasting sites (gold refinery spot), suspected uncontaminated sites in the study area (farm lands for Hg background levels in the study area) and other samples were taken from gardens in Takoradi, Cape Coast and Accra (non-mining cities to compare with the background levels in the mining areas). A detailed description of the sampling location for the soil samples is presented in Table 3.2.

Table 3.1: Description of sampling stations for water, sediment and fish samples.

River	Sampling Site	Types of samples collected	Description of Sampling Location
Aprepre	Station A (Reference Station)	Water Sediment	Up-stream of the Aprepre River in Dumasi. ASGM activities at this station involves only cyanidation of Au-rich Hg-contaminated tailings. No amalgamation. Reports of sporadic CN spillage/leakage from nearby LSGM company.
	Station B	Water Sediment	Mid-stream of the Aprepre River. ASGM operations site (situated at the river bank). Both amalgamation and cyanidation of Au-rich Hg-contaminated tailings were in operation.
	Station C	Water Sediment	Between mid- and downstream of the Aprepre River (about 100 meters from Station B)
	Station D	Water Sediment Fish	Downstream of Aprepre. Two ASGM operation sites situated close to the bank. About 300 meters from station B.
[ε]**Ankobra**	Station E	Water Sediment	Upstream of the Ankobra River at Himan (a suburb of Prestea). Evidence of ASGM activities (alluvial gold mining involving only amalgamation).
	Station F	Water Sediment	About 300 meters down-stream of the Ankobra River. Evidence of ASGM activities (alluvial gold mining involving only amalgamation).
	Station G	Water Sediment Fish	Down-stream of the Ankobra River, (at Ankobra* a suburb of Prestea). ASGM operation site situated at the river bank. Evidence of alluvial gold mining using Hg. Close to the Bogoso-Prestea main road.

*Ankobra, is a suburb/settlement of/in Prestea; named after the Ankobra River

[ε]ASGM activities along the sections of the Ankobra River used for the study were amalgamation based. The resultant Hg-contaminated tailings are sold to processing centres several kilometres away. Accordingly, there is no processing centre within the catchment area of the Ankobra River used for the study.

Table 3.2: Description of sampling location for soil samples.

Sampling Point	Description of Sampling Location
P1	ASGM operation site at Nankaba in Prestea
P1a	100 m away from P1
P2	ASGM operation site at Ankobra in Prestea, close to Station G
P3	Commercial amalgam burning site at Ankobra in Prestea
P3a	100 m away from P3
P4	Himan in Prestea about 500 m from P2
Q1	ASGM operation site at Dumasi, about 100 m to Station D
Q2	ASGM operation site at Dumasi, 50 m to Station B
Q2a	50 m away from Q2
Q3	Farmland at Dumasi, about 150 m from Q1 and 50 m from Station D
AK	Farm in Ayesukrom village in the study district, 3 km from Dumasi mining sites
TK	Garden in Takoradi, about 121 km from the study area
CC	Garden in Cape Coast, about 149 km from the study area
AC	Garden in Accra, about 300 km from the study area.

The exact sampling locations for all the sample matrices were noted with a Global Positioning System (GPS) and recorded on a sample location map (Fig. 3.1). The sampling protocol for each sample type is described below.

Water

Sampling of surface water samples was performed using "Clean hand-dirty hands sampling protocol" (USEPA Method 1669, 1996). Arm-length plastic gloves were worn. Samples were collected into 500 mL acid precleaned Teflon bottles for Hg analysis. At each sample collection point, Teflon bottles were washed a number of times with water to be collected. This was followed by immersion of the bottle under the water surface (approximately 10 cm beneath the surface of the river water) with the open end of the bottle facing upstream. The sampling container was fully filled followed by acidification (2.5 mL of 0.5% HCl [high purity low Hg content]) to stabilize the Hg present (Parker and Bloom 2005; USEPA 1631 E, 2002). Sampling in the Ankobra River was done with the aid of a canoe as shown in Fig. 3.2.

Triplicate samples were collected with 500 mL pre-cleaned high purity polyethylene bottles for BOD analysis and other parameters. Sampling containers for BOD determination were filled to the brim and tightly capped to prevent air from entering. Surface water samples for cyanide analysis were collected using opaque pre-cleaned

polyethylene sampling bottles. The cyanide present was stabilized with 10 % NaOH solution.

The pH of the water samples was measured on-site using a well calibrated Accumet Portable AP6 pH meter. Temperature, conductivity, salinity and total dissolved solids were also measured on-site with the Hach Sension 5 Conductivity Meter. Other parameters measured on-site include: colour, turbidity, total suspended solids, and fluoride using the Hach DR/890 colorimeter. Dissolved oxygen and alkalinity were determined by titrimetric method on-site. The samples were placed on ice in a cooler and transported to the laboratories of the Ghana Atomic Energy Commission.

Figure 3.2: Collection of water aboard a boat upstream of the Ankobra River at Himan, Prestea.

Sediment

Sediment samples were collected at the same locations where water samples had been taken. Surficial sediment samples were collected at the upper 4 cm depth with a pre-cleaned plastic shovel on board a locally manufactured canoe (Serfor-Armah et al., 2006; Beldowski et al., 2015). Surficial sediment (top 4 cm depth) was collected because the bulk of activity affecting MeHg production and transport occurred at the top 4cm. Samples were transferred into a precleaned tightly covered plastic container. No preservatives were added to the samples (Serfor-Armah et al., 2006). The bottles containing sediment samples were then placed in hermetically closed polyethylene bags. The bags containing the samples were stored in thermo-insulated boxes with ice packs and transported to the laboratories of the Ghana Atomic Energy Commission.

Soil

Soil samples were collected at various ASGM mining operation sites, amalgam roasting sites and farm lands. Each sample location was sub-sectioned into 0-2, 2-4, 4-6, 6-8, 8-10, 10-15 and 15-20 cm and collected into hermetically closed polyethylene bags.

Fish

Fish samples were caught randomly in the Ankobra River in Prestea and the Aprepre downstream in Dumasi by local men in the area using fish hooks as shown in Fig. 3.3.

Figure 3.3: Sampling of fish at Prestea-Ankobra by the locals.

Samples obtained were species known for consumption. The fish catches were stored in hermetically closed containers (polyethylene) and transported in a thermally insulated polyethylene receptacle. The fish samples were later stored in freezers with subsequent identification by personnel of the Department of Marine and Fisheries Sciences, University of Ghana.

3.3.3 Preparation of samples for analysis

Apparatus

A Thermo Scientific Super Modulyo Freeze Dryer (Thermo Electron Corporation) was used for lyophilization of sediment, soil and fish samples. Fritsch Pulverisette 2 was used for homogenization of sediment and soil samples. A British Standard Laboratory Metric Test Sieve (ISO 565 BS 410 ISO 3310-1) of 100 µm size mesh material (Endocotts Limited, London, England) was used for sieving lyophilized soil and sediment samples.

Water samples

Water samples were kept in a refrigerator at 4 ˚C prior to shipment by courier to the Hg laboratory of the Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia.

Sediment/soil

Macro-organisms, organic debris, stones and shelly fragments were gloved hand-picked from the sediment and soil samples (Horvat et al., 2003b; Tulasi et al., 2013; Reis et al., 2014). The samples were then placed in pre-cleaned plastic bowls and kept frozen at -20 °C. The frozen samples were then placed on the stacks of a freeze dryer and lyophilized at -40 °C and 120 mbar (Adotey et al., 2011).

After lyophilization, the samples were homogenized by milling and sieving at a pore size of 100 µm. During the entire sample preparation process, special precautions were taken to avoid cross-contamination of samples, including the sequence of sample treatment. Therefore homogenization started with the control samples, followed by less contaminated and continued with more contaminated samples (Horvat et al., 2003b).

The homogenizer and sieve were thoroughly cleaned after each sample and dried in an oven at a temperature of 40 °C for about 30 min. For each sample, the first aliquot grounded was discarded, and then the bulk of the samples was grounded (Horvat et al., 2003b). The lyophilized homogenate samples were then stored in hermetically closed polyethylene bags and kept in a freezer until shipment by courier to the Laboratories of the Department of Environmental Sciences, Jožef Stefan Institute (JSI), Ljubljana, Slovenia.

Fish

The fish samples were taken to the Department of Marine and Fisheries Sciences, University of Ghana, for identification. Nine different fish species were identified. The length, width and weight of the fishes were taken after defrosting in the laboratory (Voegborlo & Adimado, 2010). The inedible portions (intestines, fins, scales) of the fish were discarded. The edible portions were dissected and frozen at -20 °C and lyophilized at -40 °C for 48 hr. The lyophilized samples were homogenized by milling in a laboratory blender with Teflon coated parts.

3.4 Analysis of Samples

3.4.1 General scheme of analysis

The general experimental design for this study is presented in Fig 3.4. The general scheme for determination of THg and MeHg in the samples is shown in Fig 3.5 and Fig 3.6, respectively.

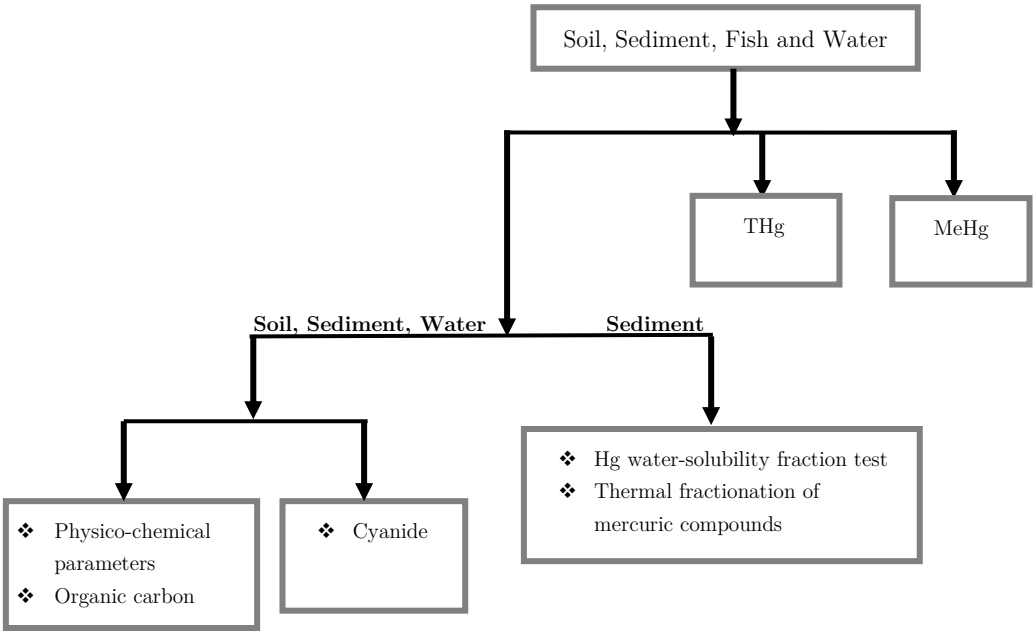


Figure 3.4: The general experimental design.

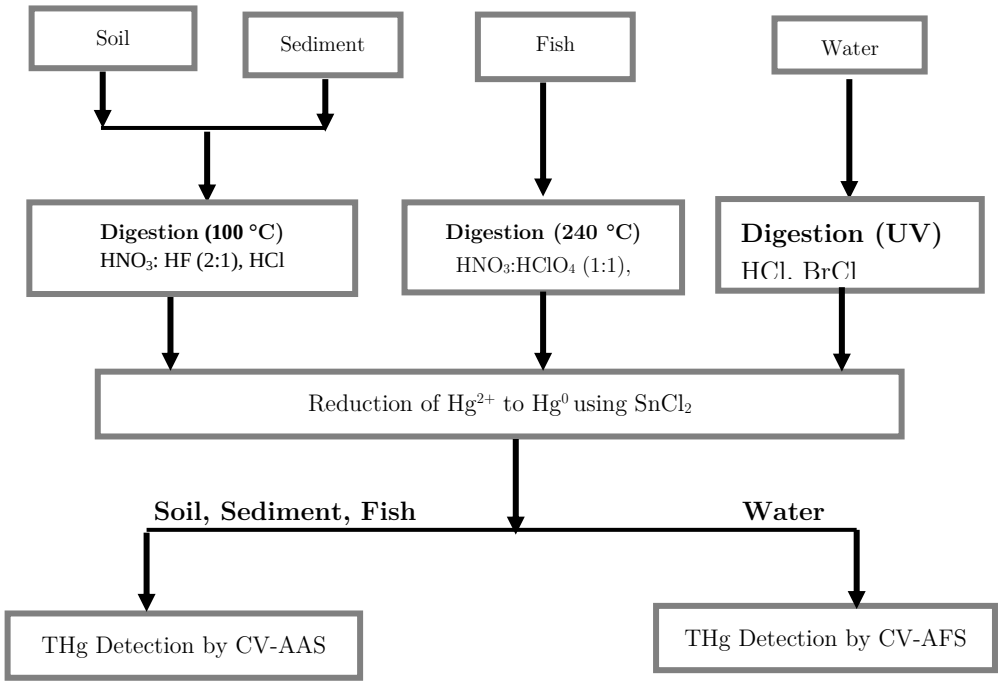


Figure 3.5: General scheme for THg determination.

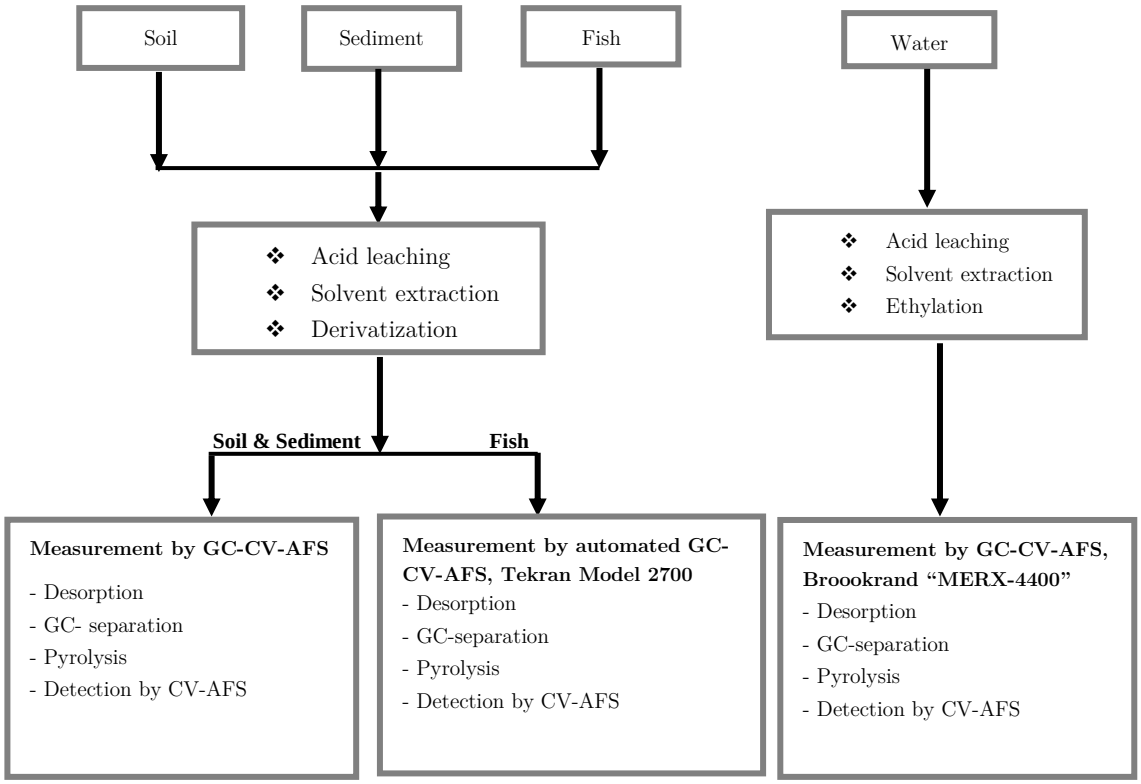


Figure 3.6**:** General scheme for MeHg determination.

3.4.2 Cleaning of laboratory glassware and Teflon

Teflons and glass vessels were soaked overnight in 2 percent Micro-90 detergent solution separately. The glass vessels were rinsed thoroughly first with tap H_2O, then by 10% $KMnO_4$ solution. They were rinsed again with tap water until the colour of KMnO4 solution was invisible. This was followed by 12% $NH_2OH \bullet HCl$ to neutralize excess $KMnO_4$; subsequently, Milli-Q H_2O was used to rinse the vessels thoroughly. The glass vessels were filled with 1% HCl solution and stored in a mercury-free storage facility. Vials were emptied just before being used for sample processing and dried at 60 °C in an oven.

Teflons were thoroughly rinsed with tap H_2O, and it was then placed in 50% (v/v) HNO_3 solution and heated at 90 °C for 48 hr. After being thoroughly rinsed with Milli-Q water, Teflons were transferred and soaked in 10% (v/v) HCl solution for 24 hr at room temperature. The Teflons were thoroughly rinsed again with Milli-Q H_2O, filled with 1% HCl and kept in sealed Hg-free plastic bags (Horvat et al., 1993b). Teflons were emptied at the point of usage and dried at 60 °C in an oven.

The 30 mL amber glass vials for THg analysis in water were washed with tap H_2O, followed by Milli-Q. They were heated in an oven at 500 °C for 5 hours. The vials were then placed in a clean paper box and kept in a mercury-free storage environment.

3.4.3 Analysis of THg

3.4.3.1 Chemicals and reagents for THg analysis

The chemicals used were of analytical grade: nitric acid (HNO_3, 65 %), hydrochloric acid (HCl, 37 %), hydrofluoric acid (HF, 40%), boric acid (H_3BO_3, 99.5-100.5%), sulfuric acid (H_2SO_4, 95-97 %), perchloric acid ($HClO_4$, 70%), Tin (II) chloride dehydrate ($SnCl_2$. $2H_2O$, 96.0-101.0 %), potassium bromide (KBr, ≥ 99.5%), potassium bromate ($KBrO_3$, 99.9%), hydroxylamine hydrochloride ($NH_2OH \bullet HCl$, 99%), hydrochloric acid, suprapur (HCl, 30%), and were all obtained from Merck, Darmstadt, Germany.

Stock solutions of standard inorganic mercury (1 µg/mL and 5 µg/mL as Hg^{2+} in 5 % [v/v] HNO_3) were prepared from NIST SRM 3133 (Mercury Standard Solution, 10 g/mL).

Working solutions were freshly prepared by diluting an appropriate aliquot of the stock solution through intermediate solutions using blank solution. 5% H_3BO_3 was used for neutralizing excess HF during acid digestion for total mercury determination, and was prepared by dissolving an appropriate amount of H_3BO_3 in deionized-distilled water (DDW).

Stannous chloride ($SnCl_2$) solution (10% w/v in 10% HCl) was prepared by dissolving the appropriate amount of $SnCl_2 \bullet 2H_2O$ in 10 % HCl. The solution was purged with nitrogen gas at a flow rate of 60 mL/min for an hour to get rid of any elemental mercury. This was used for reduction of Hg^{2+} to Hg^0 in soil, sediment and fish samples for THg analysis on a semi-automated cold vapor atomic absorption spectrophotometry mercury analyzer – CVAAS (Sanso Seisakusho Co., Ltd, Tokyo, Japan) model HG-201. Stannous chloride solution (20% w/v in 10% HCl) was prepared by dissolving the appropriate amount of $SnCl_2 \bullet 2H_2O$ in 10 % HCl. The solution was aerated with nitrogen gas at a flow rate of 60 mL/min for an hour to get rid of any elemental mercury. This was used to reduce Hg^{2+} to Hg^0 in water samples for THg determination on TEKRAN, model 2600 CVAFS Hg Analyzer.

Bromine monochloride (BrCl) solution was prepared by dissolving the appropriate amount of KBr and $KBrO_3$ in 20 mL of DDW. Followed by addition of 100 mL of HCl. Oxidation of all the Hg species in the water samples was done with the BrCl solution.

Hydroxylamine hydrochloride (30% w/v) was prepared by dissolving the appropriate amount of $NH_2OH \bullet HCl$ in 100 mL milli-Q water. The solution was purified by adding 0.1 mL $SnCl_2$ solution and aerated with Hg free N_2 gas (60 mL/min., for 60 min.). This solution was used to neutralize excess halogen in the water sample after oxidation.

3.4.3.2 Apparatus for THg determination

Semi-automated mercury analyser (Sanso Seisakusho Co., Ltd, Tokyo, Japan) model HG-201 was used for THg measurement in soil, sediment and fish samples.

Flow-Injection, Cold Vapour Atomic Fluorescence Spectrometer (CV-AFS), Tekran model 2600 was used for THg measurement in H_2O samples.

3.4.3.3 THg determination in soil, sediment and fish samples

Hg species in the samples were oxidized by acids digestion and detected by the cold vapour atomic absorption spectrometer (CV AAS) which is based on the reduction of the oxidized mercury (ionic mercury) in the solution to the elemental state and its

subsequent transfer into the absorption cell of the CV AAS for measurement at a wavelength of 253.7 nm. The description of the measurement system was based on the open-air flow system which requires clean ambient air as a carrier gas which makes the apparatus easy to operate (Akagi 1997). Fig 3.7 shows a flow chart for the determination of THg in soil, sediment and fish.

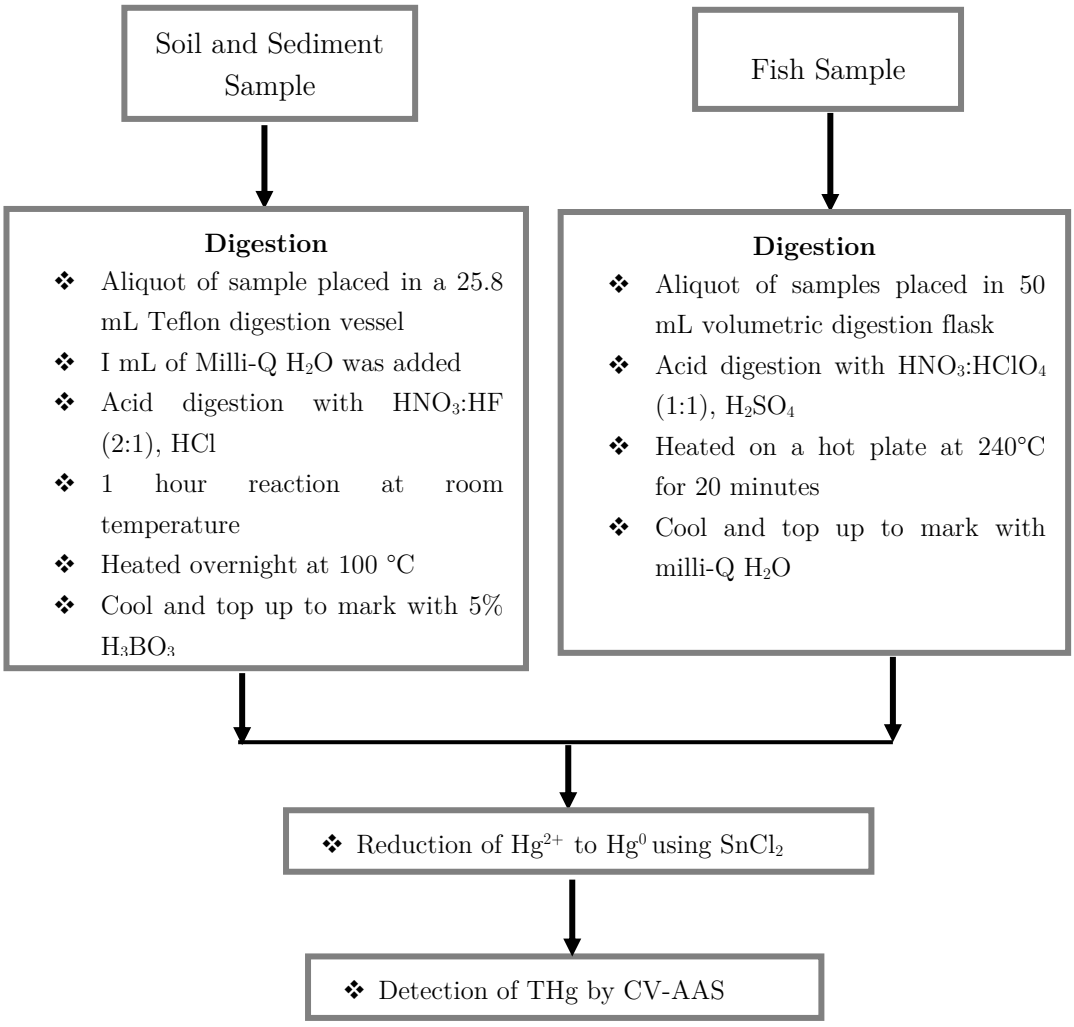


Figure 3.7**:** Schematic flow chart for determination of THg in soil, sediment and fish.

Instrumentation

Cold vapor atomic absorption spectrometric (CVAAS) technique [circulation-open air flow system] was used in THg determination in soil, sediment and fish. A semi-automated mercury analyzer (Sanso Seisakusho Co., Ltd, Tokyo, Japan) model HG-201 was used. Prior to the introduction of the sample into the CVAAS equipment, samples were acid digested to convert all the Hg species into $Hg^{2+.}$ After the digestion, the samples were introduced into the equipment for reduction of Hg^{2+} ions in the sample test solution to elemental mercury vapour (Hg^0) with stannous chloride ($SnCl_2$). The vapour generated was introduced into the photo-absorption cell for the measurement of absorbance at 253.7

nm wavelength of Hg. The apparatus is a closed system that consists of an air circulation pump, a reaction vessel for reduction, $SnCl_2$ dispenser, acid gas trap, moisture trap (ice bath), and a 4-way cock. During its operation, the elemental vapor generated by the addition of stannous chloride is circulated through the 4-way cock at a flow rate of 1-1.5 L/min. for 30 seconds to homogenize the concentration in the gas phase. The 4-way cock is rotated by 90° to introduce the gas phase into the photo-absorption cell all at once. The measurement is completed within one minute per sample with this apparatus, which can measure even 0.1 ng of mercury with high accuracy and precision. Signals were obtained on a strip chart recorder (Akagi 1997; Voegborlo and Akagi, 2007). A schematic diagram of the system is shown in Fig. 3.8.

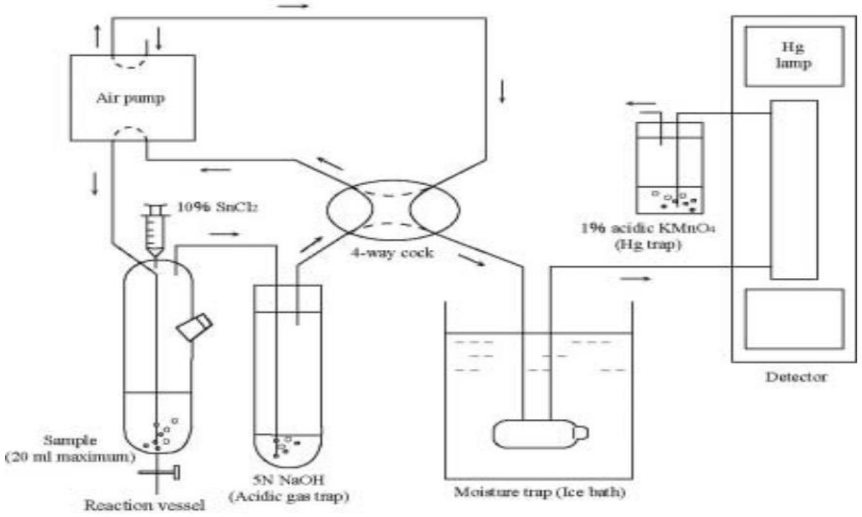


Figure 3.8: Schematic diagram of Reduction/CVAAS [Circulation-Open Air Flow System] (Akagi 1997).

Digestion of soil/sediment samples

About 200 mg of freeze-dried sediment and soil samples were weighed directly in a Teflon digestion vessel and a mixture of 5 mL HNO_3/HF (2:1) and 1 mL of HCl was added. The Teflon digestion vessel was tightly closed and the mixture was allowed to react at room temperature for an hour. Digestion was completed by heating the mixture on a hot plate at 100 °C overnight. The digestate was cooled and diluted with 5% H_3BO_3 to the 25.8 mL mark. The following were subjected to the same treatment (for quality control purposes): 30 and 20 µL of standard Hg solution (1 and 5 µg/mL respectively), a blank, and certified reference materials of matrix and concentration match (Horvat, et al., 1986, 1991; Kocman et al., 2005).

Digestion of fish samples

The fish samples were acid digested for total mercury determination by an open flask procedure (Akagi and Nishimura, 1991). About 200 mg of lyophilized homogenized fish samples were weighed directly into 50 mL volumetric digestion flask. One (1) mL of

Milli-Q H_2O was added. A mixture of 2 mL $HNO_3/HClO_4$ (1:1) and 5 mL H_2SO_4 was then added in turn and digested on an aluminum block heater at 240 °C for 20 minutes. The digest was left to cool and topped up with Milli-Q to the 50 mL graduated mark. Blank, standard working solutions of 4 ng/mL and 10 ng/mL prepared from 5 µg/mL stock NIST 3133 standard Hg solution, and certified reference materials of matrix and concentration match were subjected to the same treatment.

Measurement of THg in soil/sediment and fish samples

Determination of THg in the digest was carried out by a semi-automated cold vapor atomic absorption spectrophotometry mercury analyzer – CVAAS (Sanso Seisakusho Co., Ltd, Tokyo, Japan) Model HG-201 (Horvat et al., 1991). A photograph of the automated mercury analyzer (CV AAS) Model HG-201 is shown in Fig. 3.9. Aliquot of the digest was pipetted into the reaction vessel and 0.5 mL $SnCl_2 \cdot 2H_2O$ (10 % (w/v) in HCl (10%) was added from the dispenser for the reduction of Hg^{2+} to Hg^0 [Eq. 3.1]. After reduction, the elemental mercury vapor generated was circulated through the 4-way stop cock to homogenize the concentration in the gas phase. After 30 s, the 4-way cock was rotated through 90^0 and the mercury vapour was swept into the photo-absorption cell. Signals were obtained on a Strip Chart Recorder as peaks (Horvat et al., 1991; Voegborlo and Akagi, 2007).

$$Hg^{2+} + Sn^{2+} \rightarrow Sn^{4+} + Hg^0 \tag{3.1}$$

Figure 3.9: CVAAS Semi-automated Hg analyzer (Circulation-Open Air Flow System).

Calculation of analytical results

The peak heights (mm) obtained after measurement of fixed volumes V in mL of each of the blank, the standard, and the sample test solutions (or their diluted solutions) for the total mercury analysis are labeled Pblank (blank), Pstd (standard), and Ps (sample),

respectively. The total mercury concentration in the sample is calculated with the following formula [Eq. 3.2]:

$$C_{sample} = \left(\frac{P_{sample} - P_{blank}}{P_{std} - P_{blank}} \right) \cdot F. \frac{C_{std}}{m_{sample}} \tag{3.2}$$

C_{sample} - Concentration of Hg in the sample (ng/g)
C_{std} - Concentration of Hg in the standard (ng/mL)
P_{sample} - Peak height in mm for the digested sample
P_{std} - Peak height in mm for the standard solution
P_{blank} - Peak height in mm of the blank test solution
F - Dilution factor
M_{Sample} - Mass of the sample in g

Quality control (QC)

Certified Reference Materials (BCR-580, BCR-320R, ERM-CC-141) for mercury of matrix match, at concentrations typical for the concentration range measured in the sample were analysed. Each sample was analyzed in duplicates. The sample was reanalysed if the parallel analysis differed for more than 10%. In each set of analysis, two blanks and duplicates of the quality control material (reference material) were analyzed and quality control charts (Schewart) prepared. Analysis of the blanks was made to avoid uncontrolled contamination.

The Limit of Detection (LOD) is the smallest concentration from which it is possible to deduce the presence of the analyte. It was calculated as three times the standard deviation (σ) of the blank: $LOD = \sigma * 3$. For a given mass or volume of the sample, the LOD is calculated as [Eq. 3.3]:

$$LOD = \frac{\sigma * 3}{V_{sample}(m_{sample})} \tag{3.3}$$

The Limit of Quantification (LOQ) was calculated as five times the LOD, $LOQ = 5 * (LOD)$. For a given mass or the volume of the sample, the LOQ is calculated as [Eq. 3.4]:

$$LOQ = \frac{5 * (LOD)}{V_{sample}(m_{sample})} \tag{3.4}$$

3.4.3.4 THg determination in water samples

Instrumentation

Analysis for THg in H_2O was done by Cold Vapor Atomic Fluorescence Spectrometric (CVAFS) technique. A flow-based automated mercury analysis system (Tekran Model 2600) which provide the implementation of US EPA 1631E (2002) was used. The instrument consists of an autosampler (Tekran Model 2621), peristaltic pump (Tekran Model 2610), gas liquid separator and Dual-Stage gold amalgamation system for preconcentration of mercury, an inbuilt two heating coils for desorption of Hg^0 from gold traps. Detection based on atomic fluorescence technique. Detector used was the photomultiplier tube. Data processing, pump flow, and autosampler were controlled by the Tek-MDS-2 software. During its operation, the elemental vapor generated by the addition of stannous chloride is separated from the solution by the vapour/liquid separator and swept onto the gold trap using N_2 gas as the carrier gas. Hg^0 is thermally desorbed from the gold trap into an inert gas stream (Argon gas) that carries the released Hg^0 to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the Atom cell of a cold-vapour atomic fluorescence spectrometer (CVAFS) for detection. Here the Hg^0 absorbed UV light at a wavelength 253.7 nm, and excited to a higher energy level. The fluorescence radiation emitted due to de-excitation of the excited atom is detected by the photomultiplier tube of the AFS. A schematic diagram of the Flow Injection, Cold Vapour Atomic Fluorescence Spectrometer (CVAFS) System is presented in Fig. 3.10.

The intensity of the fluorescence radiation increases with increasing atom concentration, providing the basis for its quantitative determination. Thus, the fluorescence intensity is directly proportional to the concentration of mercury in the sample solution. The concentration of mercury present in the sample is determined quantitatively by using calibration curve of response versus concentration. Fig. 3.11 shows Flow Injection, Cold Vapor Atomic Fluorescence Spectrometer (Tekran Model 2600).

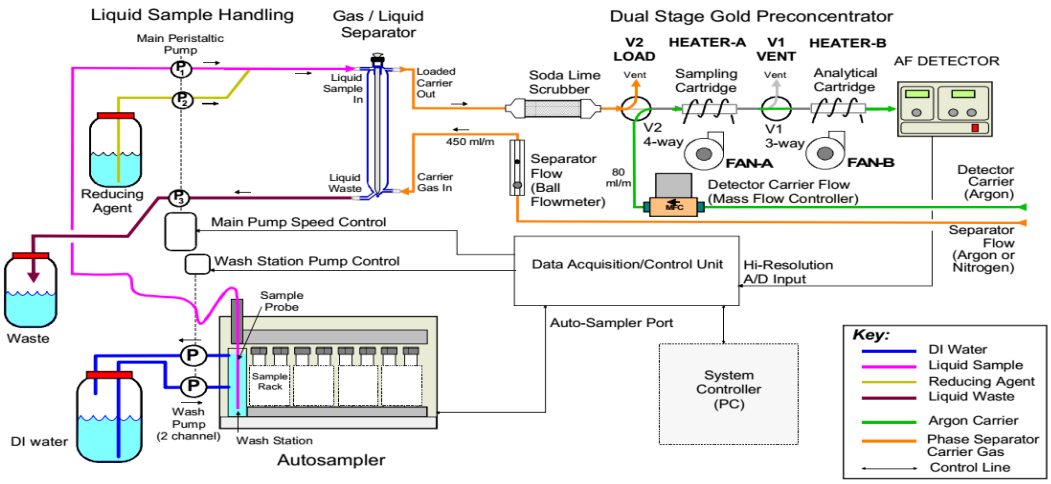


Figure 3.10: Schematic Diagram of the Flow Injection, Cold Vapour Atomic Fluorescence Spectrometer (CVAFS) System (USEPA 1631E, 2002).

Digestion procedure

THg in H_2O samples was determined following the procedure described by US EPA 1631E. About 39.4 mL of H_2O sample was weighed into a digestion bottle. The water sample was digested by the addition of 0.4 mL of HCl and oxidation of all the Hg species by BrCl [Eq. 3.5], and sample solution exposure to Ultra Violet light overnight. To neutralize excess halogens, 30% $NH_2OH \cdot HCl$ (40 µm) was added. Seven (7) sample blanks and calibration standards of concentrations 0.5, 1.0, 5, 10, 15, 20, and 25 ng/L prepared from 1 µg/L NIST 3144 were prepared in the same way as the samples.

$$Hg + 2Brcl \rightarrow Hg(Br)_2 + Cl_2 \tag{3.5}$$

Measurement of THg

The oxidized Hg in the samples was then reduced to gaseous elemental Hg (Hg^0) by $SnCl_2$ solution [Eq. 3.6]. The reduced Hg^0 was aerated from the aqueous sample by nitrogen gas through phase separator and pre-concentrated on the gold trap. Hg is thermally desorbed from the gold trap and detected by CV-AFS, Tekran Model 2600. During each batch of sample analyses, 3 blanks were also measured.

$$Hg^{2+} + Sn^{2+} \rightarrow Sn^{4+} + Hg^0 \tag{3.6}$$

Figure 3.11: Flow Injection, Cold Vapor Atomic Fluorescence Spectrometer (Tekran Model 2600).

Quality assurance and quality control

Analysis of blanks was critical to the reliable determination of Hg at low levels. Standard curve was created by analyzing a series of analytical standards of 0.5, 1.0, 5, 10, 15, 20 and 25 ng/L. A graph of response verse concentration of the standards was plotted and a correlation coefficient was calculated. This was automatically done by Tek-MDS-2 software. A standard curve must have a correlation coefficient greater than or equal to 0.995. Additional set of standards were analyzed to rule out operational error, when a correlation coefficient falls below 0.995. Samples with higher concentration exceeding the upper limit of the standard curve were diluted and reanalyzed. Ongoing Precision and Recovery (OPR) using a working standard of concentration 5 ng/L was analyzed after every six (6) measurements. The recovery of OPR must be between 90 and 110% of 5 ng/L. Additional OPR was run if at any batch it fails to meet these criteria. Certified reference materials of matrix and concentration match were analyzed prior to and after sample analysis of each batch of samples.

3.4.4 Determination of MeHg and EtHg species

Chemicals and reagents

The chemicals used were of analytical grade: nitric acid (HNO_3, 65 %), hydrochloric acid (HCl, 37 %), sulfuric acid (H_2SO_4, 95-97 %), dichloromethane (CH_2Cl_2, 99.8%), Potassium hydroxide (KOH, 85%), glacial acetic acid (CH_3COOH), and were all obtained from Merck, Darmstadt, Germany. Individual stock solutions of standard organomercury chloride (1 µg/mL as Hg) were prepared by dissolving appropriate amounts of methylmercury chloride (CH_3HgCl, ≥ 98 %) and ethylmercury chloride (C_2H_5HgCl, ≥ 97%) in 5% (v/v) HNO_3 respectively. The organomercury compounds were purchased from Merck, Darmstadt, Germany. Mixed working standards (1 ng/mL) of MeHg and EtHg were prepared by appropriate dilution of the stock standard solutions (1 µg/mL) in 1% HNO_3 daily.

For calibration on Tekran 2700 CV-AFS used for MeHg measurement in fish samples, standards of 500 pg/mL were prepared by dilution of stock standard solution (1 µg/mL of MeHgCl) in 0.2 and 0.3 % (v/v) trace metal grade hydrochloric acid (HCl) and ultrapure glacial acetic acid (CH_3COOH), respectively, daily. 10 pg/mL standard was prepared by appropriate dilution of 500 pg/mL also in 0.2 and 0.3 % of HCl and CH_3COOH respectively. Calibration standards of 0.02 ppt, 0.05 ppt, 0.1 ppt, 0.5 ppt, 1.0 ppt, 2.0 ppt and 4.0 ppt were prepared by dilutions of 10 pg/mL (for 0.02 ppt, 0.05 ppt, 0.1 ppt) and 500 pg/mL (for 0.5 ppt, 1.0 ppt, 2.0 ppt and 4.0 ppt).

1 M Copper (II) Sulfate pentahydrate ($CuO_4S.5H_2O$) solution was used to displace methylmercury from its strong, natural inorganic and organic sulphur bonds. It also prevents the decomposition of dimethylmercury if present. This was prepared by dissolving appropriate amounts of $CuO_4S.5H_2O$ (99.0-100.5%, Merck, Darmstadt, Germany) in distilled-deionized water (DDW). This solution was always prepared freshly. 5% (v/v) sulphuric acid (H_2SO_4) solution was prepared in DDW. This solution was used to acidify the extraction solution.

The free methylmercury was converted to bromide salt by the addition of excess 18 % (w/v) potassium bromide to a solution strongly acidified with sulfuric acid (pH > 0.5). This was prepared by dissolving appropriate amounts of KBr (99.5%, Merck, Darmstadt, Germany) in DDW.

Freshly prepared 1% (w/v) Sodium (tetra-n-propyl) borate [$NaB(C_3H_7)_4$] solutions were used as a derivatization reagent. This reagent (propylation reagent) converts the ionic mercury species to nonpolar mercury species which is more volatile for gas chromatographic separation. This was prepared by emptying 1g of the $NaB(C_3H_7)_4$, (99 % ABCR Gmbh & Co) into 1% potassium hydroxide (KOH, 85.0 %, Merck, Darmstadt, Germany). This was used in MeHg and EtHg determination in soil and sediment samples.

1% (w/v) ethylating reagent of sodium tetraethylborate ([$NaB(C_2H_5)_4$], 99% ABCR Gmbh & Co) was prepared by dissolving 1 g of sodium tetraethylborate into 1% w/v potassium hydroxide. This reagent was used in MeHg determination in fish samples.

Derivatization reagents (propylation and ethylation reagents) were stored in the freezer and were mostly thawed just prior to use. Derivatization reagent should not be used if they show any discolorization. The frozen reagent has been proven to be stable for several months if not thawed.

The pH of the extracted organomercury compounds from the soil and sediment samples, and the organomercury mixed standards of methyl and ethyl mercury were adjusted to 4.2 prior to derivatization (propylation) of Hg species using 1M sodium acetate buffer solution. This was prepared by dissolving appropriate amounts of the tri-sodium citrate dihydrate salts ($C_6H_5Na_3O_7 \bullet 2H_2O$, 99.0-101.0%, Merck, Darmstadt, Germany) with DDW and appropriate amount of crystalline citric acid monohydrate ($C_6H_8O_7 \bullet H_2O$).

2 M sodium acetate buffer was also prepared for adjusting the pH of extracted monomethyl mercury from fish samples, as well as MeHg standards prior to ethylation of Hg species in extractant and standards.

Standard solutions and all other solutions were prepared with ultra-pure water (resistivity, 18.2 MΩ cm) obtained from a Milli-Q purification system (Millipore Co., Bedford, MA, USA).

3.4.4.1 MeHg and EtHg determination in soil and sediment

The organomercury species (MeHg and EtHg) were determined after acid leaching and extraction into organic phase, and back extraction into water phase, derivatization by aqueous propylation, followed by pre-concentration by purging and trapping onto Tenax, and detected after heat desorption and GC- separation by CVAFS detector (Horvat et al., 1993a; Liang et al., 1994). This procedure used by the Jožef Stefan Institute (JSI), Slovenia, complies with US EPA Method 1630. A scheme for the determination of the organo-Hg species is presented in Fig 3.12.

Instrumentation

Measurement of organo-mercury species (MeHg and EtHg) after extraction from the matrix combined the following main steps: **(a)** aqueous phase derivatization by $NaBPr_4$ (propylation); **(b)** pre-concentration of derivatized species on tenax trap; **(c)** chromatographic separation by gas chromatography-thermal desorption **(d)** detection by Atomic Fluorescence Spectroscopy; **(e)** signal processing by a desktop computer equipped with mercury Guru System Software. This is based on the implementation of USEPA 1630. The set-up consist of a 50 mL Teflon derivatization and purge vessel (bubbler),

coiled 1 mm diameter nickel-chrome heating wires for desorption and pyrolysis. multifunction heater controller, (Model GPC 145), an 'in-house' constructed Gas Chromatographic Column (60 cm X 4 mm i.d.), which was packed with OV-3 (15%) on chromosome W, AW, DMCS substrate at 80 °C in a Kambic laboratory oven, Model ST-45E. The GC column is coupled to an Atomic Fluorescence Spectrophotometer (AFS), Brooks Rand model III (Horvat et al., 1993a; Liang et al., 1994). All the connections between components are made with Teflon tubing and Omnifit Teflon variable-bore connectors (two and three way valve connector). The operation of this analytical set-up is described in the steps below:

(i) The Derivatization Step: Mercury species in the sample extract solution were derivatized in the Teflon reaction vessel by the addition of $NaBPr_4$ solution (Demuth and Heumann, 2001). Volatile propylated mercury species were formed. Derivatization enables transformation of ionic (polar character) mercury species in the sample solution to non polar. The nonpolar forms are more volatile than the ionic forms; and besides they do not decompose on the column (chromatographic) as a result of high heat stability.

(ii) The Purge and Trap Step: The N_2 gas valve is then opened and the flow rates regulated by a flow meter (cole parmer) at 60 mL min-1 through the sample solution in the reaction vessel. Fritted Teflon bubbler was used to obtain a maximum surface contact with the sample solution in the reaction vessel. The derivatized volatile mercury species are purged from the reaction medium with the N_2 carrier gas flow onto a tenax trap which adsorbs the mercury species.

(iii) Desorption and Gas Chromatographic Separation Step: The tenax trap is placed through the coiled nickel-chrome wire and connected to the GC column and Ar gas flow with Teflon tubing and connectors. The heater controller of the power supply is switched on and the nickel-chrome wire is heated. Mercury species is thermally desorbed at a temperature of 200 °C for 30 seconds from the tenax trap onto the isothermal GC column. Ar was used as the carrier gas for transporting the Hg species to the GC column and the detector at a flow rate of 50 mL min-1. The flow rate is regulated by a flow meter. Separation of the mercury species was performed on a temperature (80 °C) programmed GC column. The Hg species were eluted based on their weight.

(iv) Pyrolysis and Atomic Fluorescence Detection Step: The eluted Hg species from the GC column were carried in an Ar gas stream into the pyrolysis system. Which is a quartz glass tube wrapped with nickel-chrome wire and heated electrothermally by a temperature controller. The eluted Hg species were thermally decomposed at 800 °C into the atomized state. The atomized species were carried by Ar gas into the AFS and excited by absorption of UV light at a wavelength of 253.7 nm. The fluorescence radiation emitted due to de-excitation of the atoms is detected by the photomultiplier tube of the AFS. The output from the detector was quantified using a standard GC plotter/integrator for peak height measurement. The analytical signal is processed by a computer equipped with mercury Guru system software which is recorded and stored in a file.

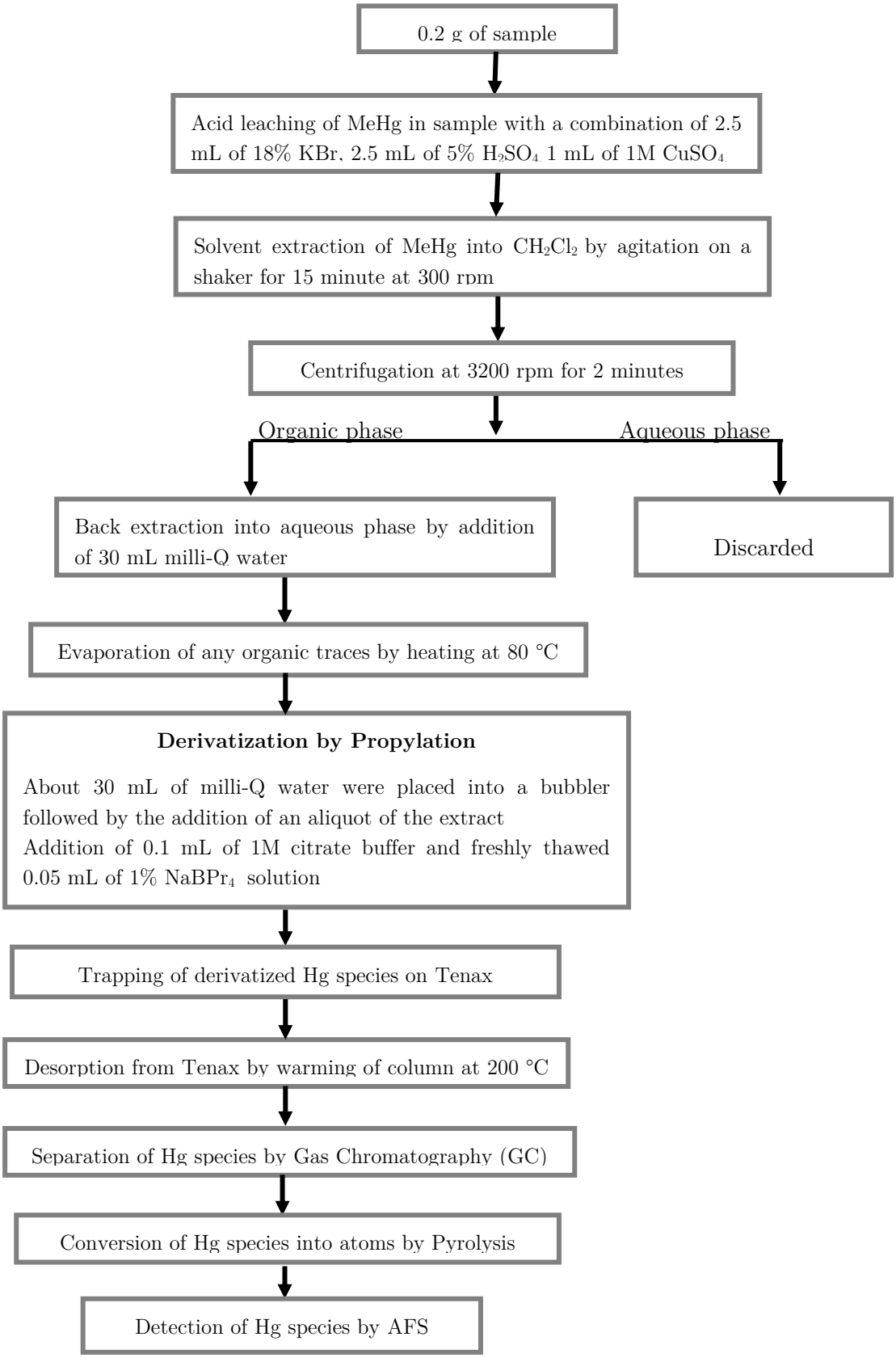


Figure 3.12**:** Scheme for the determination of MeHg and EtHg.

Extraction procedure

The samples were weighed into acid precleaned Teflon bottles and acid leached with 18% KBr, 5% H_2SO_4 and 1 M $CuSO_4$ solution (Bloom et al., 1997; Hintelman, 1999; Leemakers et al., 2003). The bottles were tightly closed and agitated over a shaker for 15 minutes at 300 revolution per minutes (rpm). $CuSO_4$ displaces the organo-mercury compound from its strong, natural inorganic and organic sulphur bonds. The free organo-mercury was converted to bromide salt. This was followed by solvent extraction of MeHgBr and EtHgBr into organic solvent [15 mL CH_2Cl_2] (Bloom et al., 1997) by shaking for 15 minutes and then back extraction into aqueous phase (30 mL milli Q water) by heating on a hot plate at 80 °C to evaporate CH_2Cl_2. The extract was purged for 5 minutes with Hg free N_2 to quantitatively remove any traces of CH_2Cl_2. The extract was stored in a freezer until analysis.

Derivatization and pre-concentration

About 50 mL derivatization reaction vessel was half filled with milli Q water and about 200 µL of the extracted sample solution was pipetted into the reaction vessel. The sample was acetate buffered to pH of 4.2. Methyl and ethyl mercury in the samples were derivatized (propylated) by the addition of 50 µL of 1% $NaBPr_4$ solution and the reaction vessel was tightly capped immediately. The mixture was allowed to react without bubbling for 15 minutes at room temperature. The propylated reaction results in the formation of propylmethylmercury (MePrHg), propylethylmercury (EtPrHg) and dipropylmercury from inorganic mercury [Eq. 3.7; 3.8; 3.9]. After the reaction period, the volatile propylated mercury species were pre-concentrated by purging from the solution onto a tenax trap (adsorbent) for 15 minutes with Hg-free, high purity nitrogen gas at a flow rate of 60 mL min^{-1}. Fig 3.13 shows a scheme for the process of mercury species derivatization and preconcentration on tenax trap. After the sample was purged, dry nitrogen was flushed for 5 minutes at a flow rate of 60 mL min^{-1} through the tenax trap to remove traces of condensed water vapour, which could cause an interference during chromatographic elution and CV-AFS detection. It was then dried from traces of water by purging again with N_2 for 5 minutes. Propylation was used as the preferred derivatization reagent to ethylation due to its ability to distinguish Hg^{2+} from EtHg (Demuth and Heumann, 2001; Rahman et al., 2014).

$$Hg^{2+} + 2NaBPr_4 \rightarrow Pr_2Hg + 2BPr_3 + 2Na^+ \quad (3.7)$$

$$MeHg^+ + NaBPr_4 \rightarrow MePrHg + BPr_3 + Na^+ \quad (3.8)$$

$$EtHg^+ + NaBPr_4 \rightarrow EtPrHg + BPr_3 + Na^+ \quad (3.9)$$

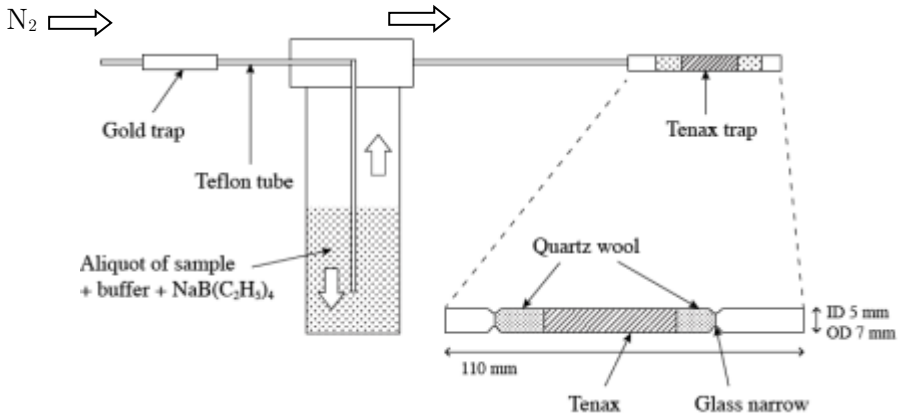


Figure 3.13: Schematic diagram illustrating the process for mercury species derivatization and preconcentration on tenax trap (Horvat et al., 1993a).

Gas chromatographic separation and detection

Mercury species on the tenax trap were thermally (200 °C) desorbed onto the GC column (isothermal) at 80 °C. Species of Hg were transformed to Hg^0 through pyrolysis (800 °C) to atomize Hg and the organo-mercury species (MeHg and EtHg) were measured by cold vapour atomic fluorescence spectrophotometer (CVAFS) detector. A scheme of desorption, GC-separation of mercury species, pyrolysis and CVAFS detection is shown in Fig 3.14. Fig 3.15 shows the analytical system of thermal desorption, GC-separation of mercury species, pyrolysis and CVAFS detection for this study.

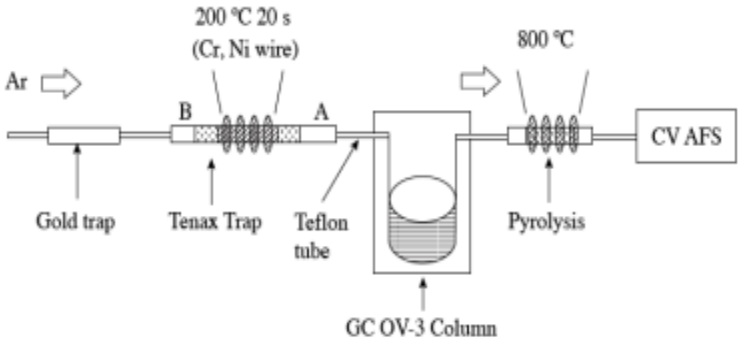


Figure 3.14: A scheme of desorption, GC-separation of mercury species, pyrolysis and CVAFS detection (Horvat et al., 1993a).

Figure 3.15: Analytical system for thermal desorption, GC-separation of mercury species, pyrolysis and CVAFS detection.

Quality control

Certified reference materials (BCR 580, IAEA 433) for mercury of matrix and concentrations matching the samples were analysed for consistent check on the validity of the analytical method, and quality control charts prepared. Each sample was prepared in parallel and each was analyzed in duplicates. The sample was reanalysed if the parallel analysis differ for more than 10%. In each set of the analysis, three blanks were analyzed. Analysis of the blanks (both method and bubbler blank) were to avoid uncontrolled contamination.
The limit of detection (LOD) was calculated as three times the standard deviation of the blank.

Calculation of the analytical results

The peak heights (mm) obtained after measurement of volumes V in mL of each of the blanks, the standard, and the sample test solutions (or their diluted solutions) for the methyl and ethyl mercury analysis are labeled P_{sblank} (sample blank), P_{blank} (Bubbler blank), P_{std} (standard), and P_s (sample), respectively. The organo-mercury concentration in the sample is calculated with the following formula [3.10]:

$$C_{sample} = \left(\frac{P_{sample} - P_{sblank}}{P_{std} - P_{blank}} \right) \cdot F . \frac{C_{std}}{m_{sample}} \qquad (3.10)$$

C_{sample} - Concentration of Hg in the sample (ng/g)
C_{std} - Mass concentration of Hg in the standard (pg)
P_{sample} - Peak height in mm for the extracted sample
P_{std} - Peak height in mm for the standard solution
P_{blank} - Peak height in mm of the bubbler blank

P_{blank} - Peak height in mm of the sample or method blank test solution
F - Dilution factor
M_{Sample} - Mass of the sample in g

3.4.4.2 Methylmercury determination in fish samples

Methyl mercury in fish was determined by acid leaching and solvent extraction, followed by aqueous phase ethylation, preconcentration on tenax trap, heat desorption, GC separation and detection by CV AFS.

Instrumentation

MeHg in fish samples were determined by a fully automated aqueous phase ethylation, purge and trap, desorption, GC separation and CV AFS detection after acid leaching and solvent extraction. An automated MeHg analysis system (Tekran Model 2700) based on the implementation of USEPA 1630 was used in the measurement of MeHg in fish samples. Tekran 2700 is a fully integrated gas chromatography cold-vapour atomic fluorescence spectrophotometer (GC-CVAFS) self-contained compact unit. The system is connected to an autosampler (Tekran 2621M) which has a sample purging probe and three sample racks with 63 position for 30 mL glass reaction vials. The Tekran 2700 compact unit consists of a built-in Infrared (IR) trap heater, Tenax trap wrapped with nickel-chrome wire, built-in GC oven, GC column with packed 15% OV-3, pyrolyzer, and CVAFS detector. Data processing and autosampler was controlled by Tek-MDS-2 software. In the course of its operation, ethylated volatile mercury species formed by the addition of $NaBEt_4$ solution is purged with the autosampler probe onto the Tenax. The Hg species are desorbed from the trap at 200 °C into the isothermal (80 °C) GC column and separated. The eluted species undergoes pyrolysis to form Hg^0. The Hg^0 is detected by CV AFS. The operation of the instrument is similar to the manual system which is described detail in Section 3.4.4.1. A flow diagram of Tekran Model 2700 is shown in Fig 3.16.

Extraction procedure

The fish samples were acid leached and extracted into an organic solvent, and back extracted into the aqueous phase by evaporating the organic solvent. The fish samples were extracted using the same procedure as the soil and sediment.

Derivatization by ethylation

About 30 mL glass reaction vial was filled with about 29.6 mL milli Q water and about 100 µL of the extracted sample solution was pipetted and added. About 300 µL of 2 M acetate solution was used to buffer the sample solution to pH of 4.2 (since ethylation reaction is maximum at pH within 4 to 5). MeHg in the sample was then ethylated by the addition of 50 µL of 1% $NaBEt_4$ solution making a sample volume of 30 mL; immediately the reaction vials were tightly capped. The ethylated reaction results in the

formation of ethylmethylmercury (MeEtHg), and diethyl mercury from inorganic mercury (Tseng et al., 1997). The ethylated volatile species were purged with the sample purging probe of the autosampler onto the Tenax trap of the Tekran 2700 (Fig. 3.17). N_2 was used as the carrier gas.

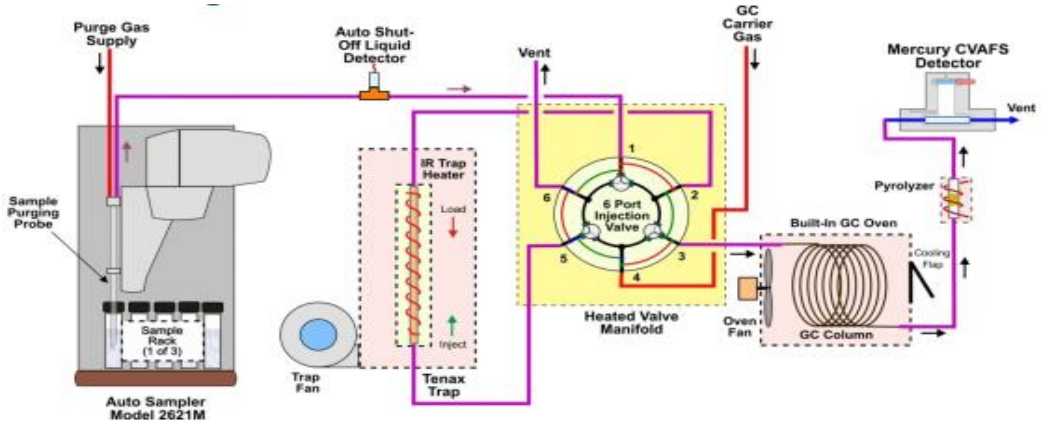


Figure 3.16: Flow diagram of Tekran 2700 (www.tekran2700.com).

Gas chromatographic separation and detection

The ethylated species on the Tenax trap was desorbed onto the isothermal GC-column for separation. Under a flow of Ar the eluted species were converted to Hg^0 by pyrolytic decomposition and then detected by CV-AFS.

Figure 3.17: Tekran 2700 MeHg Analyzer for MeHg measurement in fish.

Quality control

Analysis of blanks was crucial in avoiding contamination. Seven (7) calibration blanks were analyzed. Standard curve was created by analyzing a series of analytical standards of concentrations: 0.02 ppt, 0.05 ppt, 0.1 ppt, 0.5 ppt, 1.0 ppt, 2.0 ppt and 4.0 ppt. A graph of response versus concentration of the standards was plotted, and the correlation coefficient was calculated. This was automatically done by Tek-MDS-2 software. On-going Precision and Recovery (OPR) using a working standard of concentration 0.5 ppt was analyzed after every six (6) measurements. Certified Reference Materials (Dorm-4 and ERM-CC-464) of matrix and concentration match were analyzed prior to analysis of each batch of samples. Each sample was prepared in parallels and each was analyzed in duplicates. The sample was reanalysed if the parallel analysis differed for more than 10%. In each set of the analysis, three blanks were analyzed.

The Limit of Detection (LOD) was calculated as three times the standard deviation of the blank. Matrix spike was prepared by the addition of a known mass of MeHg standard to two of the fish samples and analyzed. The unspiked aliquot of the spiked samples was analyzed and the percentage recovery of the matrix spike was calculated as [Eq. 3.11]:

$$\%\,\mathrm{Recovery} = 100 * \frac{A - B}{T} \tag{3.11}$$

A = Measured concentration of analyte after spiking
B = Measured concentration of analyte before spiking
T = True concentration of the spike

3.4.4.3 MeHg determination in water samples

MeHg in water samples was determined using aqueous ethylation, purge, trap, and gas chromatography-cold vapor atomic fluorescence spectrometry detection after acid digestion and solvent extraction (USEPA 1630, 1998).

Instrumentation

MeHg in water samples was determined by gas chromatography-cold vapour atomic fluorescence spectroscopy (GC-CVAFS) with the Brooksrand "MERX-4400" Automated Methylmercury Analytical System. The instrument is a closed analytical system that comprised four interconnected modules that include: MERX Autosampler, MERX Purge and Trap Module, MERX GC and Pyrolysis Module and Brooksrand Model III Atomic Fluorescence Spectrophotometer detector. The analytical system is controlled along with data processing by the Brooksrand Mercury Guru software. Schematic diagram of Brooksrand "MERX" GC-CVAFS Automated MeHg Analytical System is shown in Fig 3.18. The system is based on the implementation of USEPA Method 1630. The principle of operation is the same with the manual GC-CVAFS (found in Section 3.4.4.1) except for the addition of auto sampler in this system. During its operation, extracted and ethylated mercury species solutions in 40 mL amber glass vials with sealed Teflon lined septa cap placed in the autosampler. Each vial is pierced with the auto-sampler probe and the ethylated sample solution is automatically transferred through N_2 gas pressure to the purge vessel of the MERX Purged and Trap module. The volatile ethylated species are stripped of the liquid phase onto the Tenax traps using N_2 carrier gas. Three traps

(collecting trap, drying trap and desorption trap) were processed simultaneously to attain quick sample analysis. While the first trap is drying, the second trap is collecting ethylated mercury species from the next vial. After the first trap dried, another vial with ethylated Hg species is purged onto the third trap while the second trap is dried; the first trap is heated to desorb the Hg species using Ar carrier gas onto a temperature-programmed packed GC column for separation based on their masses. The separation and pyrolysis was performed on the MERX GC and Pyrolysis module. The eluted species are decomposed into atomized form and detected by the Brooks Rand Model III Atomic Fluorescence Spectrophotometer detector (Žickovic et al., 2017a, 2017b; Nerentorp Mastromonaco et al., 2017). The instrumental conditions for MeHg analysis are presented in Table 3.3.

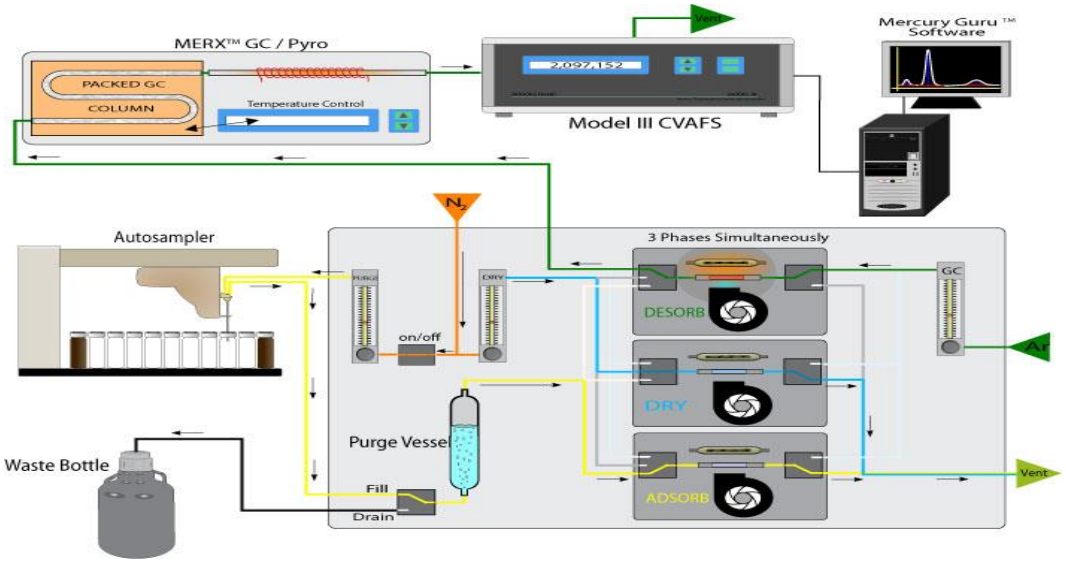


Figure 3.18: Schematic diagram of the Brooksrand "MERX" GC-CVAFS Automated MeHg Analytical System (www.brookrandinc.com).

Table 3.3: Instrumental conditions of MERX GC-CVAFS for MeHg analysis in water.

Parameter	**Condition**
Desorption Coil Temperature	200 ˚C
Pyrolytic Coil Temperature	800 °C
Nitrogen flow Rate (Purge & Trap carrier gas)	47 mL s^{-1}
Argon Flow Rate (GC and Detector carrier gas)	35 mL s^{-1}
Gas Chromatography Column	15% OV-3
GC Temperature	36 °C
Light Source	UV Lamb
Wavelength of UV Radiation	253.7 nm
Run Duration	6 minutes

Extraction procedure

Methyl mercury was determined following the procedure described by Horvat et al., (1993b, 2003a) and Liang et al. (1994, 1996). H_2O sample (70 mL) was placed into an acid-precleaned 125 mL PTFE bottle. About 5 mL of concentration HCl and 30 mL of CH_2Cl_2 were added. The bottle was tightly closed and overnight extraction of MeHgCl into the organic solvent was performed through digital shaking. The aqueous layer was pipetted off, leaving the methylene chloride, containing MeHg chloride. MeHgCl was back-extracted into aqueous phase by the addition of 40 mL of Milli-Q H_2O and evaporating CH_2Cl_2 at 80°C in a water bath. The sample solution (the extractant) was then purged with mercury-free N_2 for 5 minutes to quantitatively remove residual methylene chloride.

Derivatization

The extracted solutions were transferred into amber glass vials and the pH adjusted to 4.2 with 300 µL of 2 M acetate buffer to ensure optimum reaction of ethylating agent. MeHg in the sample solutions was ethylated by the addition of 50 µL of 1% $NaBEt_4$ solution. The solutions were quickly topped up with milli-Q H_2O to the final volume of 40 mL. The vials were tightly sealed with Teflon-lined Septa Caps and gently shaken. The immediate capping of the vial was done to prevent loss of volatile ethylated mercury species to ensure accurate measurement.

GC separation and AFS detection

The sample solutions were loaded into the auto-sampler of the automated MERX GC-AFS mercury analyser by Brooks Rand. Each vial was pierced with the auto-sampler probe and the ethylated sample solutions were transferred through gas pressure to a purge vessel of the MERX Purge and Trap module. The volatile ethylated Hg species were purged from the liquid phase with N_2 onto a Tenax trap and thermally desorbed at 200 °C onto a temperature programmed GC column (36 °C). The eluted Hg species were converted to Hg^0 by pyrolytic decomposition at 800 °C and detected by CVAFS. The automated MERX GC-CVAFS MeHg Analytical System is presented in Fig 3.19.

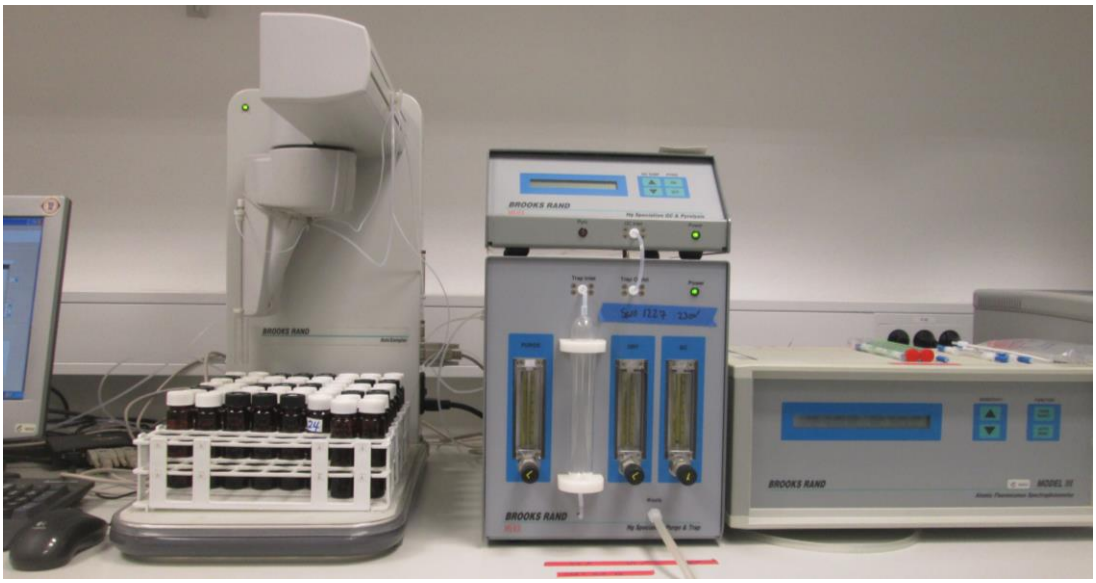


Figure 3.19: Brooksrand "MERX" GC-CVAFS Automated MeHg Analytical System for MeHg measurement in water.

Quality control

Three blanks and three standards of 5 pg were analyzed after every six samples. Three samples were spiked with 50 µl of 100 pgmL-1 MeHg standard and analyzed. The recovery was calculated.

3.4.5 Determination of water-soluble fraction of Hg from sediment

The water-soluble fraction of Hg in sediment samples was determined after water leaching of the soluble Hg, followed by filtration, acid digestion of the filtrate and total mercury measurement.

Instrumentation

Total mercury of the water-soluble fraction (filtrate) was determined using Tekran Model 2600. This analytical instrument is described in detail in Section 3.4.3.4.

Extraction of water-soluble fraction of Hg

The sediment samples were shaken with 100 mL of milli-Q and also with rain water in an end-over-end shaker for 24 and 48 hrs. After shaking, the samples were centrifuged at 3200 rpm and the supernatant filtered through a 0.2 µm pore size nylon filter.

Analysis of the water-soluble fraction of Hg

Total mercury in the water-soluble fraction of the filtrate was determined following the procedure described in USEPA 1631E (2002). Digestion of filtrate, total mercury measurement and quality assurance and control measures are all described in Section 3.4.3.4. A scheme for the determination of water-soluble fraction of Hg is presented in Fig. 3.20.

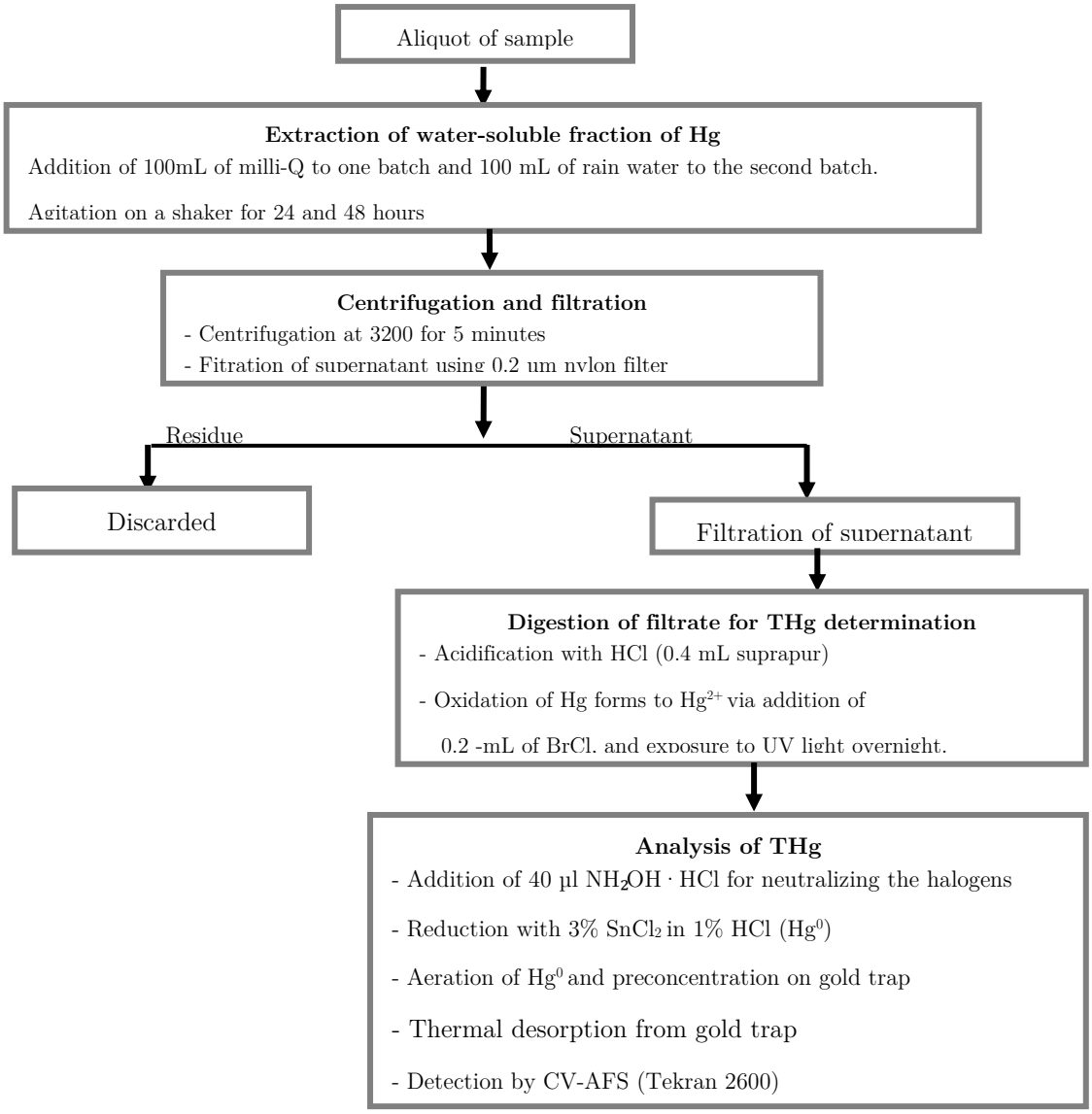


Figure 3.20**:** Scheme for determination of water-soluble fraction of Hg in sediment.

3.4.6 Thermal fractionation of mercury compounds

Determination of Hg binding forms by solid phase Hg pyrolysis was carried out by an in-house designed apparatus.

Apparatus

The analytical instrument for temperature fractionation was a device constructed in-house (Fig. 3.21) by the Jožef Stefan Institute, Ljubljana, Slovenia. It consist of LUMEX PYRO-915+ PUMP **(1)** for the supply of a carrier air at a flow rate of 1 L/min. The PYRO PUMP consists of a Hg filter. **(2)** Quartz tube with an outer diameter of 20 mm and inner diameter of 16 mm, was placed in an electric tube furnace **(3)**. Quartz boat **(4)** used to hold samples was positioned in the quartz tube in the middle of the electric

tube furnace Nerbertherm RT 50/250/11 where the thermocouple was located. The quartz boat is semi-circular, with an inner diameter of 5 mm and length of 35 mm. The tube furnace consists of a ceramic tube, inner diameter of 50 mm, and two plugs of ceramic fiber on each side. Each plug has a drilled hole in the middle with a tight fit to a quartz tube to reduce heat loss. The Lumex Pyro 915+ pyrolysis unit, (350 x 350 x 120 mm) **(5)**, ensured decomposition of volatile Hg compounds. Hg^0 detected using the Lumex RA-915+ atomic absorption detector **(6)** with Zeeman correction (detector: 470 x 210 x 110 mm), directly connected to a computer **(7)** for the collection of data (Sadlar et al., 2015).

Thermal fractionation of mercury compounds in sediment

Samples were weighed into a quartz boat which was positioned into a quartz tube and placed in the electric tube furnace and connected to the pyrolytic cell. The samples were heated by slow progressive temperature increase (about 2.2 °C min^{-1}) to 800 °C by the electric furnace to transform mercury compounds in the samples into volatile Hg [elemental] (Biester et al., 1998). Continuous flow of air by the pyrolytic cell pump unit carries Hg to the pyrolytic cell for additional decomposition of residual volatile Hg compounds. The released elemental Hg was then detected by Lumex CV-AAS. The Hg signal and the sample temperature were simultaneously processed by a personal computer.

The results are depicted as Hg thermo-desorption curves (TDC), which shows Hg released (ng/m^3) and temperature plots. Mercury compounds were categorized by released temperature range (thermal-released commencement through maximum peak to the point where the curve returns to the baseline).

Fig 3.21: Apparatus for thermal fractionation of Hg.

3.4.7 Determination of THg in soil and sediment using k_0 Instrumental Neutron Activation Analysis (k_0-INAA).

Aliquots (varying from 150 to 210 mg) of soil and sediment samples were placed into a pure polyethylene ampoule (SPRONK System, Lexmond, The Netherlands) and sealed. For the determination of intermediate/median (mercury) radionuclides, samples and standards (Al-0.1% Au alloy IRMM-530R disc of 6 mm in diameter and 0.1 mm thick) were stacked together and fixed in the polyethylene ampoule in sandwiched form and irradiated for 12 hours in the Carousel Facility (CF) of the 250 kW TRIGA Mark II Nuclear Reactor of the Jožef Stefan Institute, Slovenia, at a thermal neutron flux of 1.1×10^{12} cm $^{-2}$ s^{-1}.

After irradiation the samples and standards were allowed to 'cool' for 3, 6 and 21 days. After the cooling time each sample was measured three times on an absolutely calibrated HPGe detector (Canberra, USA) with 40 % and 45% relative efficiency. Measurements were carried out at such distances that the dead time was kept below 10 % with negligible random coincidences. The detector was connected to an EG&G ORTEC Spectrum Master high-rate multichannel analyzer [zero dead time (ZDT) mode] with Maestro®-32 Spectroscopy Software.

The HyperLab 2002 program was used for peak area evaluation. For the determination of f (thermal to epithermal flux ratio) and α (a parameter which represents the epithermal flux deviation from the ideal 1/E distribution), the "Cd-ratio" method for multi monitor was applied (Jacimovic et al., 2003). The values obtained for f = 28.79 and α = -0.0023 were used to calculate the element concentrations. The mercury concentrations and effective solid angle calculations were carried out on the

KayWin® software package. Fig. 3.21 shows the scheme for the determination of elemental composition of soil and sediment by k_0-INAA.

For QA/QC during the k_0-INAA Certified Reference Material BCR-320R Channel Sediment was used.

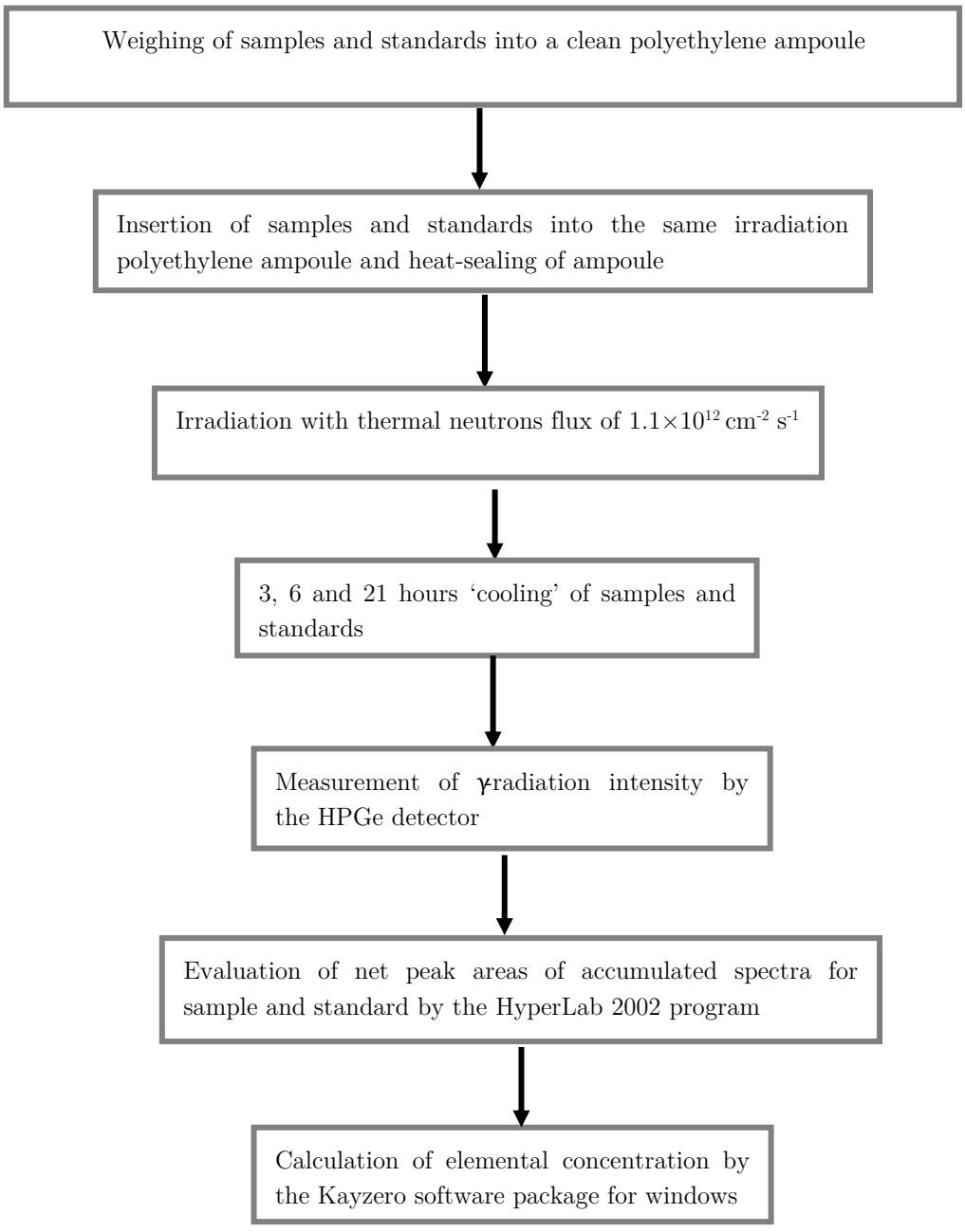


Figure 3.21**:** Scheme for the determination of elemental composition of soil and sediment by k_0-INAA.

3.4.8 Total cyanide determination

Total, free and WAD (weak acid dissociation) cyanide in water and sediment were determined by the Skalar segmented flow auto-analyzer (Skalar Method 295). The method was derived from Standard Methods for the Examination of Water and Waste Water, 23rd Edition (2005), Methods 4500-CN. The cyanide species in the water samples were directly determined in this method. While that in the lyophilized sediment samples were determined after samples have been digested and solubilized. Free cyanide was analysed using the colorimetric method. WAD cyanide was analysed using the colorimetric method with a distillation step, while total cyanide was analysed using the colorimetric method with coupled distillation and UV digestion. For total cyanide determination, cyanide in the solution was released from all cyanide complexes. This was achieved by UV digestion and distillation. The cyanide was converted to cyanogen chloride by reaction with chloramine-T which subsequently reacts with isonicotinic and barbituric acid to form a blue-coloured complex. The intensity of this complex is concentration-dependent and measured at 600 nm.

Free cyanide and WAD cyanide were determined the same way as total cyanide; however, the UV lamb was switched-off for free cyanide, and instead $ZnSO_4$ solution was added to precipitate any iron cyanide present. In the determination of WAD cyanide, the UV digestion lamb was switched off and the samples were deionized.

Ten (10) sediment samples (six from the Aprepre River and four from the Ankobra River) were randomly selected for cyanide determination. The same samples were used for TOC determination as well as the test for Hg solubility in water.

3.4.9 Organic carbon determination

Total organic carbon content in sediment and soil and dissolved organic carbon in water were achieved by the dry combustion method using the Leco CS844 Carbon/Sulphur determinator (Santi et al., 2006) and Skalar analyzer, respectively.

3.5 Statistical analysis

To explore the similarity between the parameters (THg, MeHg, OC, CN and solubility) determined with respect to the sampling sites, hierarchical cluster analysis was used. Hierarchy Cluster Analysis (HCA) was performed on a mean centered data set using Wards linkage with squared Euclidean Distance between data for similarity groupings of sampling sites with respect to parameters. The results obtained are reported as dendrograms in Fig. 4.8 and Fig. 4.9.

Chapter 4

Results and Discussion

4.1 Preamble

The results obtained are presented and discussed in this Chapter. The presentation and discussing of results have been divided into eight sections (Sections 4.1 to 4.8). Section 4.1 focuses on discussion of analytical performances. Sections 4.2 to 4.8 consist of presentation and discussion of the following: THg and MeHg in soil, sediment, water and fish; water-solubility of Hg in sediment; thermal fractionation of mercuric compounds in soil and sediment. Results are presented in the form of Tables and Figures.

4.2 Analytical Performance

THg and mercury species

The atomic absorption technique of a semi-automated cold vapor atomic absorption spectrophotometry mercury analyzer (Sanso Seisakusho Co., Ltd, Tokyo, Japan) model HG-201 used for THg determination in the solid samples (soil, sediment and fish) gave absorption peak heights on a strip chart.

The species of mercury were determined using a propylation agent to convert all Hg species to volatile mercury compounds, preconcentration of the volatile compounds on Tenax, and separation of the species on an isothermal gas chromatographic column and detection by atomic fluorescence technique produced chromatographic peaks (Fig. 4.1). Four peaks were observed, with an order of elution: Hg^0, MeHg (the peak of interest), EtHg, and Hg^{2+}. The retention time (min.) for the peak of interest (MeHg) was 2.71±0.04. It was observed that an increase in weight (eg. from 0.02 g to 0.2 g) of highly contaminated samples for the extraction of Hg species, separation and detection gives the same concentration levels (no matrix effect). This was because there was no observed MeHg artifact produced by the analytical method for high quantity contaminated samples extracted.

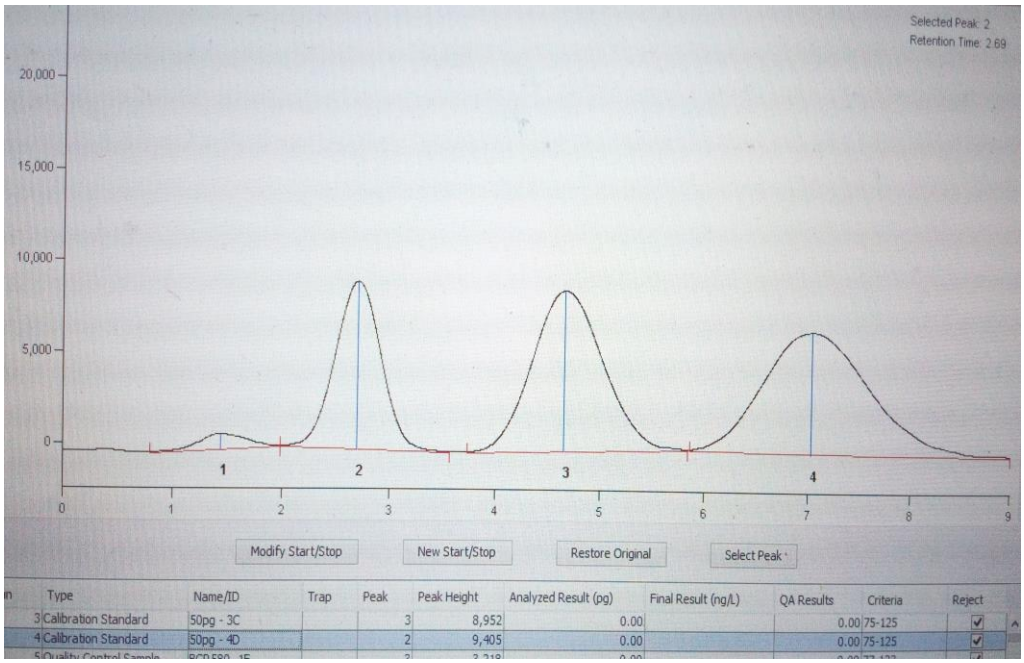
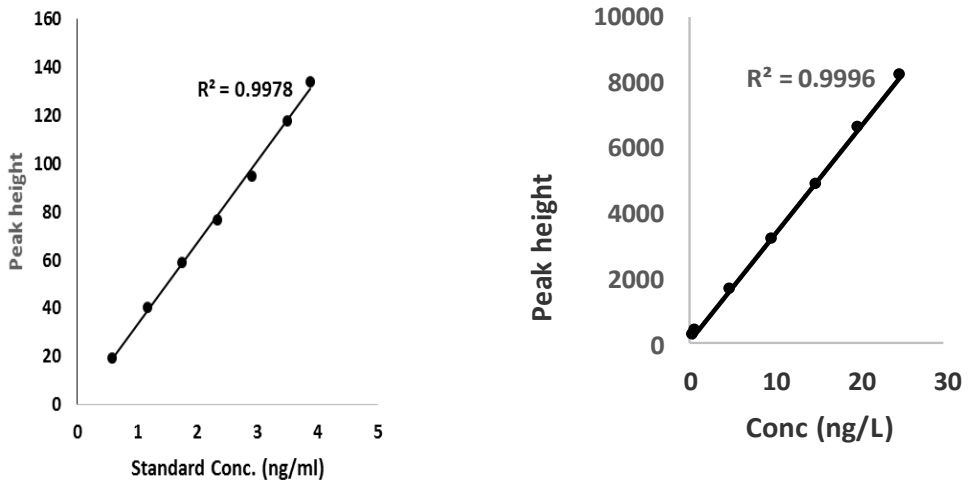


Figure 4.1: Chromatogram of Hg species produced by the GC-CVAFS analytical technique.

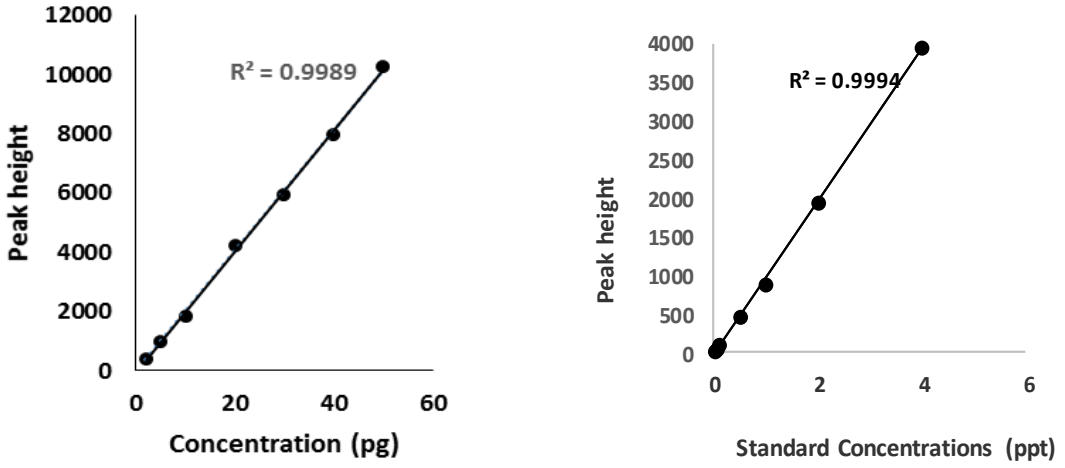
4.2.1 Linearity of calibration curves

The linearity of a calibration curve is usually expressed as the variance of the slope of the regression line. Mathematical linear regression equation, y = mx + C, relating to the results should have an intercept and slope that gives a correlation coefficient. The calibration curves created based on the response value of the respective concentrations are shown in Fig. 4.2 & 4.3. The relationship that exists between the concentrations of Hg standards and the corresponding response predict the unknown concentrations of the analyte in the various matrices. All the calibration curves in this study were linear with r^2 of at least 0.997 achieved daily. This indicated good analytical performance during measurement.



(a) **(b)**

Figure 4.2: Calibration curve of peak height versus standard Hg concentrations for THg measurement in **(a)** soil sediment and fish using CV-AAS HG-201 semi-automated Hg analyzer and **(b)** water samples by CV-AFS Tekran model 2600.



(c) **(d)**

Figure 4.3: Calibration curve of peak height versus standard concentrations of MeHg in **(c)** soil and sediment using manual GC-CV-AFS after aqueous phase propylation and (d) fish by GC-CV-AFS Tekran model 2700.

4.2.2 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and the Limit of Quantification (LOQ) of the analytical technique used for total and methyl mercury determination in sediment, soil water and fish are presented in Table 4.1.

Table 4.1: LOD and LOQ for total and methyl Mercury.

Matrix	THg			MeHg		
	Analytical Technique	LOD (ng/g)	LOQ (ng/g)	Analytical Technique	LOD (ng/g)	LOQ (ng/g)
Sediment & Soil	CV-AAS	0.05	1.25	GC-CV-AFS	25	63
Fish	CV-AAS	0.05	1.25	GC-CV-AFS	0.06	0.15
Water	CV-AFS	0.04 ng/L	0.2 ng/L	GC-CV-AFS	2 pg/L	6 pg/L

4.2.3 Quality control and quality assurance

The validity of the analytical methods, consistency and reliability of the results were assessed by analysing Certified Reference Materials (CRM) of matrix and concentration match. These were analyse together with the samples. CRM's analyzed for THg were ERM-CC-141 (calcareous loam soil) and BCR-320R (channel sediment). Both THg and MeHg were analyzed in BCR-580 (estuarine sediment). Also analysed were DORM 4 (fish protein), a reference product on fish protein by National Research Council Canada (NRCC) and ERM-CE-464 (tuna fish). IAEA Reference Material, IAEA-433 (marine sediment) was analyzed for MeHg.

Table 4.2: Analysis of THg and MeHg in Certified Reference Materials/Reference Material (n=12).

CRM	**Matrix**	**THg**			**MeHg**		
		Present study	**Certified value 95% CI**	**% Recovery**	**Present study**	**Certified value 95% CI**	**% Recovery**
BCR-580 (mg/kg)	Estuarine sediment	131 ± 1.43 [CV-AAS]	132 ± 3	99.2	75.7 ± 2.5 [GC-CV-AFS]	75 ± 4	101
BCR-320R (mg/kg)	Channel sediment	0.79 ± 0.01 [CV-AAS]	0.85 ± 0.09 [CV-AFS; CV-AAS]	92.9	-	-	-
ERM-CC-141 (mg/kg)	Calcareous loam soil	0.079 ±1.51 [CV-AAS]	0.083 ± 0.017	-	-	-	-
IAEA 433 (µg/kg)	Marine sediment	-	-	-	0.17 ± 0.07 [GC-CV-AFS]	0.17 ± 0.008	100
DORM-4 (mg/kg)	Fish protein	0.366 ± 0.01 [CV-AAS]	0.410 ± 0.055	89.3	0.354 ± 0.031 [GC-CV-AFS]	0.354 ± 0.009	100
ERM-CE-464 (mg/kg)	Tuna fish	5.23 ± 0.04 [CV-AAS]	5.24 ± 0.10	99.8	5.49 ± 0.08 [GC-CV-AFS]	5.50 ± 0.17	99.8
BCR-579 (ng/kg)	Coastal sea water	2.02 ± 0.08 [CV-AFS]	1.9 ± 0.5	106.3	-	-	-

Measured value is given as mean ± SD n = (number of determination)

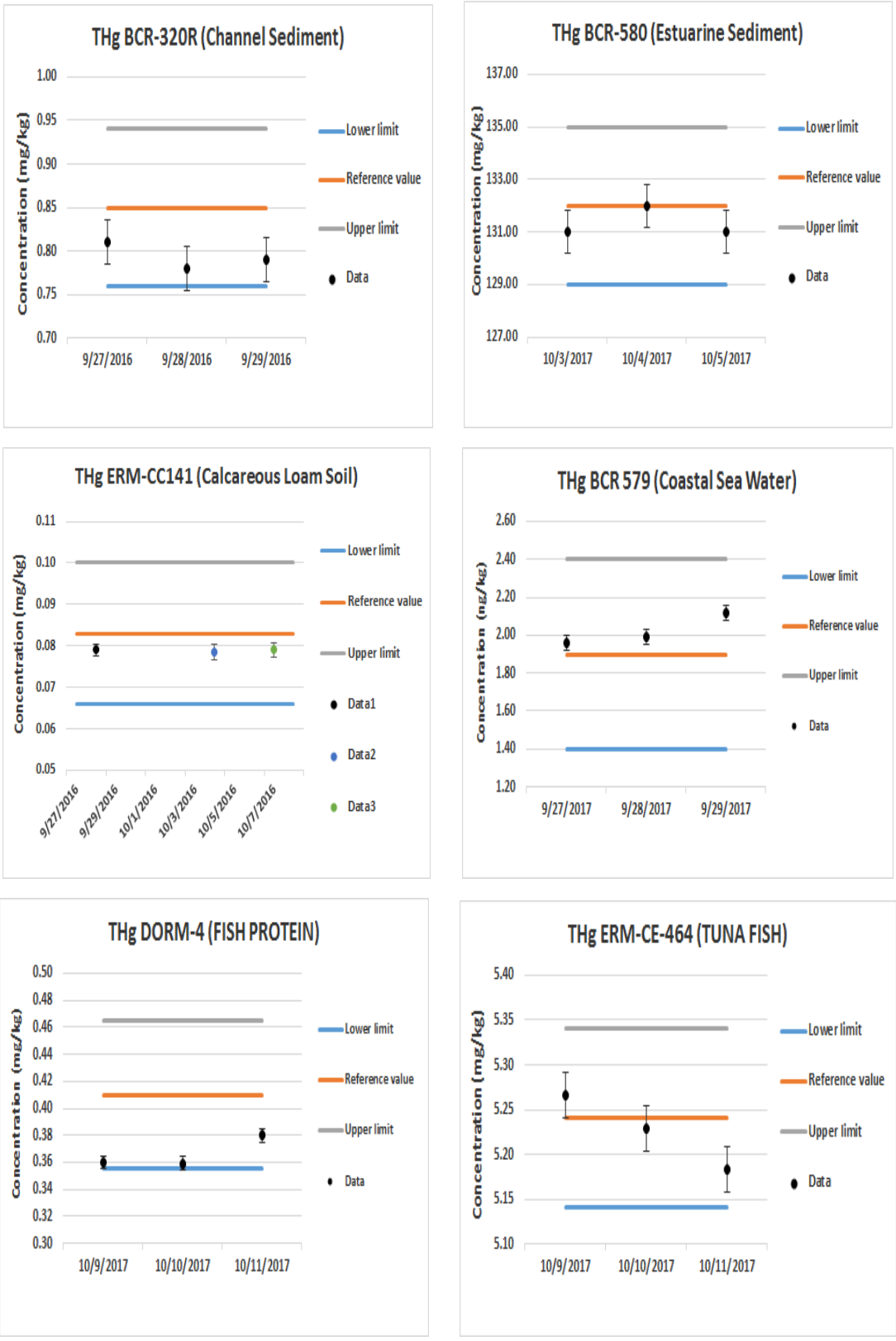


Figure 4.4: Schewart control charts for THg in CRM's.

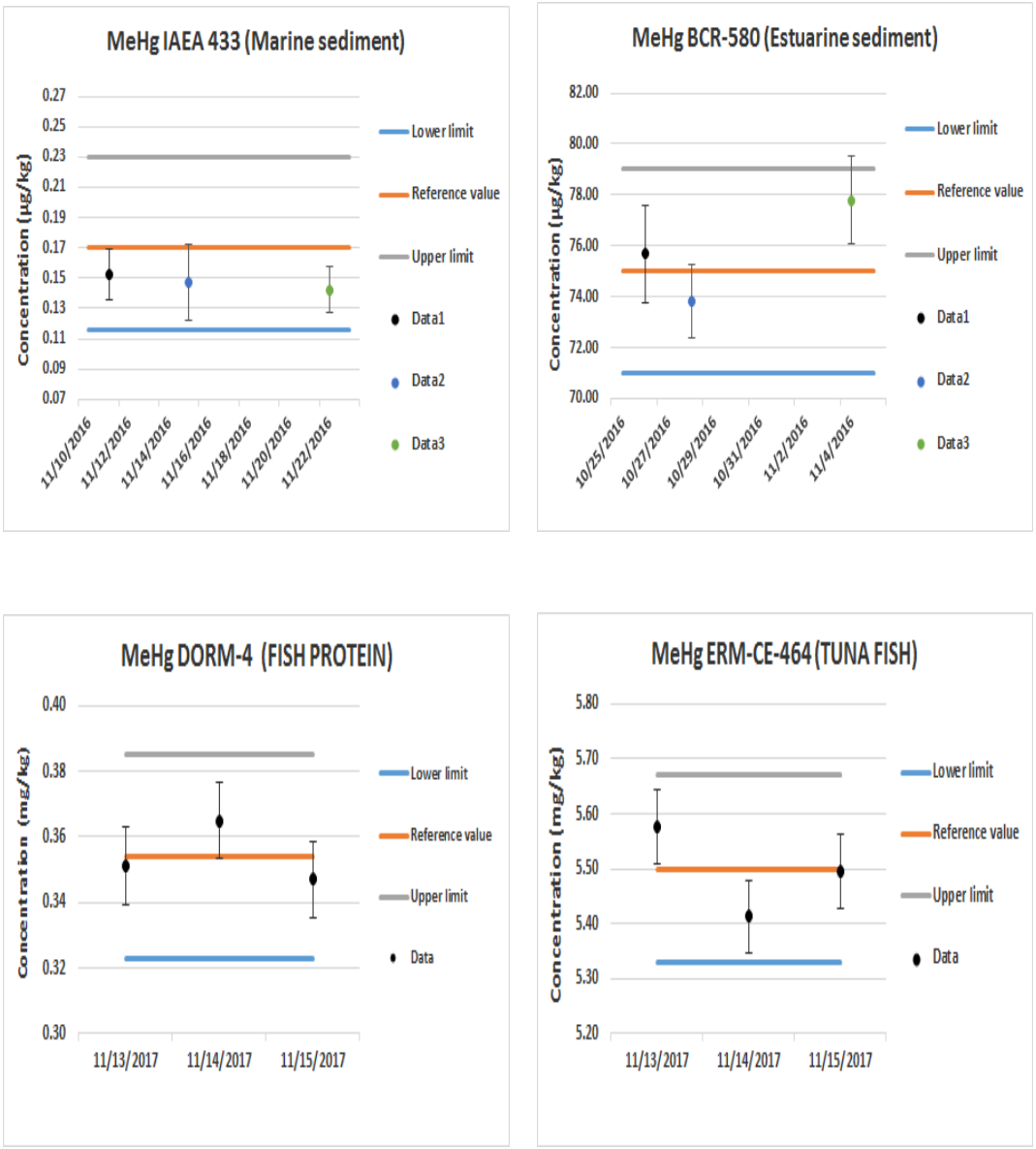


Figure 4.5: schewart control charts for MeHg in CRMs.

The results obtained are presented in Table 4.2 and on the Schewart control charts (Fig. 4.4 and 4.5). From the control charts it is evident that all the values obtained are within the expended uncertainty k=2 of the certified reference materials and also within $\bar{x} \pm \sigma$. This confirms that the analytical methods used for this study are reliable.

4.2.3.1 Comparison of THg levels determined by CV-AAS with that obtained by k_0-INAA in some selected sediment and soil samples from the study area.

THg levels for some selected soil and sediment samples were analysed using CV-AAS and re-analysed with k_0-INAA as a quality control check. Comparison of THg levels in the selected soil and sediment samples analysed with both CV-AAS and k_0-INAA are presented in Table 4.3. Results obtained by CV-AAS are similar to that found using k_0-

INAA. The results obtained between CV-AAS and k_0-INAA techniques have a relative standard deviation below 10% (1.5 to 9.3%). This shows that the analytical method used for THg determination in the solid samples in the study is valid, and hence results obtained are reliable.

Table 4.3: Comparison of THg levels determined by both CV-AAS and k_0-INAA in some selected sediment and soil samples.

Matrix	Sampling Station	Sampling Code	THg (µg/g)		RSD (%)
			CV-AAS	k_0-INAA	
Sediment	Station D	S3	415	495	9.3
		S4	364	362	0.4
		S5	306	315	2.0
		S6	324	344	4.2
		SB	162	179	7.1
Soil		P1	10695	12700	4.5
		P2	7001	7440	4.3
		Q1	10229	11400	7.7
		Q2a	314	321	1.5

4.3 Vertical distribution of THg and MeHg in soil samples

The depth (2, 4, 6, 8, 10, 15 and 20 cm) distributions of THg, MeHg, cyanide and organic carbon in the soil samples are presented in Fig. 4.6. The THg concentration in the upper 2 cm and lower 20 cm ranged from 80 to 457931 ng/g and 56 to 3778 ng/g, respectively. Higher THg levels (7000 to 457931 ng/g) in the upper 2 cm soil were found in samples collected within 50 m of amalgam burning site (P3) and ASGM operation site (P1, P2, Q1, Q2). The highest concentration (457931 ng/g) of total mercury at the upper 2 cm was observed at the commercial amalgam burning site (P3) in Prestea. All the soil samples collected beyond 50 m to 100 m from ASGM operation site had THg levels (314 to 3277 ng/g) above the background THg levels of 78.3 ng/g in a village called Ayesukrom, within the study area. Topsoils collected further away (>150 m up to 3 km) from the ASGM site in the study area (Table 4.4) recorded lower Hg concentrations (78 to 80 ng/g) just around the background level.

Table 4.4: Total and methyl mercury concentration in 2 cm top soil.

Sample Point	THg (ng/g)	MeHg (ng/g)	MeHg (%)	OC (mg/kg)	TCN (mg/kg)
P1	10695	5.27	0.05	3.12	<0.001
P1a (100 m from P1)	800	0.69	0.09	1.57	<0.001
P2	7001	9.85	0.14	1.39	<0.001
P3	457931	59.4	0.01	2.09	<0.001
P3a (100 m from P3)	3277	1.76	0.05	1.08	<0.001
P4 (500 m from P2)	80	0.44	0.55	1.78	<0.001
Q1	10229	68.9	0.67	1.49	0.06
Q2	19620	1.27	0.01	0.48	0.01
Q2a (50 m from Q2)	314	0.63	0.25	0.11	0.01
Q3 (150 m from Q1)	80	0.78	0.98	1.56	0.03
AK	78	0.17	0.22	0.97	<0.001
TK	50	0.13	0.26	1.05	<0.001
CC	41	0.21	0.51	1.26	<0.001
AC	28	0.34	1.21	2.42	<0.001

Vertical distribution of THg concentrations in the soil was not uniform. The levels varied among the vertical layers. In general, THg concentrations decreased with depth. Thus, the higher THg levels were observed in the upper layers and lower levels in the deeper layers of the soil core. However, soil samples from non-contaminated area and site further away from ASGM and amalgam burning site shows less variations in THg levels with respect to depth.

THg and MeHg levels in this study were comparable to reported values in literature (Table 4.5) [e.g. UNIDO, 2001; Donkor et al., 2006; Gerson et al., 2018]. Only Gerson et al have examined soil MeHg concentrations in ASGM sites. THg values in this study were within the range reported by Donkor et al., (2006) in Ghana ASGM site around the Offin River. Their maximum value of 2147 µg/g exceeded that obtained in this study by a factor of about 5. UNIDO (2001) reported THg level of 23.3 µg/g in an amalgam burning site in the study area. This value falls below the level (457931 ng/g) reported in the commercial amalgam burning site for this study by a factor of about 20. This is possibly due to the intensity of operation at the site for this study, since it is a commercial refinery site.

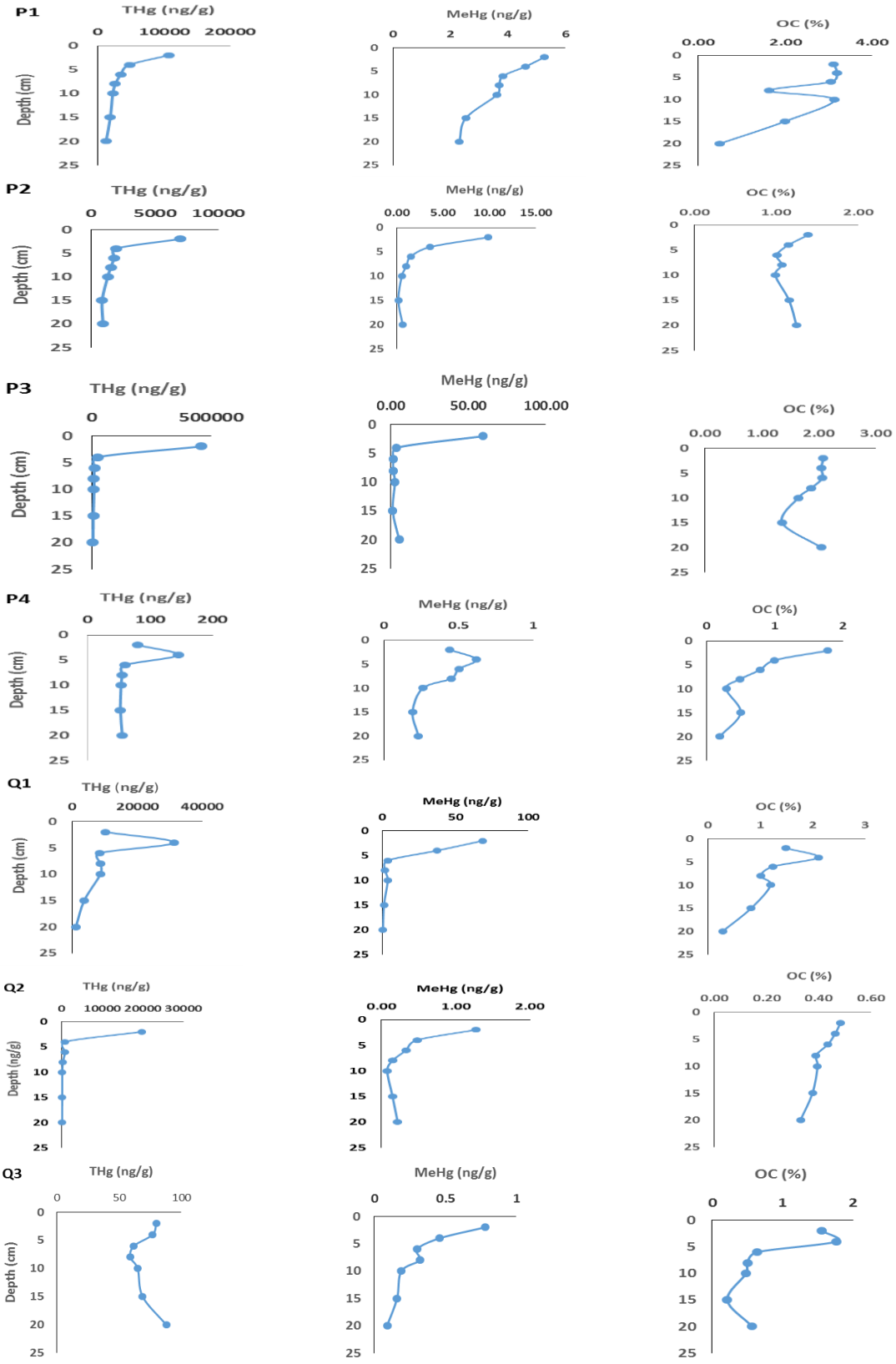


Figure 4.6: Vertical variations of THg, MeHg and organic carbon (OC) in soil core.

Table 4.5: Comparison of THg and MeHg results obtained in soils with literature data from other ASGM areas.

	Present Study	**Literature**		
		Dunkwa On-Offin[a]	**Dumasi**[b]	**Senegal**[c]
THg in Soil (µg/g)	80 – 458	1.56-2147	23.3	0.05-130
MeHg in soil (ng/g)	0.4 -69	-	-	0.05-48

[a]Donkor et al., 2006 [b]UNIDO, 2001 [c]Gerson et al., 2018

THg levels reported in other nations ASGM site, for example in Senegal by Gerson et al. (2018) ranged from 0.05 to 130 µg/g. The levels are within the range obtained in this study, while the maximum levels in this study are above that reported in Senegal by a factor of about 3. THg levels in this study, at all the ASGM sites, amalgam burning site, and the site that is 100 m away from the amalgam burning site (P3a), are above the probable effect concentration (PEC) of 1.06 µg/g and USEPA (2013) Residential Soil Screening Level (SSL) of 2.3 µg/g. This reveals high anthropogenic release of Hg during amalgam burning at ASGM sites (Tulasi et al., 2019). Hence resulting in high surface contamination of Hg in these sites, which is subsequently washed by rain into river bodies in the study area. About 60% of the top 2 cm soils collected at a distance (>50 up to 3 km) from the ASGM sites and the amalgam burning site exceeds the USEPA (1985) THg soil standard of 0.1 µg/g and the background concentration of 78.3 ng/g in the study area. THg levels in composite 2 cm topsoil samples collected in Takoradi, Cape Coast and Accra, non- mining areas (about 120, 149 and 300 km from the study area, respectively), recorded 49.7, 41.3 and 28.0 ng/g, respectively. This reveals that THg level decreases considerabley with distance from the source of pollution. This therefore shows that the elevated levels of Hg in the study area are due to anthropogenic mercury released from ASGM activities that use mercury in gold extraction (Tulasi et al., 2019b).

MeHg level in the upper 2 cm and the lower 20 cm of the soil core ranged from 0.4 to 68.9 ng/g and 0.09 to 2.3 ng/g, respectively. Cyanide levels in soils from the Dumasi village (a village close to LSGM company) ranged from 0.01 to 0.06 mg/kg; while that from Prestea were all below the detection limit of <0.001 mg/kg. It was observed that MeHg levels (0.17 to 68.9 mg/kg) from the cyanide contaminated-mercury loaded soils from Dumasi were higher than the levels (0.04 to 54.9 mg/kg) found in Prestea despite higher THg levels (80 to 457931 mg/kg) from Prestea. MeHg concentrations show variations with depth. Organic carbon (OC) in the upper 2 cm and lower 20 cm in the soil samples varies from 0.48 to 3.13% and 0.21 to 2.05%, respectively. MeHg and OC show similar verticals variations, where MeHg concentrations at the surface were higher (first few centimeters) and decreased in deeper layers.

In general, positive correlations were observed between MeHg and OC in most of the soil cores (Table 4.6), except for P3 which had a correlation coefficient of only 0.1371, $p>0.05$. The correlations between MeHg and OC in this study are in agreement with affinity of MeHg for OC; and similar to results: $r^2=0.57$, $p<0.001$ and $r^2 = 0.46$, $p<0.01$ found in studies by Gray et al. (2015) and Tomiyasu et al. (2011), respectively. OC contents (1.08 to 3.12 mg/kg) in the upper 2 cm soils from Prestea were higher than levels (0.11 to 1.56) from Dumasi. Hence, the high MeHg levels from Dumasi are due to the presence of cyanide.

Table 4.6: Correlation of organic carbon with methyl mercury in soil core.

Sample code	Correlation coefficient
P1	0.5843, $p<0.01$
P2	0.5387, $p<0.05$
P3	0.1371, $p>0.05$
P4	0.3461, $p<0.05$
Q1	0.4025, $p<0.05$
Q2	0.5612, $p<0.05$
Q3	0.7057, $p<0.05$

MeHg levels were comparable to levels reported in literature. MeHg levels (0.052 to 48 ng/g) reported by Gerson et al. (2018) in Senegal ASGM sites are within levels obtained in this study. Maximum concentration (68.86 ng/g) in this study exceed that reported by Gerson et al., 2018. MeHg levels were all below the USEPA (2013) MeHg residential Soil Screening Level (SSL) of 0.78 µg/g. The percentages of MeHg as Hg were generally below 1% (Table 4.4), which is consistent with data obtained in studies from other parts of the world (Hines et al., 2000; Gerson et al., 2018).

4.4 Distribution of Total and Methyl Mercury in Sediment Samples

The concentrations of THg and MeHg in all the surface sediment samples from the Aprepre and Ankobra Rivers (Stations B, C, D, E, F and G) varied from 11 to 624 ngTHg/g with a mean range of 147 to 316 ng/g (Fig. 4.7); and 0.07 to 14.8 ngMeHg/g with a mean range of 0.11 to 3.45 (Fig. 4.7), respectively. THg concentration in sediment from the reference station (Station A) ranges from 31 to 80 ng/g with a mean of 50.8 ng/g. The reference station (control site) was chosen upstream of the Aprepre River. This was because the location was a non-ASGM site with almost the same forest cover as the other stations, and closest to receiving effluent containing cyanide from LSGM activities (which uses cyanidation for gold extraction). Higher THg levels were found in Stations B, D and G having maximum levels of 624 ng/g, 432 ng/g and 490 ng/g respectively.

About 75 and 90% of THg levels in sediment from sampling stations B and D, respectively, of the Aprepre stream exceeded the USEPA THg sediment standard of 0.2 µg/g (USEPA, 1985) and the average background level (50.8 ng/g). The elevated levels of THg in sediment from these 3 stations were as a result of their closeness (short distance) to ASGM operation sites, which is the likely source of Hg contamination in the area; occurring during amalgamation and amalgam burning. All the samples from Station C, E and F were all below the USEPA standard guideline of 0.2 µg/g due to their distances from the source of pollution; with the exception of one sample from Station C with THg level of 220 ng/g.

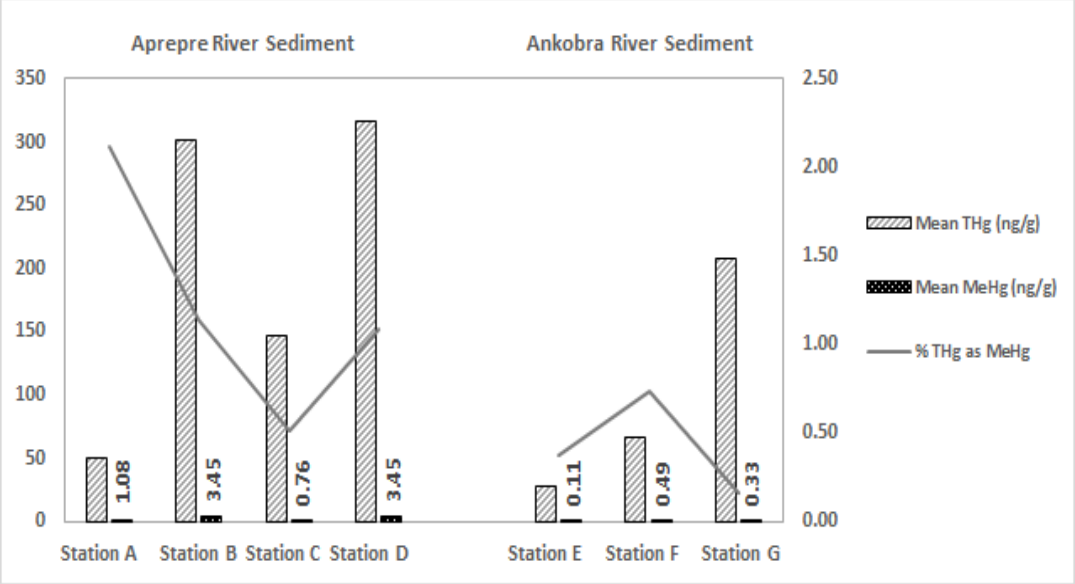


Figure 4.7: Distribution of mean THg, MeHg and % THg as MeHg in sediment samples from the Aprepre and Ankobra River.

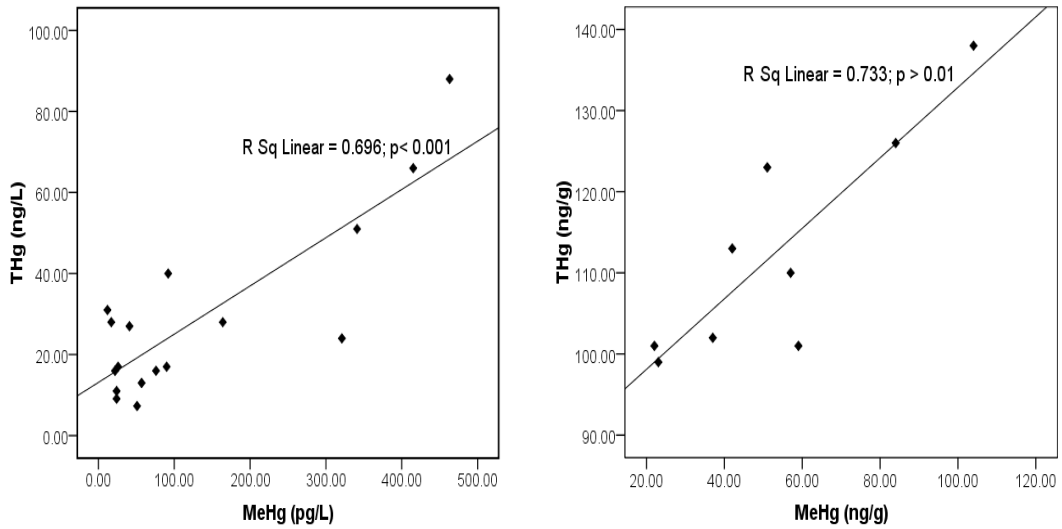
Comparison of the THg levels obtained in this study and values reported in scientific literature for both Ghana and ASGM sites in other countries [Table 4.7] shows THg concentrations ranging from 640 to 8500 ng/g were reported for the Aprepre River by UNIDO (2001). These values far exceeded the values reported for the present study by a factor of 10. This difference could be attributed to the period of sampling for this study. At the time of sampling (2017-2018), the Government of Ghana had placed a ban on the operations of ASGM. Accordingly, the intensity of ASGM operations was low since most of the miners had folded up. Notwithstanding, some ASGM activities were still on-going. THg levels from the Ankobra River (11-490 ng/g) were comparable to values (63-270 ng/g) reported by Akabzaa and Yidana (2011). The maximum THg level in the present study exceeded values reported by Akabzaa and Yidana (2011) by a factor of about two. Literature data on four mining villages (Bantako, Kharahenna, Kolya, and Sabodala) in Senegalese ASGM sites (southeastern region of Kedougou) reported THg of 59 to 3400 ng/g with a median value of 820 ng/g (Gerson et al., 2018). The maximum values were reported in Kolya. The values reported for Senegal were higher than that reported in this study by a factor of about four. MeHg levels reported by Gerson et al. (2018) were 0.13 to 19 ng/g with a mean value of 4.3 ng/g (Table 4.7). These values are fairly comparable to MeHg levels of 0.07 to 14.83 ng/g obtained in the present study.

Table 4.7: Comparison of THg and MeHg results obtained in sediment and water samples with literature data from ASGM sites.

	Present Study		**Literature**		
	Aprepre River	**Ankobra River**	**Aprepre[a] River**	**Ankobra[b] River**	**Senegal[c]**
THg in sediment (ng/g)	31- 624	11 - 490	640 - 8500	63 - 270	59-3400
MeHg in sediment (ng/g)	0.07 – 15	0.07 – 1.8	-	-	0.13 -19
THg in H_2O (ng/L)	2.0 - 88	99 - 138	140 -760	1000-8000	2.5-2400
MeHg in H_2O (pg/L)	17 - 463	22 - 104	-	-	0.13 -19

[a]UNIDO (2001) [b]Akabzaa and Yidana (2011) [c]Gerson et al. (2018)

In order to establish that the levels of THg are not the only determining factor influencing the levels of MeHg, the relationship between THg and MeHg was assessed using Pearson's Correlation performed with SPSS 16. THg and MeHg from the Aprepre and Ankobra Rivers showed a positive correlation (Aprepre River: $r^2 = 0.696$; $p > 0.001$; Ankobra River: $r^2 = 0.733$; $p > 0.01$; Fig. 4.8; both Ankobra and Aprepre Rivers: $r^2 = 0.359$; $p > 0.001$). However, these correlation coefficients are not so strong implying that THg concentrations are not the sole factor influencing the MeHg levels. This, therefore, suggests that contributions from other influences, such as factors (CN and OC) that affect the bioavailability of Hg to sulphate-reducing bacteria, and the activity and structure of the microbial community [Ullrich et al., 2001]) play key roles in influencing mercury methylation.



(a) **(b)**

Figure 4.8: Correlation between THg and MeHg levels in sediment samples from the Aprepre and Ankobra Rivers (Aprepre River: a; Ankobra River: b).

4.4.1 Effect of cyanide on methylmercury levels

The levels of THg, MeHg, percentage (%) THg as MeHg, organic carbon as well as cyanide levels obtained in ten (10) randomly selected sediment samples (sampling points S1 to S6; and SA to SD from the Aprepre and Ankobra Rivers, respectively) are presented in Table 4.8. Sampling points S1 to S6 represent samples taken from Stations B and D of the Aprepre River (known to receive effluent from both the LSGM and ASGM activities [through application of cyanidation by LSGM, amalgamation and cyanidation of Hg-contaminated tailings by the ASGM]). SA to SD are samples taken from Station G of the Ankobra River (close to the ASGM operation site [exclusively amalgamation]; but several kilometres away from processing sites engaged in cyanidation of Hg-contaminated tailing and from LSGM).

MeHg levels in sediment from the Aprepre River (S1 to S6) vary between 4.58 and 14.8 ng/g (1.41 to 3.66% of THg as MeHg; with 241 to 415 ng/g dry mass of THg). MeHg from the Ankobra River (SA to SD) ranged from 0.24 to 1.21 ng/g (0.08 to 0.35% of THg as MeHg; with THg levels ranging from 162 ng/g to 490 ng/g). The stations (SA to SD) are further away from cyanide exposure due to the absence of processing sites engaged in cyanidation of Hg-contaminated tailing and may be the absence of a LSGM company within the catchment.

Free cyanide, weak acid dissociation (WAD) cyanide and total cyanide (TCN) levels in sediment samples from the Aprepre River vary from 0.05 to 0.21; 1.05 to 3.90; and 4.76 to 13.9 mgCN/kg sediment, respectively, and are presented in Table 4.8. About 60% of measured free cyanide (ranging from 0.11 to 0.21 mg/kg) were above the USEPA (1977) sediment standard of 0.1 mg/kg; and within 0.1 to 0.25 mg/kg USEPA (1977) sediment standard for moderate pollution. Hence the sediment from the Aprepre River is contaminated with cyanide. This may be due to the continuous increasing number of processing sites engaged in cyanidation of Au-rich Hg-contaminated tailings, and to a lesser extent the proximity of the tailing dam and sump of a LSGM processing facility at Dumasi (Tulasi et al., 2021). Free, WAD, and TCN levels were all below the instrumental detection limit of 0.001 mg/kg in the Ankobra River. The insignificant levels of cyanide in the Ankobra River may be attributed to the absence of processing sites engaged in cyanidation of Hg-contaminated tailings around the catchment area of the Ankobra River where the study was undertaken. This may also be as a result of natural degradation of cyanide by means of oxidation or photodecomposition (Pereira and Neto, 2007), and long distance from the source of cyanide pollution. Generally, it was observed that the level of cyanide decreases with distance from the source of pollution. It was therefore observed that samples from the Aprepre River with high cyanide levels compared to the Ankobra River show higher MeHg levels, as well as high %THg as MeHg. This could possibly be attributed to the interaction between cyanide and mercury. Which leads to the formation of mercuric cyanide [$(Hg(CN)_2$] and causes Hg to be highly soluble and bioavailable (Marshall et al., 2020) for methylation (Velasquez-Lopez et al., 2011; Veiga et al., 2006; Coles and Cochrane, 2006). Guimaraes et al. (2011) reported low MeHg in free cyanide- contaminated stream. This is contrary to what was found for this study, probably due to relatively low free cyanide (about an average of 6.3% of WAD); with about 40% of free cyanide (in samples) below the USEPA (1977) guideline value.

MeHg levels in the river sediments showed a positive relationship with free, WAD and total cyanide (Free-CN: $r^2 = 0.5974$, $p < 0.01$; WAD: $r^2 = 0.632$, $p < 0.05$ [Fig. 4.9]; TCN: $r^2 = 0.788$, $p < 0.0\ 1$). Also, the percentage of THg as MeHg shows a positive relationship with all the cyanide species (Free-CN: $r^2 = 0.773$, $p < 0.01$; WAD: $r^2 =$

0.749, p <0.05; TCN: r^2 =0.777, p < 0.01) in all the selected sediment samples from both rivers.

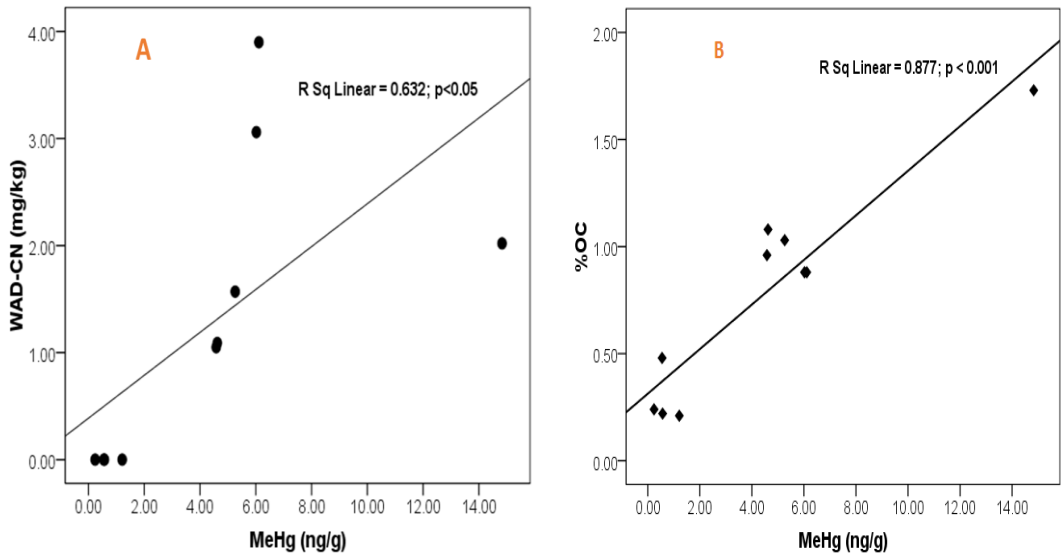


Figure 4.9: Correlation of MeHg with: (A) WAD cyanide, (B) organic carbon.

MeHg correlated positively (r^2 = 0.877; p < 0.001; n = 10; Fig. 4.9) with organic carbon in all ten (10) randomly selected sediment samples from the Aprepre and the Ankobra Rivers. The Aprepre River sediments are characterized by higher organic carbon content (0.9 to 1.73%) compared to the Ankobra River (0.21 to 0.48 %). An increase in MeHg levels in sediment with increasing organic carbon has also been observed in several studies (Wright and Hamilton, 1982; Warner et al., 2005; Chavon et al., 2011; Tulasi et al., 2017). Sediment from the Aprepre River (mid to downstream) recorded significantly higher MeHg levels with a THg as MeHg maximum fraction of 3.7% compared to 0.35% for the Ankobra River (Table 4.8). Naturally in sediment, only about 1 to 1.5% of total mercury content occurs as methylmercury, and even much lower (typically <0.5%) in estuarine and marine sediment (Kelly et al., 1995; Ullrich et al., 2001). Though an increase in both cyanide and organic carbon increases MeHg levels, organic carbon is not the main factor for the higher MeHg level in the Aprepre River. This is because mean organic carbon level of 0.99 % in sediment from the Reference Station shows no difference from that reported in Stations B and D of the Aprepre River. However, the reference station reported the highest cyanide level of 0.49 mg/kg which is higher than that reported for Station B and D by a factor of about 2. Mean MeHg levels for the reference station vary from 0.23 to 1.83 ng/g; with 0.74 to 3.98% of THg occurring as MeHg. Hence, the high percentage of THg occurring as MeHg shows Hg methylation is the highest at the reference station due to high levels of cyanide. Also normalizing MeHg with OC (MeHg-OC) and correlating with FCN, WAD and TCN shows a positive correlation of r^2 = 0.6227, p<0.05; r^2=0.747, p>0.01; and r^2=0.798, p<0.01, respectively. Hence, the presence of cyanide is a major factor for the high MeHg levels in the Aprepre River.

Table 4.8: Mercury water-soluble fraction in sediment.

			Physico-chemical parameters of River water									Water-soluble Hg (Milli-Q)		Water-soluble Hg (Rain H2O)	
River Body	**Location**	**Sample code**	**Temp (°C)**	**pH**	**salinity**	**OC (%)**	**FCN (Mg/kg)**	**WAD (mg/kg)**	**TCN (mg/kg)**	**THg (ng/g)**	**MeHg (ng/kg)**	**24 hr (ng/g)**	**(%)**	**24 hr (ng/g)**	**(%)**
Aprepre	Station B &D	S1	26.5	7.3	0.3	0.88	0.21	3.90	8.3	241	6.11	0.67±0.06	0.28	1.04± 0.1	0.43
		S2	25.2	7.4	0.1	1.73	0.16	2.02	10.7	405	14.8	1.67±0.1	0.4	2.4±0.1	0.6
		S3	27.4	7.1	0.1	0.88	0.14	3.06	13.9	415	6.02	1.38±0.06	0.3	1.21±0.1	0.3
		S4	27.1	7.0	0.0	1.03	0.08	1.57	7.84	364	5.26	0.84±0.06	0.2	1.22±0.04	0.3
		S5	27.1	7.2	0.0	1.08	0.11	1.09	4.95	306	4.62	1.60±0.09	0.5	1.42±0.09	0.5
		S6	27.0	7.0	0.0	0.96	0.05	1.05	4.76	324	4.58	1.51±0.08	0.47	1.16±0.14	0.36
Ankobra	Station G	SA	27.3	7.7	0.0	0.21	<0.001	<0.001	<0.001	490	1.21	1.60±0.07	0.33	1.73±0.13	0.35
		SB	27.3	7.5	0.0	0.22	<0.001	<0.001	<0.001	162	0.57	0.57±0.02	0.35	0.60±0.04	0.4
		SC	27.2	7.7	0.0	0.48	<0.001	<0.001	<0.001	175	0.55	0.16±0.03	0.09	0.31±0.1	0.2
		SD	27.5	7.5	0.0	0.24	<0.001	<0.001	<0.001	307	0.24	0.48±0.03	0.16	0.87±0.1	0.28

TCN: Total cyanide OC: Organic carbon FCN: Free cyanide WAD: Weak acid Dissociation cyanide

4.5 Mercury Water-Solubility Fraction in Sediment

To ascertain the potential biological uptake for Hg methylation, the Hg water-soluble fractions in sediment from each river were assessed. The solubility test was performed using Milli-Q water and rain water (Rodrigues et al., 2010; Reis et al., 2014). Twenty-four (24) hours of extraction was selected for the test. This is because beyond 24 to 48 hours of extraction, the amount of leachable Hg was the same. The results obtained for the Hg water-soluble fraction in sediment samples from the Aprepre and Ankobra Rivers are presented in Table 4.8 (expressed in absolute values as well as in percentage of total content). The milli-Q Hg water-soluble fraction in the sediment ranged from 0.16 to 1.67 ng/g; for rain water extraction, the fraction of Hg soluble in rain water ranged from 0.60 to 2.4 ng/g.

A critical assessment of the percentage of Hg water-soluble leached using Milli-Q and rain water (Table 4.8) shows that in general, solubility of Hg was slightly higher in rain water than Milli-Q water. On the whole, the percentage of the water-soluble fraction of Hg in the samples was below 1% (that is 0.09 - 0.6%). Milli-Q water is usually used in various schemes designed to extract water-soluble fractions from soils and sediment. In this study, rain-water was also used as an extractant alongside Milli-Q water, because it reflects the natural conditions more realistically. In the case of river sediments, the influence of rain-water is most important after precipitation events and consequent elevated water levels of the river that in turn can affect mobility and availability of Hg. In addition, due to the presence of various minerals, rain-water can be slightly acidic, which might contribute to better extraction efficiency of Hg compared to milli-Q water. This approach was used due to the absence of clean river water matrix that had no particulates. In addition, in-situ river water was probably already enriched with Hg.

Weak positive correlation ($r^2 = 0.3067$; $p < 0.05$; $n = 10$) was observed between MeHg levels and water-soluble mercury content. Also, the percentage of THg as MeHg also shows a weak positive relationship with water-soluble mercury content ($r^2 = 0.2079$; $p < 0.01$; $n = 10$). The highest Hg water-soluble fraction obtained in this study was observed in sediments from the Aprepre River (0.67 -1.67 ng/g with milli-Q; and 1.04 – 2.4 ng/g for rain-water, respectively) with corresponding high MeHg levels. Therefore, Hg would be potentially bioavailable in sediment from the Aprepre River rather than the Ankobra River. This is in agreement with similar observations by Wahle and Kordel (1997) and Reis et al. (2014).

4.6 Hierarchical Cluster Analysis

The dendrograms obtained using both variables and sampling sites show two main clusters (Cluster 1 in Fig. 4.10 consisting of MeHg, CN, OC and solubility; and Cluster 2 consisting only of THg. The grouping of solubility, MeHg, CN and OC in Cluster 1 can be attributed to the influence of CN, OC and Hg solubility on MeHg contents. Also MeHg together with CN shows that this system is contaminated with both Hg and CN. Cluster 1 in Fig. 4.11 consists of sampling sites S1, S2, S3, S4, S5, S6 (Aprepre River) and SA (Ankobra River) which receives effluent containing both CN and Hg. The existence of only Hg in Cluster 2 of Fig. 4.10 shows that there is no CN contamination in this system and could be attributed to the Ankobra River consisting of sampling sites SB, SC and SD.

Interestingly, sampling site SA in the Ankobra River clustered with the sampling sites in the Aprepre River. This could be a result of the mobility of CN from the Aprepre River into the Ankobra River (through the Mansi River, a tributary of the Ankobra River). There is a likelihood that traces of cyanide may be present at the SA sampling site due to its closeness to the Aprepre River before the CN finally degrades completely as it transports to sampling site SB, SC and SD. Cyanide was below the detection limit (Table 4.8) in the samples from the Ankobra River as a result of degradation in the presence of sunlight as it travels down the stream.

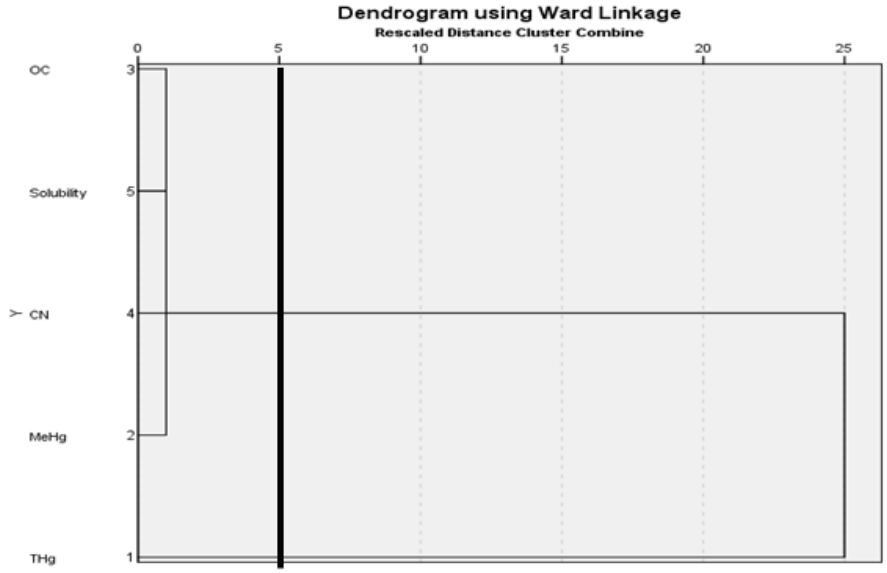


Figure 4.10: Dendrogram showing clustering of parameters.

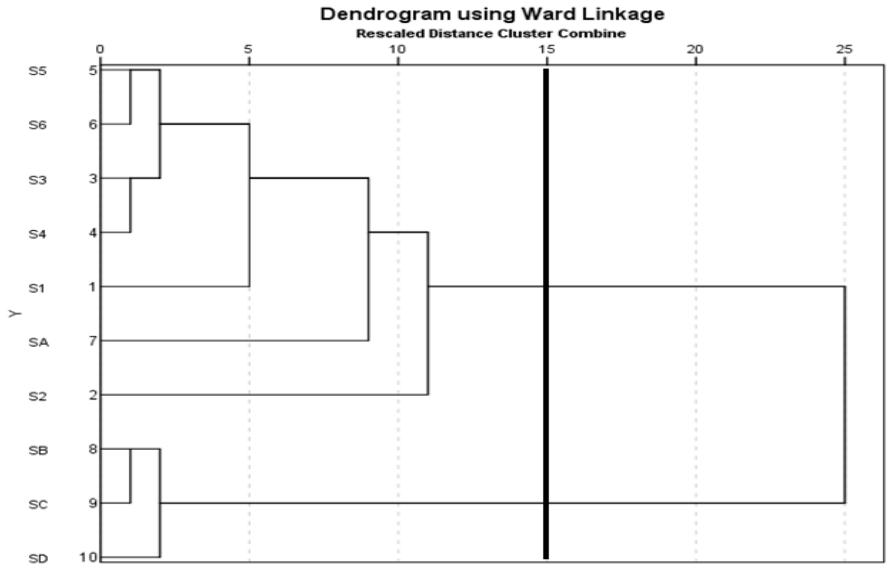


Figure 4.11: Dendrogram showing clustering of sampling sites.

4.7 Thermal Fractionation of Mercury Compounds in Sediment

The possible mercuric compounds present in the sediment samples from the two rivers were determined using temperature fractionation. The results for thermal release of Hg compound from sediment samples are presented in thermo-desorption curves in Fig. 4.12. The mercuric compounds and the corresponding temperatures obtained from this study will be discussed in relation to the work done by Rumayor et al. (2013) on pure mercury compounds and their temperatures (Table 4.9).

The thermograms for all the samples analysed generally show two clear distinguishable peaks (Fig. 4.12). The first peak on each thermogram (Fig. 4.12 a-d), released at 20-360 °C, suggests the presence of complexes of Hg with one or a combination of the following; humic material, $HgCl_2$ and $Hg(CN)_2$ with reference to thermal desorption temperature by Rumayor et al. (2013) [Table 4.9]. The second peak for each thermogram (Fig. 4.12 a-d) was released at 420-600 °C, which is most likely to contain Hg_2SO_4 (Rumayor et al., 2013) [Table 4.9]. It was observed that the first peaks on thermograms from sediment samples from Hg-contaminated cyanide-loaded areas (Fig. 4.12 a-b) were distinct with a maximum peak height temperature of 270 °C.

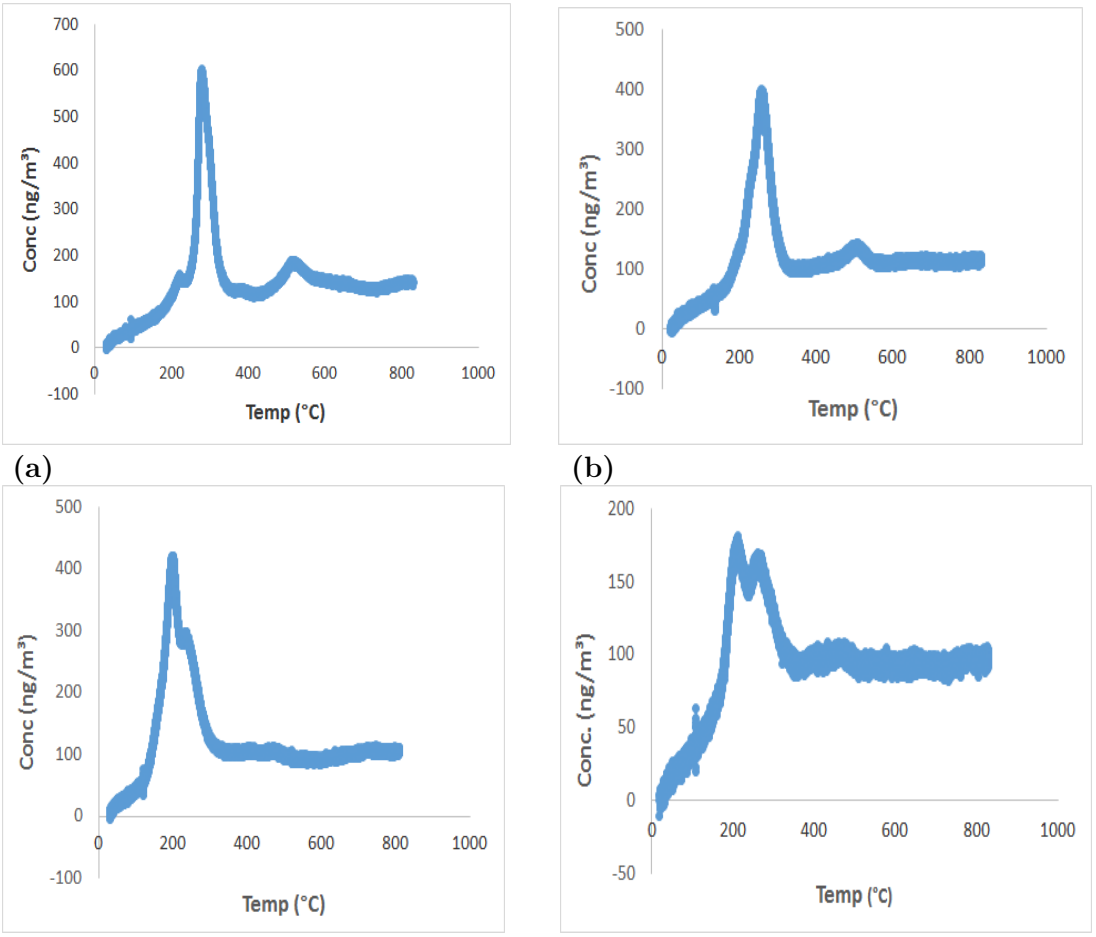


Figure 4.12: Thermograms of sediment samples (Aprepre: a, b; Ankobra River: c, d).

This is comparable to the maximum peak height of 267 °C reported for $Hg(CN)_2$ by Rumayor et al. (2013) [Table 4.9]. Hence, the composition of the first peaks (Fig. 4.12 a-b) is likely to contain a high proportion of $Hg(CN)_2$. The first peak on the thermograms for the sediment from the Ankobra River occurs at 220 °C. This maximum temperature does not correspond to the temperature for $Hg(CN)_2$ reported by Rumayor et al. (2013). This is a confirmation of the absence of cyanide in the Ankobra River.

Table 4.9: Thermal dissociation temperature corresponding to the pure mercury compounds (Rumayor et al., 2013).

Mercury Compound	**High Peak Temp. (°C)**	**Start Temp. – End Temp. decomposition Peak (°C)**
HgI_2	100 ± 12	60-180
$HgBr_2$	110 ± 9	60-220
Hg_2Cl_2	119 ± 9	60-250
$HgCl_2$	138 ± 4	90-350
HgS_2 red	305 ± 12	210-340
HgF_2	234 ± 42; 449 ± 12	120-350; 400-500
HgO red	308 ± 1; 471 ± 5	200-360; 370-530
HgO yellow	284 ± 7; 469 ± 6	190-380; 320-540
Hg_2SO_4	295 ± 4; 514 ± 4	200 -400; 410-600
$HgSO_4$	583 ± 8	500-600
$Hg(SCN)_2$	177 ± 4; 288 ± 4	100-220; 250-340
$Hg(CN)_2$	267 ± 1	140-360
$Hg(NO_3)_2 \cdot H_2O$	215 ± 4; 280 ± 13; 460 ± 25	150-370; 375-520
$Hg_2(NO_3)_2 \cdot 2H_2O$	264 ± 35; 427 ± 19	120-375; 376-500
$HgCl_2O_8 \cdot H_2O$	273 ± 1; 475 ± 5; 590 ± 9	154-360; 380-510; 520-650

4.8 Total and Methyl Mercury Distribution in Water Samples

Water samples from 7 stations were collected. In each station, an average of about 4 samples were collected. The results for the mean THg and MeHg levels in all sampling stations (Station A, B, C, D, E, F and G) from the Aprepre and Ankobra Rivers are shown in Fig 4.13. THg concentration in all the sampling stations of the surface water samples varies between 2.0 to 138 ng/L, with a mean range of 2.2 to 121 ng/L (Fig. 4.13). The highest THg values (99 – 138 ng/L) were found in the Ankobra River (Station

E to Station G). Station A, the reference station located upstream of the Aprepre River has the lowest concentration ranging from 2.0 to 2.4 ng/L. From mid- to down-stream of the Aprepre River THg levels range from 7.3 to 88 ng/L with the highest values found at Station D due to its distal location to two ASGM operation sites (Hg pollution sources through amalgamation and amalgam roasting).

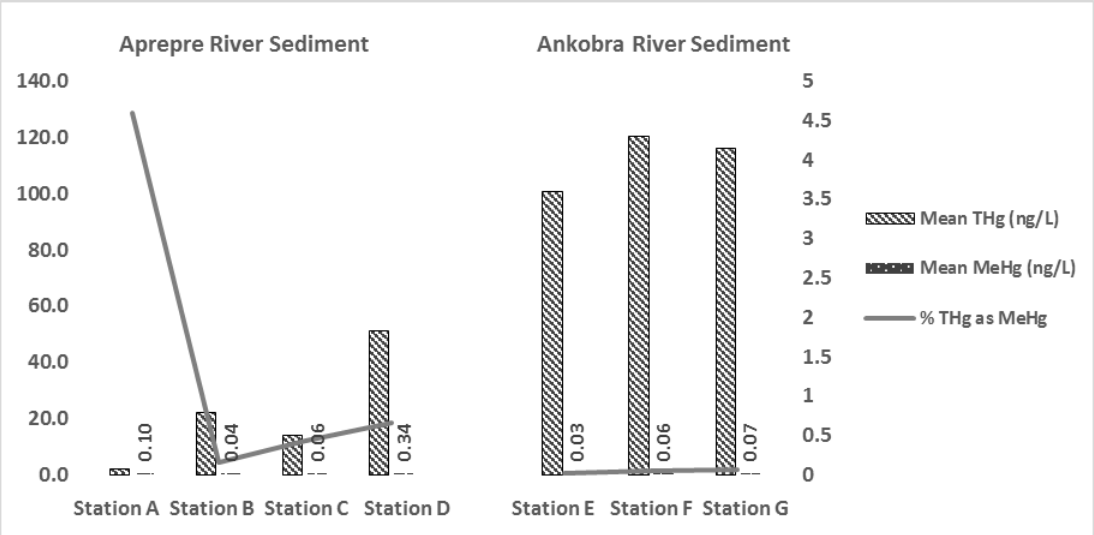


Figure 4.13: Distribution of mean THg and MeHg levels in water samples.

In general, THg level in the Ankobra River is higher than in the Aprepre River, which was contrary to what was observed with the sediment samples. This is because the Ankobra River has high average total suspended solids (TSS) of 830 mg/L while that of Aprepre is 2 mg/L. High TSS is due to the activities of alluvial miners in the Ankobra River (Station E, Station F, and Station G). This therefore confirms the fact that mercury tends to be bound to particulate matter in H_2O samples, hence the much higher concentrations in the Ankobra River. Results obtained in this study are comparable to literature data (Table 4.7). UNIDO (2001) observed higher THg concentration varying from 140 to 760 ng/L in the Aprepre River. The highest THg level was higher than that observed in this study by a factor of about 9. Literature data from the Ankobra River also established a high THg level from 1000 to 8000 ng/L (Akabzaa and Yidana, 2011). The maximum level is extremely higher than that recorded in this study by a factor of about 11. THg levels from all the stations in this study were far above the reference station A. Also about 26% of the water samples from the Aprepre River were below the USEPA standard of 12 ng/L for protection against toxic levels of bioaccumulation in aquatic organisms (USEPA, 1985). While all the samples from the Ankobra River were all above the USEPA standard of 12 ng/L. The concentrations of THg in all the water samples from this study were much below water quality guidelines for THg including: (a) WHO international drinking water Hg guideline of 6,000 ng/L (WHO, 2005), (b) WHO THg standard of 1000 ng/L (WHO, 1976), (c) USEPA drinking water THg guideline of 2,000 ng/L (USEPA, 2009), (d) USEPA guideline of 770 ng/L recommended to protect aquatic wildlife against chronic effects of Hg (USEPA, 1995). MeHg levels in all 7 stations vary from 12 to 463 pg/L with a mean range of 30 to 340 pg/L (Fig 4.13). Higher MeHg levels were found in the Aprepre River. These levels are quite comparable to those reported in literature. MeHg levels in surface natural waters are generally in the

range of 20 to 100 pg/L (Bloom et al., 1989). This is quite similar to that reported in this study. However, Bloom et al., (1989) reported 4000 pg/L in the bottom of a pristine lake water. Also, Gerson et al., (2018) found MeHg level ranging from 6.6 pg/L to 68000 pg/L. This is extremely higher than that reported in this current study. Though the Ankobra River recorded higher THg levels in the water samples than in the Aprepre River, it has the lowest MeHg concentration, which implies that MeHg production is low in the Ankobra River.

The percentage of THg as MeHg ranges between 0.06 to 4.84% and 0.04 to 0.09% in water samples from the Aprepre and Ankobra Rivers, respectively (Table 4.10). Fractions of THg as MeHg are generally higher in water samples than in sediment samples (Ullrich et al., 2001). These were also observed in this study with the maximum proportion of THg as MeHg (4.8%) in the water samples higher than that in the sediment samples (3.7%). This study observed <5% of total mercury as MeHg, though up to about 30% has been found in other studies in freshwaters (Kudo et al., 1982; Bloom et al., 1989). The reference station from the Aprepre River with the least (background) THg levels recorded the maximum fraction of THg as MeHg ranging from 4.38 to 4.84%. This is probably because it recorded the highest CN levels (ranging from 0.77 to 0.82). Located up-stream of Aprepre River in Dumasi, the reference station has ASGM activities involving exclusive cyanidation of Au-rich Hg-contaminated tailings. Sporadic CN spillage/leakage from LSGM company due to proximity has also been reported.

A strong positive correlation (r^2 = 0.8615; p = 0.001; Fig. 4.14) was found between MeHg and CN. Hence the presence of cyanide in the Aprepre River influence methylation and the higher MeHg level in that river as discussed above for the sediment samples. DOC and methyl mercury also show a positive correlation (r^2 = 0.613; p = 0.001; Fig. 4.14).

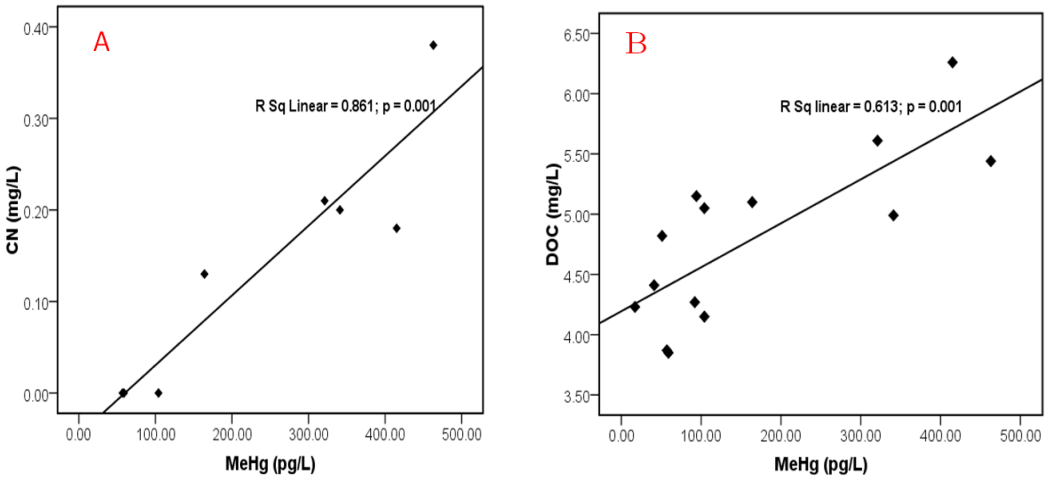


Figure 4.14: Correlation of MeHg with: (A) cyanide (B) dissolve organic carbon (DOC).

Numerous studies (Gray et al., 2015; Alpers et al., 2013, Chavon et al., 2011) observed a similar (positive) correlation between MeHg and DOC. It was suggested that MeHg level is positively influenced by the presence of DOC. Although a positive correlation was found between DOC and MeHg in this study, it was not the main determining factor for the MeHg level. Cyanide has a greater positive influence on methylation. This is because

OC content in the reference station (upstream of Aprepre River) was similar to that obtained downstream of the Aprepre River. However, cyanide levels in the reference station were higher than those obtained in the other site of the Aprepre stream by a factor of about 4 and recorded the highest percentage of THg occurring as MeHg. This suggests that methylation is higher with high cyanide as was also observed for the sediment samples.

Table 4.10**:** Sampling locations with physicochemical parameters, together with concentration of THg, MeHg and % of Hg as MeHg in non-filtered water samples.

			Physical Parameters							Chemical Parameters			Hg Contents		
River Body	**Location**	**Sample Code**	**pH**	**Temp (°C)**	**Cond. (µs/cm)**	**Salinity**	**Alkalinity (mg/L)**	**TDS (mg/L)**	**TSS (mg/L)**	**BOD (mg/L)**	**DOC (mg/L)**	**TCN (mg/L)**	**THg (ng/L)**	**MeHg (pg/L)**	**% MeHg**
Aprepre	Station A (Reference station)	WA	6.9	25.2	1048	0.5	194	512	4	3.45	5.61	0.82	2.4	104	4.38
		WB	7.0	25.0	1021	0.4	88	525	5	2.03	4.82	0.77	2.0	94	4.84
	Station B	WC	7.5	27.9	526	0.2	120	264	0	3.45	5.15	0.47	7.3	51	0.70
		WD	7.3	28.6	524	0.2	368	265	0	3.05	4.99	0.28	28	17	0.06
		WE	7.4	28.2	523	0.2	294	265	0	2.64	6.26	0.33	27	41	0.15
		WF	7.5	28.2	524	0.2	250	264	0	2.27	5.10	0.41	40	92	0.23
	Station D	W1	7.1	27.4	296	0.1	294	148	0	3.45	544	0.21	66	415	0.63
		W2	7.0	27.0	261	0.0	306	131	0	3.05	427	0.20	51	341	0.67
		W3	7.0	27.1	287	0.0	290	137	0	3.18	505	0.13	28	164	0.58
		W4	7.2	27.0	245	0.0	320	122	0	3.45	441	0.38	88	463	0.53
		W5	7.0	27.0	241	0.0	312	118	0	3.05	385	0.18	24	321	1.36
Ankobra	Station G	WR	7.7	27.2	105	0.0	362	52.7	800	3.05	423	<0.001	138	57	0.04
		WS	7.5	27.3	105	0.0	346	52.4	871	3.45	415	<0.001	110	104	0.09
		WT	7.7	27.3	105	0.0	352	52.7	742	2.64	387	<0.001	101	59	0.06

4.9 THg and MeHg Level in Fish Species

Total and methyl mercury levels in edible muscle tissue of different fish species from downstream of the Aprepre and Ankobra Rivers are presented in Table 4.11. Thirty-three fish samples were analysed (covering nine fresh water fish species). THg and MeHg levels in all the fish species vary from 196 to 4618 ng/g (49 - 1169 ng/g ww) and 162 to 4213 ng/g dw (40 - 1067 ng/g ww), respectively. There was a strong positive relationship (r^2=0.9792; $p < 0.001$; n=33) between THg and MeHg. Several studies have proven that virtually if not all of the mercury present in fish mussel tissue is in the form of MeHg (WHO, 1976; Bloom, 1992; Storelli et al., 2005). Storelli et al. (2005) reported a percentage ranging from 60% to 100% in the mussel of two fish species. The % of THg as MeHg in all the fish samples ranges from 60 to 97% with about 83% of these above 80% of the THg occurring as MeHg. The mean percentage of THg occurring as MeHg is 84%. Hence mercury levels in fish are regarded to be an ideal indicator of human exposure to MeHg contamination in this study.

THg levels of fish species from the Aprepre and Ankobra Rivers exceeded the FAO/WHO recommended maximum limit of 500 ng/g (FAO/WHO, 2004) by 33.3% and 8.3% respectively. Mean values of MeHg levels of the various fish species from the Aprepre and Ankobra Rivers are shown in Fig. 4.15. Mean MeHg level of fish species from the Ankobra River is below the USEPA methyl mercury guideline of 300 ng/g (USEPA, 2001) for human health protection and exceeded the wildlife limit of 100 ng/g by 50% (mean MeHg = 64 to 256 ng/g ww; wt: 27 to 251 g). While 33.3% of mean MeHg level of fish species from the Aprepre River is above the USEPA guideline of 300 ng/g ww and 100% above the 100 ng/g wildlife limit (mean MeHg = 148 to 741 ng/g ww; wt = 23 to 223 g). On the whole, MeHg levels were higher in fish species from the Aprepre River than from the Ankobra River, with Parachanna Obscura (snake-head fish) recording the highest. This is anticipated, since the highest MeHg levels in water and sediment (with the higher Hg-water-soluble fraction, and the main site for Hg methylation) were measured in samples from the Aprepre River (Fig. 4.7; Table 4.10). The Hg contaminated CN-loaded Aprepre River has the higher THg and MeHg concentrations in fish species. The lower MeHg levels found in fish species from the Ankobra River despite containing the highest THg levels in sediment than that from the Aprepre River are due to low MeHg production in the sediment and also because fish accumulate more methylmercury than inorganic mercury.

Table 4.11: Total mercury (THg), methylmercury (MeHg) concentrations (ng/g dry wt) and percentage of methylmercury (%MeHg) with respect to total mercury in fish species.

River Body	Sample Code	Species Name	Common Name	Weight (g)	MC (%)	THg (ng/g) dw	MeHg (ng/g) dw	THg (ng/g) dw	MeHg (ng/g) dw	MeHg (%)
Aprepre	FA	*Parachanna Obscura*	Snake Head Fish	221	74.3	3230	2877	830	739	89.1
River	FB	*Parachanna Obscura*	Snake Head Fish	316	74.7	4618	4618	1169	1067	91.3
	FC	*Parachanna Obscura*	Snake Head Fish	156	75.2	2030	2030	504	484	96.0
	FD	*Parachanna Obscura*	Snake Head Fish	200	75.0	2984	2984	745	680	91.3
	FE	*Heterobranchus bidorsalis*	Cat Fish	76	70.1	1690	1690	506	319	66.2
	FF	*Heterobranchus bidorsalis*	Cat Fish	53	70.5	1125	1125	331	250	75.3
	FG	*Heterobranchus bidorsalis*	Cat Fish	36	71.9	577	577	162	157	96.6
	FH	*Heterobranchus bidorsalis*	Cat Fish	29	72.3	740	740	205	197	96.0
	FI	*Heterobranchus bidorsalis*	Cat Fish	22	70.1	325	325	97	85	87.4
	FJ	*Heterobranchus bidorsalis*	Cat Fish	30	70.8	817	817	239	191	80.2
	FK	*Oreochromis niloticus*	Nile Tilapia	48	72.4	1904	1904	525	319	60.8
	FL	*Oreochromis niloticus*	Nile Tilapia	32	72.1	1306	1306	365	256	70.3
	FM	*Limbochromis sp*	Tilapia	27	73.4	1944	1944	517	471	91.2
	FN	*Limbochromis sp*	Tilapia	23	75.1	1749	1749	436	384	88.2
	FO	*Limbochromis sp*	Tilapia	24	70.3	1652	1652	491	403	82.1
	FP	*Limbochromis sp*	Tilapia	18	72.3	1551	1551	429	353	88.2
	FQ	*Brycinus nurse*		29	73.7	667	667	175	157	89.4
	FR	*Brycinus nurse*		31	74.0	758	758	197	188	92.1
	FS	Tilapia zillii	Red belly Tilapia	67	74,9	503	503	126	108	85.3
	FT	Tilapia zillii	Red belly Tilapia	73	71.2	789	785	226	200	88.6
	FU	Tilapia zillii	Red belly Tilapia	59	70.9	527	529	154	140	91.0
Ankobra	F1	*Penaeus species*	Schrim	26	73.5	356	356	212	56	59.6
River	F2	*Penaeus species*	Schrim	33	74.2	408	408	277	71	68.0
	F3	*Penaeus species*	Schrim	21	73.5	370	370	244	65	66.0
	F4	*Chrysichthys nigrodigitatus*	Bagrid Catfish	63	70.6	1087	1087	927	273	85.1
	F5	*Chrysichthys nigrodigitatus*	Bagrid Catfish	50	72.3	902	902	803	222	88.6
	F6	*Schilbe mystus*	African butter catfish	13	74.9	297	297	278	70	93.6
	F7	*Schilbe mystus*	African butter catfish	26	73.2	504	504	461	124	91.5
	F8	*Schilbe mystus*	African butter catfish	130	76.5	2553	2553	2319	545	90.8
	F9	*Tilapia zillii*	Tilapia	345	72.4	260	260	241	67	92.8
	F10	*Tilapia zillii*	Tilapia	262	71.9	738	738	667	188	90.4
	F11	*Tilapia zillii*	Tilapia	230	71.3	555	555	489	140	88.2
	F12	*Tilapia zillii*	Tilapia	166	75.1	196	196	162	40	82.4

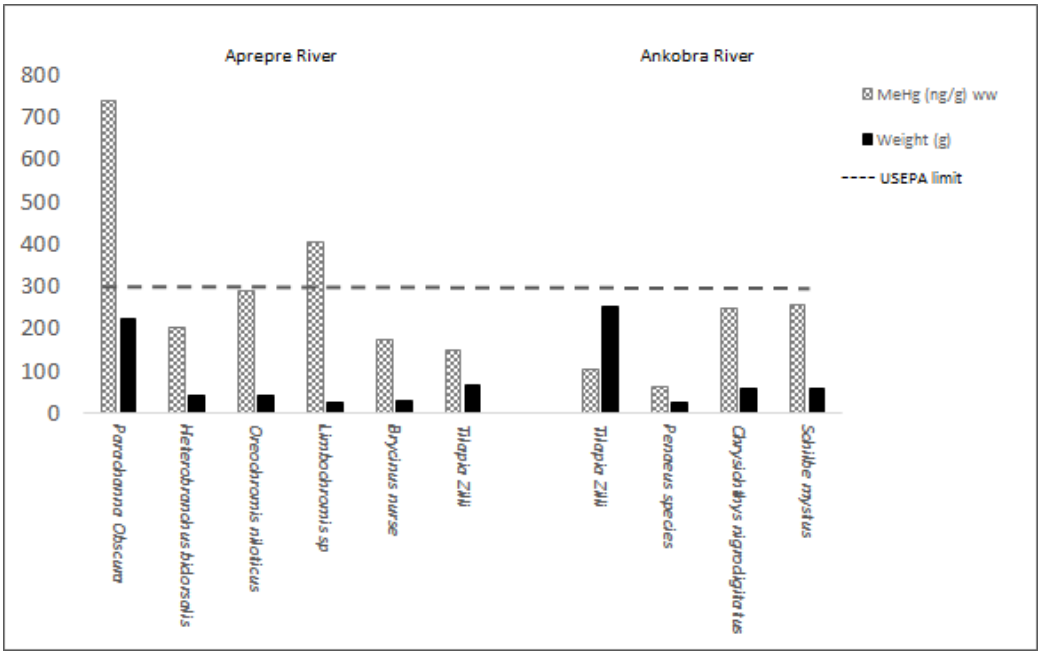


Figure 4.15: Mean values of MeHg concentrations and weight of fish species by locations.

From this study, the results in Fig. 4.15 reveal that the difference in Hg content in the fish samples analysed depends on the fish species, the weight, feeding habit and location of the fish. This has already been established by several studies that the variations of Hg levels in different species of fish is according to their weight, habitat, trophic levels and feeding habits (UNIDO 2001; Storelli et al., 2005; Voegborlo and Adimado, 2010; Tulasi et al., 2019a). Diet is generally recognized as the primary way of Hg exposure to aquatic organism. Since MeHg bioaccumulates in fishes, the highest Hg levels are mostly found in larger or older fishes at the end of the food chain such as in large carnivorous species (Castihos et al., 1998; Voegborlo and Adimado, 2010; UNIDO, 2001; Storelli et al., 2005; Tulasi et al., 2019a) due to long time exposure. In this study, Parachuna obscura (mudfish) with the highest MeHg levels is a carnivorous fish (predatory fish) and also benthopelagic which have close contact with the sediment (site for mercury methylation). Hence, the reason for its highest MeHg level in all the fish species. The majority of fish species (catfish and the various tilapia species) collected in this study are omnivorous and show high MeHg levels. Tilapia zillii, however, are herbivorous.

A positive correlation existed between methyl mercury levels in the carnivorous and some of the omnivorous fish species in the individual water body and fresh weight (Table 4.12; Fig. 4.16).

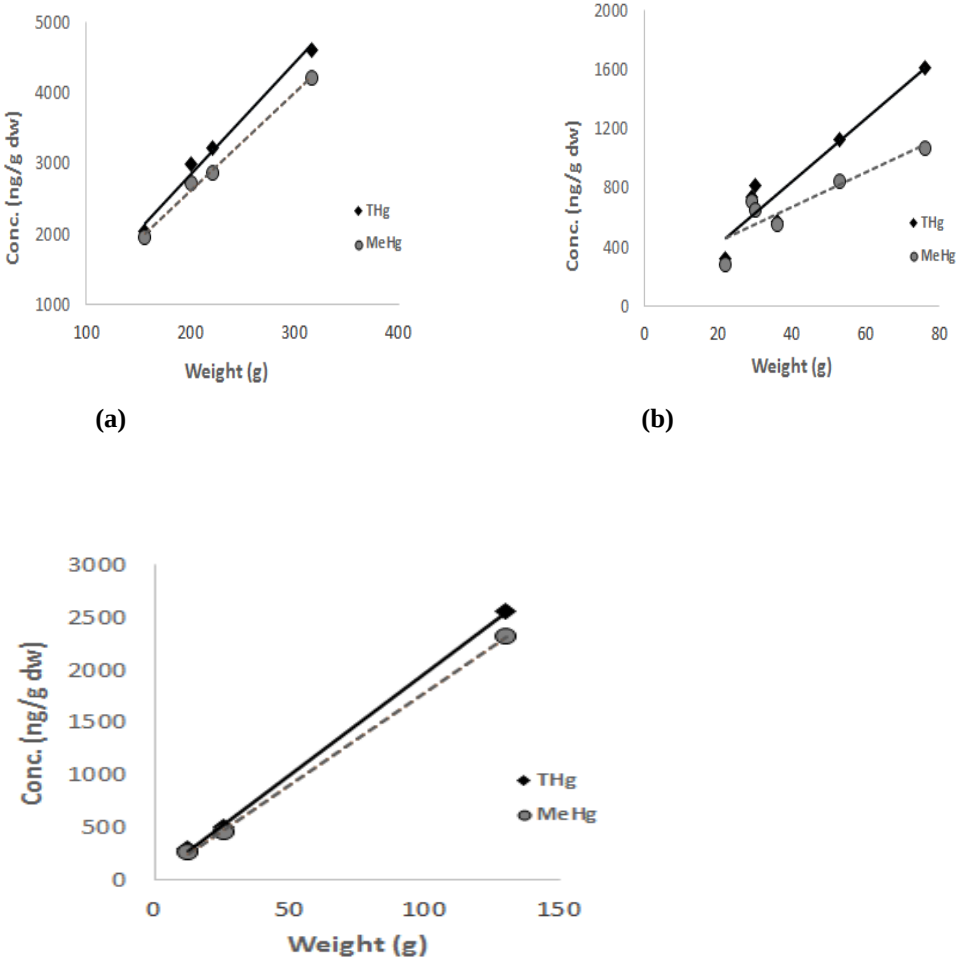


Figure 4.16: Correlation between methylmercury levels in dry weight and weight of fresh fish species (Aprepre River: a - Parachanna obscura, b - Heterobranchus bidorsalis; Ankobra River: c - Schilbe mystus.

A weak positive correlation was however, observed for Tilapia zillii. Several studies have reported a positive correlation between Hg levels in fishes and weight (Storelli et al., 2005; Lange et al., 1994). Carnivorous species usually show a good correlation while a poor relationship is are observed in herbivorous species (Voegborlo et al., 2006; Voegborlo and Adimado, 2010). In these studies, the sample size for each species is small, and hence not much difference was observed between the only carnivorous fish species (Parachanna obscura) and the other omnivorous fish species. Almost all the fish species show a good positive correlation between Hg levels and weight. The positive correlations observed confirm a greater accumulation in larger size fishes than in smaller fishes.

Table 4.12: Correlation between MeHg, THg and weight of fresh fish species.

River Body	Fish Species	Correlation coefficient	
		THg	MeHg
Aprepre River	*Parachanna obscura*	0.9883, $p < 0.01$	0.994, $p < 0.01$
	Heterobranchus bidorsalis	0.8984, $p < 0.01$	0.7838, $p < 0.05$
Ankobra River	*Schilbe mystus*	0.9997, $p < 0.05$	0.9996, $p < 0.05$

Nonetheless, this might also show differently if the same fish species are found in different locations. Hence, the outcome for comparison of MeHg levels between the same fish species (Tilapia zillii) from different locations (Aprepre and Ankobra River) is shown in Fig. 4.17. MeHg levels were higher in species from the Aprepre River than those from the Ankobra River. Although species from the Ankobra River were of larger sizes having an average weight of about four times bigger than that from the Aprepre River. This wide variation is a result of the differences in MeHg levels found in sediment and water samples from Station D (downstream) of the Aprepre River and Station G (downstream) of the Ankobra River (Fig 4.7 and Table 4.10) where these fish species were collected.

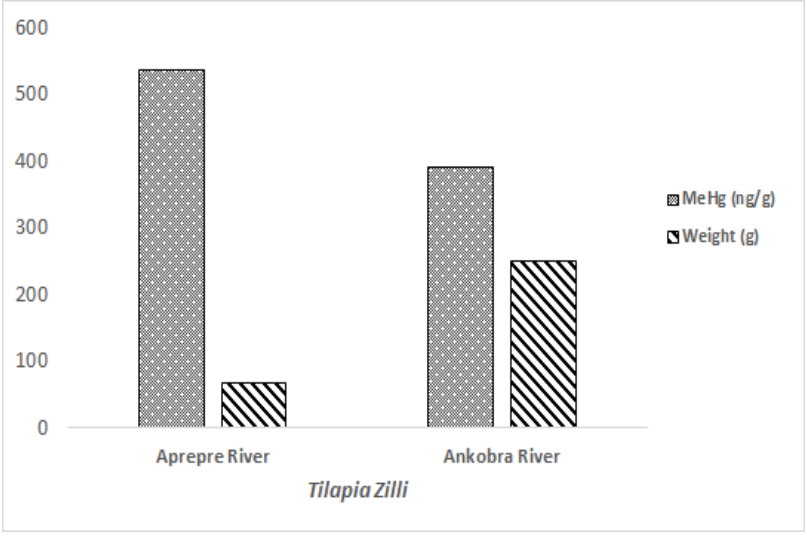


Figure 4.17: Comparison of MeHg levels in dry weight in Tilapia zillii in the two fresh water bodies.

THg levels of fish species in this study are comparable to previous studies reported by UNIDO (2001) from the Aprepre River (same study location). Literature levels reported

in all the fish species (mudfish, catfish, tilapia, lobster and schrim) range from 460 to 6420 ng/g dw. This was slightly higher than that observed in this study. They (UNIDO, 2001) reported the mean THg level of 5125 ng/g dry weight for Parachanna obscura (madfish). This was above the mean THg level of 3216 ng/g dry weight observed in this study for the same species. Mercury levels from this study are markedly higher than those reported by Kwaansa-Ansah et al. (2013) in a non-gold mining region by a factor of about four (4). The THg and MeHg levels (THg: 3.38 – 238 ng/g ww; MeHg: 3.08 – 215 ng/g ww) reported by Kwaansa-Ansah et al. (2013) from the Volta River located at the southern part of Ghana (non-gold mining region) were all below the WHO and USEPA Hg guidelines. This is because the Volta River has not been extensively impacted by anthropogenic deposition of Hg. This shows that the higher levels of Hg in fishes for the current study are as a result of the release of Hg into the environment by the activities of ASGM causing Hg contamination in river bodies in the study area.

Estimation of health risk associated with fish consumption

The public health risks associated with the consumption of fish species were estimated based on the FAO/WHO Provisional Tolerable Weekly Intake (PTWI). The Joint Food and Agriculture Organization and World Health Organization (FAO/WHO) experts on food additives have established a guideline concerning dietary Hg intake. It recommends a Provisional Tolerable Weekly Intake (PTWI) of 300 µg of THg per 60 kg body weight. PTWI for MeHg is recommended not to exceed 100 µg per 60 kg body weight. These guidelines are equivalent to 5 µg/kg body weight for THg and 1.6 µg/kg body weight of MeHg (WHO, 2003b). The Estimated Weekly Intake (EWI) of mercury for each fish species in grams and in µg/kg of body in this study using mean THg and MeHg levels is presented in Table 4.13. The estimated weekly intake of THg and MeHg in µg per 60 kg body weight calculated ranged from 0.04-3.01 µg/kg and 0.03-2.75 µg/kg, respectively, in all the fish species from the Aprepre and Ankobra Rivers. With the exception of Parachanna obscura (with EWI of MeHg as 2.75 µg/kg body weight) from the Aprepre River, the estimated weekly intake of THg and MeHg in all the fish species from both rivers was below the established PTWI limit of 5 µg/kg and 1.6 µg/kg, respectively. The calculated weekly intake of THg concentrations in the Aprepre and Ankobra Rivers is 370-1775 g and 1056-3030 g of fish, respectively, which have to be consumed to attain the PTWI limit of 300 µg. Considering the amount of fish from this study to be consumed to attain the PTWI threshold, compared to 51.7-270.7 kg of fish from the Volta River by Agorku et al. (2009), fishes from this study area, especially from the Aprepre River, are likely to constitute a significant exposure of Hg to the inhabitant. The EWI (g) of fish was calculated using the formula [Eq. 4.1]:

$$EWI(g) = \frac{W(kg) * PTWI(\mu g / kg body weight)}{C(\mu g / g)} \qquad (4.1)$$

W = average body weight (60 kg for adult)

$PTWI$ = 5 µg/kg for THg and 1.6 µg/kg for MeHg

C = Mean THg or MeHg concentration in fish (µg/g)

EWI = Estimated Weekly Intake

The mean number of fish that could be consumed safely in a week can be computed by dividing the EWI (g) by the mean weight of each fish species.

Table 4.13: Estimated Weekly Intake (EWI) of THg and MeHg in fish species.

Location	Fish Species	Mean Wt (g)	Mean THg (µg/g)	EWI_{THg} (g)	EWI_{THg} (µg/kg)	Mean MeHg (µg/g)	EWI_{MeHg} (g)	EWI_{MeHg} (µg/kg)
Aprepre River	*Parachanna obscura*	223	0.810	370	3.01	0.741	130	2.75
	Heterobranchus bidorsalis	41	0.255	1176	0.17	0.203	473	0.14
	Oreochromis niloticus	40	0.445	674	0.30	0.288	333	0.13
	Limbochromis sp	23	0.469	640	0.18	0.404	238	0.15
	Brycinus nurse	30	0.186	1613	0.09	0.172	558	0.09
	Tilapia zillii	66	0.169	1775	0.19	0.148	649	0.16
Ankobra River	*Penaeus species*	27	0.099	3030	0.04	0.064	1500	0.03
	Chrysichthys nigrodigitatus	56	0.284	1056	0.27	0.246	390	0.23
	Schilbe mystus	56	0.281	1067	0.26	0.256	375	0.24
	Tilapia zillii	251	0.119	2521	0.50	0.104	923	0.44

*Calculation of the EWI was done using mean THg and MeHg of each fish species and an average body weight of 60 kg of an adult.

Chapter 5

Conclusions

The study investigated total and methyl Hg in sediment, water and fish from Hg-contaminated cyanide-loaded (Aprepre River) and Hg-contaminated non-cyanide (Ankobra River) aquatic environments in the Prestea Huni-valley district, southwestern Ghana. The study also assessed the Hg water-soluble fraction in sediments from both rivers, as well as identified mercuric compounds by thermal fractionation. Additionally, the depth distribution of total and methyl mercury in soils from artisanal and small-scale gold mining (ASGM) communities were also assessed. Furthermore, Kayzero Instrumental Neutron Activation Analysis (k_0-INAA) was used to assess the levels of THg in soil and sediment; and the results obtained compared with THg levels in soil and sediment determined with CV-AAS.

The use of k_0-INAA to verify the reproducibility of THg level in selected sediment and soil samples analysed with CV-AAS had a relative standard deviation of less than 10% indicating excellent agreement between the two analytical methods. This shows the reliability of the CV-AAS analytical technique used in the study.

The high THg levels in the upper 2 cm soil (7000 to 457931 ng/g) from ASGM and the commercial amalgam roasting sites compared to the levels (1307 to 3778 ng/g) in the corresponding lower 20 cm depth are an indication of anthropogenic release and deposition of Hg from the activities of artisanal and small-scale gold miners in the study area (and not from natural sources). MeHg levels in the upper 2 cm and the corresponding lower 20 cm (0.44-68.9 and 0.09-2.31 ng/g, respectively) of the soil core are all below the USEPA (2013) MeHg residential soil screening level (SSL) of 0.78 µg/g. However, these levels cannot be underestimated since MeHg is bioaccumulative.

MeHg levels (0.17 to 68.9 ng/g) were high in cyanide-contaminated mercury-loaded soils from Dumasi compared with MeHg from non-cyanide Hg-loaded samples from Prestea due to the ability of cyanide to make Hg soluble (highly) for methylation. MeHg and organic carbon (OC) have similar vertical variations with levels decreasing with depth in the soil. Nonetheless, organic carbon contents (1.08 to 3.12 mg/kg) in the upper 2 cm soils from Prestea were higher than levels (0.11 to 1.56) from Dumasi. Hence, the high MeHg levels from Dumasi are mainly due to the presence of cyanide. The high Hg levels (THg and MeHg) in the soils can be taken up by plants and livestock. Hg in the soil samples from ASGM operation sites and the commercial amalgam burning sites are the major sources of Hg in the studied aquatic environment (Aprepre and Ankobra Rivers). The Hg-contaminated effluent from gold extraction at the ASGM operation sites is not directly discharged into aquatic systems, but poured directly on soils. Soils contaminated with Hg are washed into the Aprepre and Ankobra Rivers as well as other water bodies during surface run-off's.

Sediments from the Aprepre River ranged from 4.58 to 14.83 ngMeHg/g (with 241-415 ngTHg/g, and 0.05 – 1.21 mgCN/kg). For the Ankobra River, MeHg ranged from 0.24 to 1.21 ng/g (with THg of 162-490 ng/g dw), with cyanide levels below the instrumental detection limit of <0.001 mg/kg. Accordingly, high levels of MeHg were established in the Hg-contaminated cyanide-loaded aquatic environment (Aprepre River) compared to low levels of MeHg in the Ankobra River (Hg-contaminated non-cyanide aquatic environment). The study also established high organic carbon levels in sediments from the Aprepre River; and a strong correlation between OC and MeHg in sediments from the two rivers. From the Hg water-soluble test (resulting from extraction of Hg using water) conducted on Hg-contaminated sediments from the two rivers, Hg in sediments from the Aprepre River was highly soluble. Accordingly, Hg was more bioavailable for methylation in the Aprepre River, hence the high MeHg levels. $Hg(CN)_2$ was identified as the probable mercuric compound in sediment from the Aprepre River based on thermal fractionation. This therefore indicates that although MeHg levels correlated more highly with organic carbon than cyanide, the high MeHg levels in the Aprepre River sediment are comparatively attributed to cyanide.

THg levels in water samples from the Aprepre and Ankobra Rivers were all below the USEPA and the WHO Drinking Water Hg Guidelines of 2000 ng/L and 1000 ng/L respectively. However, THg levels in almost all the water samples were above the USEPA standard of 12 ng/L for Protection Against Toxic Levels of Bioaccumulation in Aquatic Organisms (USEPA, 1985). The Aprepre River contaminated with both Hg and cyanide had higher MeHg levels than the Hg-contaminated non-cyanide loaded Ankobra River, signifying high methylation rate in the Aprepre River due to the presence of $Hg(CN)_2$.

THg and MeHg levels in the fish species were higher in the Hg-contaminated cyanide-loaded Aprepre River (the same river had the highest MeHg levels in water and sediment). THg and MeHg levels in fish species increased with size (mass) of the fish. About 47.6% and 16.7% of MeHg in fish species from the Aprepre and Ankobra Rivers, respectively, exceeded the USEPA MeHg limit of 300 ng/g for human consumption.

The calculated weekly intake (in ranges) of MeHg through the consumption of fish (for an adult weighing 60 kg [FAO/WHO, 2004]) shows that 130-649 g and 375-1500 g for the Aprepre and Ankobra Rivers, respectively, have to be consumed to attain the Provisional Tolerable Weekly Intake (PTWI) limit of 1.6 µg/kg body weight. The amount of fish from the Aprepre River is however likely to constitute a significant exposure of Hg to the inhabitant in the study area.

The Estimated Weekly Intake [EWI] (0.04-3.01 µg/kg/person for THg; and 0.03-0.44 µg/kg/person for MeHg) in all the fish species from both rivers was both below the established PTWI limit of 5 µgTHg/kg/person and 1.6 µgMeHg/kg/person; except for the fish called Parachanna obscura (snake-head fish) from the Aprepre River with EWI of 2.75 µg/kg/person for MeHg.

Recommendations

In light of the conclusions from the study, the following recommendations are proposed:

a) There is a need for an in-depth study to be conducted for thorough understanding of the processes involved in Hg-CN interaction in the aquatic environment.
b) To minimize the release of metallic Hg into the environment during almagam burning by small-scale and artisanal gold miners, there is a need for strict enforcement of the use of the Hg Distillation Retort (introduced by the Minerals

Commission of Ghana in collaboration with UNIDO) to recycle mercury safely by distilling the amalgam.

c) There should also be strict enforcement of all mining regulations regarding the discharge of cyanide and other toxic mine waste into rivers/streams within the catchment area of the mining companies. In addition, the Environmental Protection Agency should monitor all operations, discharges and the environment to detect any escape of cyanide and subsequent impact of that release through the institution of regular cyanide audits.

d) There is a need for the development of a technology for recovery of elemental Hg in liquid effluent after amalgamation for re-use by small-scale gold miners instead of disposing it into the surroundings. This will help reduce cost of gold extraction, maximize profit, and above all protect the environment from Hg contamination.

e) Sensitization and education of artisanal and small-scale gold miners, local communities within the catchment of the study area. The education should include: **(i)** symptoms associated with Hg exposure to enable the inhabitants seek early medical attention should they experience such symptoms; **(ii)** effects of long-term exposure to Hg.

f) The GEPA should acquire state-of-the art monitoring equipment for Hg and MeHg. This will help in routine monitoring of THg and MeHg levels in environmental media around the catchment area of gold mining communities.

g) The Ministry of Health in collaboration with the District and Municipal Assemblies with support from the Mineral Commission should organize frequent medical screening for the inhabitants of the mining communities.

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Publications Related to the Dissertation

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Biography

The author of this thesis, Ms. Delali Tulasi obtained her Bachelor of Science (B.Sc) degree in Chemistry from the University of Cape Coast, Ghana in 2005. Her BSc thesis was titled '*the impact of gold mining on levels of Hg, As and CN in Drinking and surface water in Bogoso, Southwestern Ghana.'* Ms. Tulasi subsequently enrolled at the University of Ghana's School of Nuclear and Allied Sciences to pursue a 2-year Master of Philosophy (M.Phil.) degree in Nuclear and RadioChemistry. She completed successfully in 2011; and the University of Ghana conferred a Master of Philosophy (Nuclear and RadioChemistry) on her. The title of her MPhil thesis was '*Studies of arsenic species in water and river sediment from Obuasi gold mines using HPLC-NAA'.* Delali was admitted on scholarship to the prestigious Jožef Stefan International Postgraduate School, Ljubljana, Slovenia, for her Doctoral studies in Eco-Technology. She is currently at the final stages of her studies.

Delali had her secondary (High School) education at the Okuapeman Secondary School, Akropong-Akwapim, Southeastern Ghana, and completed in 1998.

On complexion of her Master's degree, Delali Tulasi was appointed an Assistant Research Scientist (ARS) at the Ghana Atomic Energy Commission (GAEC) in September 2012 and posted to the National Nuclear Research Institute. She has been working with GAEC till date. Ms. Tulasi have been a Teaching Assistant at the School of Nuclear and Allied Sciences (SNAS), University of Ghana-Atomic; from August 2013 to date.

Delali had previously (2007-2008), worked as an analyst at Letap Pharmaceutical Company in Accra, Ghana. Her role included analysis of active ingredients in raw materials, intermediate and formulated drugs products. Delali worked with the Public Health Division of the Volta River Authority in Akosombo, Ghana (the sole hydro electric power generator in Ghana) from 2005 to 2006 during her mandatory Post-BSc Ghana National Service.

She has authored and co-authored about 13 articles [10 were published in international and national peer-reviewed journals; and 3 conference papers]. She is a member of the Ghana Chemical Society, and the Ghana Nuclear Society. Notable conference presentations (poster) by Delali were at: 14th International Conference on Mercury as a Global Pollutant (ICMGP 2019) Krakow, Poland; 8-13 September 2019; and the 13th International Conference on Mercury as a Global Pollutant (ICMGP 2017), Providence, Rhode Island (RI), USA; 16-21 July 2017.

Her research interest emcompasses: [1] Trace element speciation (Hg, As) in environmental media (soil, sediment, water and fish) using on-line or at-line high pressure driven separation techniques (HPLC) /gas chromatography (GC) coupled with various detection system (CV-AFS, CV-AAS, NAA) and other allied analytical techniques. [2] Environmental fate of mercury and arsenic.

Ms. Tulasi has participated in a number of international and national training courses, workshops, conferences and seminars. Notable among them are the *MercOx Project Kick Off Meeting* in 2017 at Ljubljana, Slovenia. In 2015, Delali Tulasi participated in the *IsoFood Hg Training Programme on Quality Assurance for Mercury Measurements in Food and Environmental Samples* held in Ljubljana, Slovenia. In 2014, Delali participated in the Abdus Salam International Centre for Theoretical Physics-

International Atomic Energy Agency (ICTP-IAEA) sponsored training school on Nuclear Security, held in Trieste-Italy.

Delali Tulasi was born on May 11, 1980 at Hohoe in the Volta region of southeastern Ghana to Reverend Jack Johnson Komla Tulasi and Mrs. Elizabeth Dekorlenu Tulasi. She is the fourth of seven children.