

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE



FACULTY OF PHYSICAL SCIENCES

DEPARTMENT OF CHEMISTRY

TOPIC:

**DETERMINATION OF MERCURY IN FISH FINS AS A NON-LETHAL
ASSESSMENT METHOD FOR PREDICTING MERCURY LEVELS IN FISH
MUSCLE TISSUES OF TWO SPECIES OF FRESHWATER FISH FROM THREE
DIFFERENT AQUATIC ENVIRONMENTS IN GHANA**

**‘A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY,
FACULTY OF PHYSICAL SCIENCES, COLLEGE OF SCIENCE,
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KUMASI IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF PHILOSOPHY
(ANALYTICAL/ ENVIRONMENTAL CHEMISTRY)**

By

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DECLARATION

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DEDICATION

I DEDICATE THIS DISSERTATION TO GOD ALMIGHTY AND MY FAMILY.

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ABBREVIATIONS

Toxicology Excellence for Risk Assessment	TERA
Dichlorodiphenyltrichloroethane	DDT
Polychlorinated biphenyls	PCB
United States National Academy of Sciences	USNAS
National Institute of Safety and Health	NIOSH,
Food and Agriculture Organization	FAO
World Health Organization	WHO
United States Environmental Protection Agency	USEPA
Total organic carbon	TOC
Acid volatile sulfides	AVS
Metallothioneins	MTs.
Polycyclic aromatic Hydrocarbons	PAH
Cold vapor atomic absorption spectrometry	CVAAS
National Institute for Minamata Disease	NIMD
International Atomic Energy Agency	IAEA
Certified reference material	CRM
Dogfish muscle	DORM
National Research Council of Canada	NRC
Gastro Intestinal	GI

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ABSTRACT

Mercury concentration in various tissues (dorsal fin, caudal fin, pectoral fin, pelvic fin and anal fin) of two species of fish, tilapia (*Oreochromis niloticus*) and mudfish (*Clarias gariepinus*) from three fresh water bodies in Ghana were assessed in order to determine any relationship between them. Fifteen samples each of the two species were collected from Tono Dam at Navrongo, Hydroelectric reservoir at Kpong and White Volta at Yapei.

The fish tissues were digested and analyzed by cold vapour atomic absorption spectrophotometry using an Automatic Mercury Analyzer (Model HG-5000). For all fish tissues in all species, Hg concentrations were greatest in muscle tissue (mean muscle Hg = 0.236–0.680 µg/g wet weight), followed by fin tissues (0.03–0.09 mg/kg dry weight). The coefficient of determination (r^2) derived from regression analysis of species muscle Hg against fin Hg ranged between 0.349–0.823 and 0.278–0.752 for tilapia and mudfish samples from Navrongo, 0.492–0.715 and 0.14–0.37 and 0.737–0.965 for tilapia and mudfish samples respectively from Kpong and 0.413–0.893 and 0.546–0.960 for tilapia and mudfish samples from Yapei, respectively. The examination of fin tissues as predictors of muscle Hg resulted in caudal fin being the better predictor of mercury concentration in muscle of Tilapia fishes

from all three location. The results for mudfish samples also indicated same. It is also noteworthy that the sensitivity of these nonlethal techniques was highly variable across species and dependent on specific life history characteristics of the fishes.

The mercury level in the muscle tissues were all within the WHO limit of $5.0 \mu\text{g/l}$ and hence poses no health risk to consumers. The mercury concentration in the muscle tissues of 98% of the fishes in this research recorded results lower than the WHO limit of $0.5\mu\text{g/g}$ which implies that consumption of these fishes does not pose any threat to human health.



Chapter 1

1. INTRODUCTION

Fish has been the main supply of cheap and healthy protein to a large percentage of the world's population. It is particularly valuable for providing proteins of high quality comparable with those of meat, milk or eggs, and is also a good source of omega-3 fatty acids; calcium and phosphorus, iron, trace elements like copper, and a fair proportion of the B-vitamins (Tucker, 1997; Martin *et al.*, 1982).

Additionally, fish consumption has been associated with a decreased risk of heart attack and coronary artery disease in adults by reducing cholesterol and triglyceride levels as well as inhibiting platelet aggregation (Toxicology Excellence for Risk Assessment (TERA), 1999).

Although the consumption of fish has so many benefits, it may also serve as a source of contamination to humans and other animals that feed on them due to the accumulation of chemicals such as organochlorine compounds, including pesticides such as dieldrin and DDT group, and industrial materials such as polychloro biphenyls (PCB), methylmercury and heavy metals.

The element mercury, also known as quicksilver (symbol Hg for *hydrargyrum*), and its compounds are naturally occurring in the earth's surface and present in low concentrations in all organisms and have no known normal metabolic function. (Eisler, 2010). Their presence in the cells of living organisms represents contamination from natural and anthropogenic sources; all such contamination must be regarded as undesirable and potentially hazardous (U.S. National Academy of Sciences (USNAS), 1978).

Mercury has been recognized as severe environmental pollutant, highly toxic even at low concentrations and it has the ability to enter biological systems (Porto *et al.*, 2005).

Most of the mercury compounds occur naturally in the environment (Church *et al.*, 1998), but they are also introduced into aquatic systems through anthropogenic pathways (Clarkson, 1994).

Globally, the major source of mercury in coastal systems is atmospheric deposition and anthropogenic origin such as laboratories, municipal waste, combustors in industries, chlor-alkali plants, agricultural activities, commercial and industrial boilers, construction of hydroelectric dams and artisanal gold mining activities (Heindryckx, 1974; Manahan and Stanley, 1991). Other anthropogenic sources are burning of coal and oil and the use of mercury compounds as slimicides and as antifungal agents in the paper and pulp industry and in agriculture (Graneya *et al.*, 2004). Human exposure to mercury is primarily through the consumption of fish where it is mainly present in the form of methyl mercury (Clarkson, 1994; Voegborlo and Akagi, 2007).

Emissions of mercury in air generated by coal-fired power plants and other coalburning facilities, municipal waste incinerators, and chlor-alkali plants have also contributed to environmental levels averaging 3-5x higher than those prior to 1900 (Pacyna, *et al.*, 2010). It is now widely recognized that human activities are artificially increasing mercury loads in the atmosphere on a local, regional and even global scale and enhanced atmosphere deposition of mercury is often the dominant source of mercury to aquatic systems (Hakanson *et al.*, 1990; Rolffhus and Fitzgerald, 1995).

Mercury's environmental persistence is due in part to its high affinity for particulates and organic matter. Even if mercury concentrations in sediment and water decrease over time, concentrations in organisms may not decrease due to the slow rate of elimination of the highly bioavailable methylmercury form. The physical properties,

bioavailability, and toxicity of mercury are governed by speciation into both organic and inorganic forms (Ullrich, *et al.*, 2001).

Elemental mercury, bivalent inorganic mercury, and monomethylmercury are the three most important forms of mercury occurring in natural aquatic environments (Battelle, 1987). Elemental mercury in aquatic environments has a high vapor pressure and a low solubility in water. (Major *et al.*, 1991).

Methylmercury may comprise more than 95% of the mercury in fish tissue while only 5-15% of the total mercury burden in sediments and water of contaminated lakes is methylmercury (Saroff, 1990). Exposure to methylmercury varies according to the characteristic amounts and types of fish consumed. About 95% of the methylmercury in humans originates from the ingested fish (Miettinen, 1973). Methylmercury is also readily absorbed through the skin and lungs. Once absorbed into the bloodstream, methylmercury enters the red blood cells. More than 90% of the methylmercury found in blood is bound to hemoglobin in red blood cells (Kershaw *et al.*, 1980), and some methylmercury is also bound to plasma proteins but the concentration in red blood cells is 10 times greater than that in plasma (Phelps *et al.*, 1980). About 10% of the body burden of methylmercury is found in the brain where it is slowly demethylated to inorganic mercuric Hg. Methylmercury is also readily transferred to the fetus and the fetal brain.

Due to the toxicity associated with eating fish that is contaminated with mercury it is highly recommended that routine analysis for mercury be done to ascertain the level of contamination of fish from our surrounding water bodies. This monitoring or surveillance of mercury in fish usually involves the collection of statistically representative sample of fish from targeted population, processing of the sampled organisms and analysis of fillets or whole fish (Kristofer *et al.*, 2008). There are two major ways of analyzing mercury in fish and these are the traditional or lethal methods

of monitoring the mercury levels of aquatic organisms like fish. This involves removal and subsequent killing of large number of fishes from water bodies. And the nonlethal or noninvasive tissue sampling method which involves the sampling and analyses of tissue biopsies, blood, and fish scales (Baker *et al.*, 2004; Schmitt and Brumbaugh, 2007; Lake *et al.*, 2006).

The traditional or lethal monitoring may have undesirable consequences, in that this may cause modification of food web structure, and reduction in population of the fishes, which are an important recreational and economic resource (Scheuhammer *et al.*, 2007). Moreover, removal may not be desirable or permissible for threatened or endangered species or in protected areas, such as national parks since this can cause the extinction of such species from water bodies.

Nonlethal or noninvasive tissue sampling techniques for monitoring contaminants in fish are an attractive alternative to the traditional lethal methods of obtaining muscle fillets.

The use of non-lethal methodologies for mercury analysis are particularly attractive at sites where destructive sampling methods would be detrimental to fish populations, for example, at sites where fish density is low.

Several recently published studies have demonstrated that non-lethal harvesting methods can produce accurate and reliable measures of fish muscle mercury concentrations provided appropriate analytical techniques are used (Tyus *et al.*, 1999; Baker 2002; Baker *et al.*, 2004; Peterson *et al.*, 2005.). The analyses of blood and of axial muscle obtained by biopsy provide reliable estimates of total mercury in fillets, and may require the application of anesthetics or antiseptics to maintain fish health. Baker *et al.*, (2004), reported that samples of muscle collected with a biopsy needle and dermal punch had concentrations of total mercury within 6% of those in the axial fillet

(r^2 ranged from 0.93 to 0.97, $n = 110$). Schmitt and Brumbaugh (2007), found that concentrations of total mercury in fillets of smallmouth bass (*Micropterus dolomieu*) could be accurately predicted (simple linear regression, $n = 62$) from total mercury concentrations in biopsy plug ($r^2 = 0.98$), biopsy needle ($r^2 = 0.99$), and blood ($r^2 = 0.92$). Lake *et al.*, (2006) evaluated scales of largemouth bass (*Micropterus salmoides*) as a predictor of tissue mercury concentration, and found coefficients of determination ranging from 0.67 to 0.90, depending upon preliminary washing treatments such as soap, acetone, and deionized water rinses. Overall, they concluded that scales were inherently too variable to make direct conclusions regarding fish-consumption advisories, while perhaps having a more general application as a first level screening tool (Lake *et al.*, 2006).

The clipping of pelvic or caudal fins is commonly done to mark fish in studies of fish populations. Fin clips are easily and rapidly collected with minimal harm to the organism (Gjerde and Refstie, 1998), and partially clipped fins usually regenerate (Guy *et al.*, 1996). In addition, repeated partial clipping of different fins from the same individual fish may allow monitoring of changes in mercury (Heltsley *et al.*, 2005) in threatened or endangered species or in small populations. Gremillion *et al.*, (2005), who measured total mercury in caudal fins and fillets of small numbers of walleye (*Sander vitreus*) and northern pike (*Esox lucius*) from Arizona waters, found that the mercury concentration in the caudal fin was a good predictor of that in the fillet.

Kristofer *et al.*, (2008) recently published a paper that involved the analysis of fin clips as a nonlethal method for monitoring mercury in fish and concluded that mercury concentration in fin clips was a better predictor of mercury in fillets for individual Arctic grayling ($r^2 = 0.65$, $n = 12$ and $r^2 = 0.84$, $n = 8$) and winter flounder ($r^2 = 0.94$, $n = 14$) than for individual northern pike (median $r^2 = 0.56$) or walleye (median $r^2 =$

0.22) from a given lake. In northern pike in the 400–500 mm total-length interval, the mean concentrations of total mercury in caudal fins and fillets, averaged by lake ($n = 12$), were strongly correlated ($r^2 = 0.95$).

However, the applicability of this method to all the other fins, species, aquatic systems and geographic areas is unknown.

This study will examine the potential utility of mercury concentration in fins as a predictor of mercury in axial muscle of two selected species of fish from three water bodies in Ghana.

1.1 Aims and Objective

- To determine the levels of total mercury in muscle of fish from three fresh water bodies in Ghana (Tono irrigation dam, Volta Lake at Kpong and White Volta at Yapei, Northern Region).
- To determine levels of total mercury in fish fins (anal, caudal, pectoral, pelvic and dorsal fins)
- To determine any correlation between mercury levels in the muscle and the various fins of fishes.
- To establish any relationship to enable prediction of mercury levels in muscle using levels in the fins.
- To check whether the levels of mercury concentration in the muscle tissues in the fishes are at levels of potential human health concern.

Chapter 2

2. LITERATURE REVIEW

2.1 Physical Properties of Mercury

Pure mercury is a coherent, silvery-white mobile liquid with a metallic luster (Anon., 1948) with atomic number 80, an atomic weight of 200.59 g/mol and specific gravity of 13.5 kgL⁻¹. It is the only metal which is a liquid at room temperature and this is because it has very high ionization energy. Its 1st ionization potential is 1007 kJ/mol (Lee, 1991) which makes it difficult for electrons to participate in metallic bonding. It freezes at about -39 °C with contraction, forming a white, ductile, malleable mass easily cut with a knife, and with cubic crystals. When heated, the metal expands uniformly, boiling at 357.01 °C at 760 mm, and vaporizing at about 360.0 °C. In thin layers it transmits a bluish-violet light. Mercury has a high vapour pressure (0.16 Pa at 20 °C); metallic mercury vaporizes readily under ambient conditions; a saturated atmosphere would contain approximately 15 mg Hg m⁻³ at 20 °C (Weast and Astle, 1983) Its vapor is colorless. Its vapour pressure is sufficiently high enough to yield hazardous concentrations of vapour at temperatures normally encountered both indoors and outdoors under most climatic conditions. For example, at 24 °C, a saturated atmosphere of mercury vapour would contain approximately 18 mg m⁻³- a level of mercury 360 times greater than the average permissible concentration of 0.05 mgm⁻³ recommended for occupational exposure by the National Institute of Safety and Health, USA (NIOSH, 1973). Mercury forms two well-defined series of salts namely mercurous salts derived from the oxide Hg₂O and the mercuric salts from the oxide HgO. Mercuric oxide occurs in two forms, as a bright red crystalline powder and as an orange-yellow powder. The yellow form is the most reactive and is transformed into the red when heated at 400.0 °C. Heating the red form results in a black compound, which regains its color on cooling; on further heating to 630.0 °C, it decomposes to mercury and oxygen (Anon., 1948).

2.2 Chemical Properties of Mercury

Apart from the noble gases, mercury is the only element which is a monatomic gaseous element at room temperature. However, little is known about the chemical and physical states of mercury found in the ambient air and in the air where occupational exposure occurs. Elemental mercury vapour is generally regarded as insoluble. Elemental mercury is relatively inert in dry air, oxygen, nitrous oxide, carbon dioxide, ammonia, and some other gases at room temperatures (Anon., 1948). In damp air, it slowly becomes coated with a film of mercurous oxide. When heated in air or oxygen, it is transformed into the red mercuric oxide, which decomposes into mercury and oxygen on continued heating at higher temperatures.

Chemical speciation is probably the most important variable influencing mercury toxicity, but mercury speciation is difficult to quantify, especially in natural environments (Boudou and Ribeyre, 1983). Mercury compounds in an aqueous solution are chemically complex and this is depended on factors such as pH, redox, and other variables, which result in the formation of a wide variety of chemical species having different electrical charges and solubilities. For example, HgCl_2 in solution can speciate into $\text{Hg}(\text{OH})_2$, Hg^{2+} , HgCl^+ , $\text{Hg}(\text{OH})^-$, HgCl_3^- , and HgCl_4^{2-} ; anionic forms predominate in saline environments (Boudou and Ribeyre, 1983).

In the aquatic environment, under naturally occurring conditions of pH and temperature, mercury may also become methylated by biological or chemical processes, or both (Beijer and Jernelov, 1979; USEPA, 1985; Ramamoorthy and Blumhagen, 1984; Zillioux *et al.*, 1993;) although biological methylation is limited (Callister and Winfrey, 1986). Methylmercury is the most hazardous mercury species due to its high stability, its lipid solubility, and its possession of ionic properties that lead to a high ability to penetrate membranes in living organisms (Beijer and Jernelov, 1979; Hamasaki *et al.*, 1995).

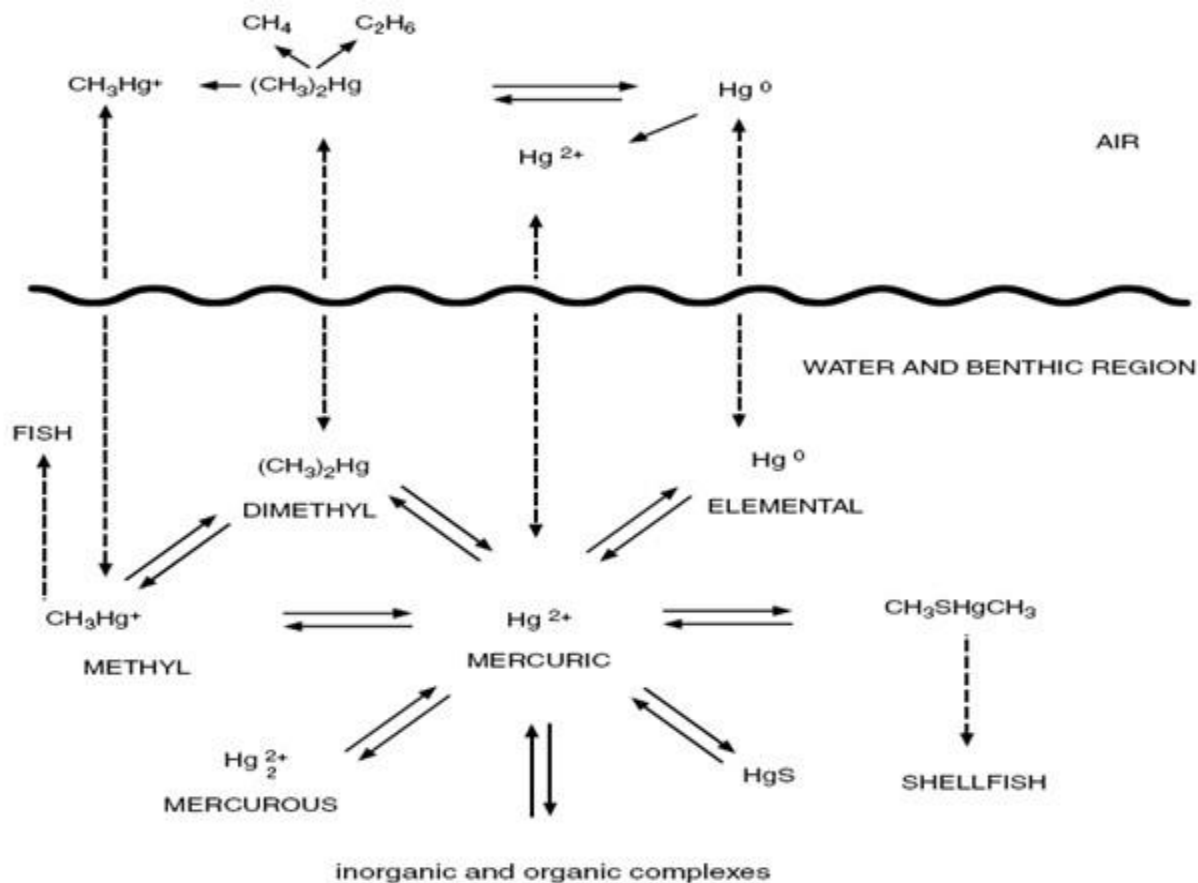


Figure 2.1: Major transformations of mercury in the environment

(Modified from Beijer and Jernelov, 1979; Nakamura, 1994; and Eisler, 2000.)

Essentially all mercury in freshwater fish tissues is in the form of methylmercury; however, methylmercury accounts for less than 1.0% of the total mercury pool in a lake (Regnell, 1994). Mercury dissolves many metals to form compounds called amalgams (Anon., 1948). Nevertheless, small amounts dissolved in water and other solvents are important from the toxicological point of view. Mercury can exist in a wide variety of physical and chemical states. This property presents special problems in assessing the possible risk to public health. The different chemical and physical forms of this element all have their intrinsic toxic properties and different applications in industry, agriculture and medicine and require a separate assessment risk.

2.3 Biochemical Properties of Mercury

Mercury binds strongly with sulfhydryl groups, and has many potential target sites during embryogenesis; phenylmercury and methylmercury compounds are among the strongest known inhibitors of cell division (Birge *et al.*, 1979).

Organomercury compounds, especially methylmercury, cross placental barriers and can enter mammals by way of the respiratory tract, gastrointestinal (GI) tract, skin, or mucus membranes (Elhassani, 1983). When compared with inorganic mercury compounds, organomercurials are more completely absorbed, are more soluble in organic solvents and lipids, pass more readily through biological membranes, and are slower to be excreted (Clarkson and Marsh, 1982; Elhassani, 1983; Greener and Kochen, 1983). Biological membranes, including those at the blood brain interface and placenta, tend to discriminate against ionic and inorganic mercury, but allow relatively easy passage of methylmercury and dissolved mercury vapor (Greener and Kochen, 1983).

In liver cells, methylmercury forms soluble complexes with cysteine and glutathione, which are secreted in bile and reabsorbed from the gastro intestinal (GI) tract. In general, however, organomercurials undergo cleavage of the carbon–mercury bond, releasing ionic inorganic mercury (Goyer, 1986). Mercuric compounds induces synthesis of metallothioneins, mainly in kidney cells. Mercury within renal cells becomes localized in lysosomes (Goyer, 1986).

2.4 Sources of Environmental Mercury Pollution

Major inputs of mercury to the environment are mainly from natural sources, with significant and increasing amounts contributed from human activities. The atmosphere plays an important role in the mobilization of mercury, with an estimated 25.0 to 30.0% of the total atmospheric burden of anthropogenic origin (USNAS, 1978). The global anthropogenic atmospheric emission of mercury is estimated at 900 to 6200 tons annually, of which the

United States contributed 300 metric tons in 1990 with 31.0% of the total from combustion of fossil fuels by power plants (Chu and Porcella, 1995). Atmospheric deposition is generally acknowledged as the major source of mercury to watersheds. In northern Minnesota watersheds, for example, atmospheric deposition was the primary source of mercury. Geologic and point source contributions were not significant. Transport from soils and organic materials may also be important, but the mercury from these sources probably originates from precipitation and direct atmospheric sorption by watershed components (Swain and Helwig, 1989; Sorensen *et al.*, 1990).

2.4.1 Natural Occurrence of Mercury

The total amount of mercury in various global reservoirs is estimated at 334.17 billion metric tons; almost this entire amount is in oceanic sediments (98.75%) and oceanic waters (1.24%), and most of the rest is in soils. Living aquatic organisms are estimated to contain only 7.0 metric tons of mercury (Clarkson *et al.*, 1984).

A review by the Joint FAO/WHO Expert Committee on Food Additives (1972) quotes the major source of mercury as the natural degassing of the earth's crust and quotes figures in the range of 25000-150000 tonnes of mercury per year. These figures originate from work by Weiss *et al.*, (1971) on concentrations of mercury in Greenland ice that was deposited prior to 1900. It was admitted that the sources of this atmospheric mercury are not yet clearly established but that volcanic gases and evaporation from the oceans are probably significant sources. The run-off of mercury from rivers having a "natural mercury" content of less than 200 ng/l would account for approximately 5000 tonnes of mercury per year. Measurements of the concentrations of mercury in air attached to aerosols (Heindryckx *et al.*, 1974) indicate that soil dispersion to the atmosphere is not an important source of mercury. Significant local contamination may result from natural sources of mercury.

Mercury from natural sources enters the biosphere directly as a gas, in lava (from terrestrial and oceanic volcanic activity), in solution, or in particulate form; cinnabar (HgS), for example, is a common mineral in hot spring deposits and a major natural source of mercury (Das *et al.*, 1982).

The global cycle of mercury involves degassing of the element from the Earth's crust and evaporation from natural bodies of water, atmospheric transport — mainly in the form of mercury vapor and deposition of mercury back onto land and water. Oceanic effluxes of mercury are tied to equatorial upwelling and phytoplankton activity and may significantly affect the global cycling of this metal. If volatilization of mercury is proportional to primary production in the world's oceans, oceanic phytoplankton activity represents about 36.0 % of the yearly mercury flow to the atmosphere, or about 2400 tonnes per year (Kim and Fitzgerald, 1986). The major natural sources of mercury are degassing of the earth's crust, emissions from volcanoes, and evaporation from natural bodies of water (National Academy of Sciences, 1978; Nriagu, 1979; Lindqvist *et al.*, 1984). Mercury emitted from volcanoes into the atmosphere, come along with large quantities of lead, cadmium, and bismuth (Hinkley *et al.*, 1999). About 6000 tonnes of mercury are discharged into the atmosphere every year from all sources (Fitzgerald, 1986); and from all volcanoes, about 60 tons or about 1.0% of the total (Varekamp and Buseck, 1986).

The most recent estimates indicate that natural emissions amount to 2700-6000 tonnes per year (Lindberg *et al.*, 1987). The earth's crust is also an important source of mercury for bodies of natural water. Some of this mercury is undoubtedly of natural origin, but some may have been deposited from the atmosphere and may, ultimately, have been generated by human activities (Lindqvist *et al.*, 1984). Thus it is difficult to assess quantitatively the relative contributions of natural and anthropogenic mercury to run-off from land to natural bodies of water.

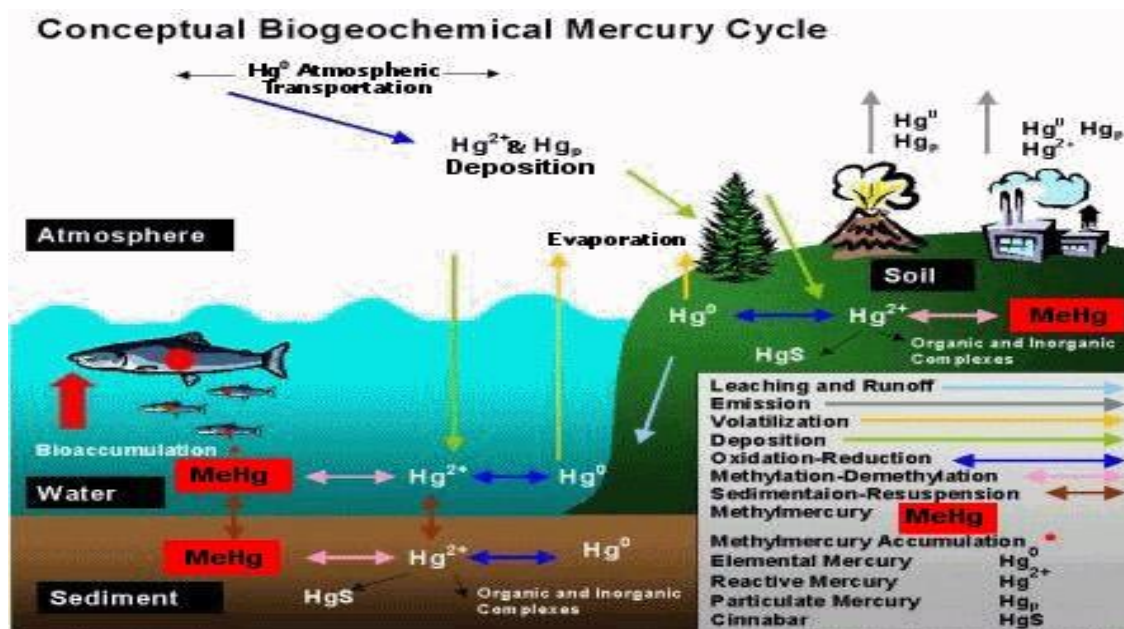


Figure 2.2: The Biochemical cycle of Mercury

Source: (<http://www.ec.gc.ca/mercure-mercury/default.asp?lang=En&n=67E16201-1>)

Terrestrial vegetation functions as a conduit for the transport of elemental mercury from the geosphere to the atmosphere (Leonard *et al.*, 1998a). In estimation of mercury emissions from plants, for example in the Carson River Drainage Basin of Nevada, an area heavily contaminated with mercury from historical gold mining activities. It was realized that over the growing season (0.5 mg Hg/m^2) add to the soil mercury emissions of 8.5 mg Hg/m^2 . And for a total landscape emission in that area of 9.0 mg Hg/m^2 , one species (tall whitetop, *Lepidium latifolium*), emits as much as 70.0 % of the mercury taken up by the roots during the growing season to the atmosphere (Leonard *et al.*, 1998a). Some factors that are known to increase the flux of elemental mercury from terrestrial plants growing in soils with high (34.0 to $54.0 \text{ mg Hg/kg soil DW}$) levels of mercury include increasing air temperature in the range 20.0 to 40.0 °C, increasing irradiance, increasing soil mercury concentrations, and increasing leaf area (Leonard *et al.*, 1998b). Table 2.1 summarizes mercury levels in some global reservoirs with their corresponding residence time.

Table 2.1: Amount of Mercury in Some Global Reservoirs and Residence Time

Reservoir	Mercury Content ^a (metric tons)	Residence Time ^b
Atmosphere	850	6–90 days
Soils	21,000,000	1000 years
Freshwater	200	—
Freshwater biota (living)	4	—
Ocean water	4,150,000,000	2000 years
Oceanic biota (living)	3	—
Ocean sediments	330,000,000,000	> 1 million years

^a From USNAS, 1978.

^b Modified from Clarkson *et al.*, 1984. Mercury. As seen in J.O. Nriagu (Ed.), *Changing Metal Cycles and Human Health*, p. 285–309. Springer-Verlag, Berlin.

2.4.2 Anthropogenic Sources of Mercury

Mining activities result in losses of mercury through the dumping of mine tailings and direct discharges to the atmosphere. The Almaden mercury mine in Spain, which accounts for 90% of the total output of the European Community, was expected to produce 1380 tonnes in 1987. Several human activities that contribute significantly to the global input of mercury include the combustion of fossil fuels; mining and reprocessing of gold, copper, and lead; operation of chloralkali plants; runoff from abandoned cinnabar mines; wastes from nuclear reactors, pharmaceutical plants, oil refining plants, and military ordnance facilities; incineration of municipal solid wastes and medical wastes; offshore oil exploration and production; disposal of batteries and fluorescent lamps; and the mining, smelting, use, and disposal of mercury (USNAS, 1978; Das *et al.*, 1982; Gonzalez, 1991; Lodenius, 1991; Facemire *et al.*, 1995; Gustafson, 1995; Atkeson *et al.*, 2003; Lacerda *et al.*, 2004; Liang *et al.*, 2004). It should be stressed that there are considerable uncertainties in the estimated fluxes of mercury in the environment and in its speciation. Concentrations in the unpolluted atmosphere and in natural bodies of water are so low as to be near the limit of detection of current analytical methods, even for the determination of total mercury. Anthropogenic releases of mercury into

confined areas can be the source of high toxicity risk even though these releases may be small relative to global emissions.

Mercury emissions from electric utilities constitute the largest uncontrolled source of mercury to the atmosphere (USEPA, 1997), and globally it accounts for up to 59.0 % of the total annual atmospheric loading of mercury from both natural and anthropogenic sources (Fitzgerald, 1986; Fitzgerald and Clarkson, 1991; WHO, 1976, WHO, 1991; Mason *et al.*, 1994; USEPA, 2000; Lamborg *et al.*, 2002). Coal-fired power plants are now considered the greatest source of environmental mercury in the United States, and the only significant source that remains unregulated (Maas *et al.*, 2004). In 1994, about 50 metric tons of mercury were emitted into the biosphere from coal-burning power plants in the United States, with lesser amounts from oil- and gas-combustion units (Finkelman, 2003). Available technologies now installed in waste combustion and medical incinerators are recommended for installation in coal-fired plants to help reduce mercury emissions from these sources by as much as 90.0 % (Maas *et al.*, 2004).

Logging and forest fires can contribute to the bioavailability of mercury (Garcia and Carignan, 2005). Watersheds impacted by clear-cut logging, or burnt forest ecosystems, release mercury into the biosphere with significant increases in the flesh of predatory fish from impacted drainage lakes when compared to reference watersheds (Garcia and Carignan, 2005).

Most of the daily intake of mercury compounds is in the form of methylmercury derived from dietary sources, primarily fish, and to a lesser extent elemental mercury from mercury vapor in dental amalgams, and ethylmercury added as an antiseptic to vaccines (Mottet *et al.*, 1985; USNAS, 2000; Clarkson *et al.*, 2003; Dye *et al.*, 2005). Dental amalgams, which may contain up to 50.0% by weight of metallic mercury, may also constitute a significant source of mercury in some cases (Summers *et al.*, 1993). Amalgam mercury is imperfectly stable, slowly leaching from the mercurysilver or mercury-gold amalgam through the action of oral bacteria and exacerbated by chewing. Following placement or removal of fillings, up to 200.0 mg

mercury is eliminated in the feces, with subsequent selection of mercury-resistant bacteria for degradation. Normal mastication may result in body accumulations of 10.0 µg daily through either intestinal uptake or respiratory intake of mercury vapor released during chewing (Summers *et al.*, 1993).

World production of mercury in recent years is estimated at 10,000 to 15,000 metric tons annually; major producers of mercury now include the former Soviet Union, Spain, the former Yugoslavia, and Italy. A review by Korrinda and Hagel (1974) realized that world production averaged about 4000 tonnes per year over the period 1900-1940. Production in 1968 was 8000 tonnes per year and, in 1973, attained 10000 tonnes per year. Although considerable yearly fluctuations were noted, the average rate of increase since 1950 has been about 2 % per year. Recent concern over environmental problems related to the use of mercury seems to have stabilized production rates and to have led to a dramatic fall in the price of mercury. For example, according to figures quoted by Korrinda and Hagel (1974), the 1966 price was \$452 per flask (a flask is 34.5 kg), the 1969 price had risen to \$510.00 but by 1972 it had fallen dramatically to \$202 per flask. It is difficult to estimate the amount of mercury released into the environment as a result of the mining and smelting of this metal. High levels of mercury in lake and stream waters have been attributed to the dumping of materials and tailings (Wallace *et al.*, 1971). Abandoned mercury mines may contribute excess mercury loadings and other contaminants to the environment. For example, mercury mines in western Turkey that were gradually abandoned owing to low demand, low prices, and increasing environmental concern over mercury adversely affected adjacent water resources (Gemici, 2004). One abandoned mine located 5 km west of Beydag, Turkey, that operated from 1958 through 1986 with a total production of 2045 metric tons of mercury during this period released metal-rich, acidic drainage affecting groundwater and adjacent stream water quality through decreasing pH, elevated levels of silicon, aluminum, magnesium, calcium, and potassium; increasing precipitation of iron oxides; and increasing sulfates, manganese, iron,

and arsenic. Most of the mine water and groundwater samples exceeded drinking water standards for aluminum, iron, manganese, arsenic, nickel, and cadmium. Mercury concentrations in all samples were below the Turkish drinking water standard of 1.0 µg/L for human health; however, two samples contained 0.3 and 0.5 µg Hg/L and were above the USEPA mercury criterion for aquatic life protection of < 0.012 µg/L (Gemici, 2004).

2.5 Environmental Transport, Distribution, and Transformation

2.5.1 Mercury Levels in the Environment

Local variations of Hg concentration are considerable, especially in coastal sea water and in lakes and rivers where mercury associated with suspended material may also contribute to the total load. The mercury content in minerals forming ordinary rock and soils is usually very low. The normal levels in igneous rocks and minerals seem to be less than 50 µg/kg, and in many cases is less than 10 µg/kg. Due to the strong binding of mercury to soil particles, including organic matter, only small amounts of the metal are present in soil solution; reported averages range between 20 and 625 µg/kg soil. Background levels in sediments are approximately the same as levels in unpolluted surface soils. Average concentrations in ocean sediments probably lie in the range between 20 and 100 µg/kg (Das *et al.*, 1980)

2.5.2 Transport and Distribution between Media

The vapour of metallic mercury (also known as mercury vapour or Hg⁰) is released into the atmosphere from a number of natural sources. Man-made emissions, mainly from the combustion of fossil fuels, forms about 25 % of the total emissions to the atmosphere. The solubility of mercury vapour in water is not high enough to account for the concentrations of mercury found in rain water. Hg²⁺ is deposited on land and water in rain. However, the putative water-soluble forms have yet to be positively identified. Particulate forms account for less than 1 % of total mercury in the atmosphere but may make an important contribution

to mercury in rain water. The residence time of mercury vapour is estimated to be between 0.4 and 3 years, and as a consequence, mercury vapour is globally distributed. The soluble form is assumed to have a residence time of the order of weeks, and therefore the distance over which it may be transported is limited. The extremely low concentrations in the atmosphere, present formidable difficulties both in the analysis of total mercury and in the identification and measurement of chemical and physical species. Mercury deposited on land and open water is, in part, re-emitted to the atmosphere as Hg^0 .

This emission, deposition, and re-emission ("ping-pong" effect) creates difficulties in tracing the movement of mercury to its source. The bottom sediment of the oceans is thought to be the ultimate sink where mercury is deposited in the form of the highly insoluble mercuric sulfide.

2.6 Uses of Mercury

Historically, mercury has been used extensively (and still is to a lesser degree) in the extraction of precious metals (for example, gold and silver) as amalgams. Mercury is used in silver amalgams in dentistry for tooth restorations. Although banned in many parts of the world, mercury iodide (3%) or mercury amidochloride (10%) are used in skin-lightening creams and soaps (Seiler *et al.*, 1994). The major use of mercury is in electrolytic cells (as the cathode) for the production of NaOH and Cl_2 . Metallic mercury is used as liquid contact material for electrical switches, in vacuum technology in diffusion pumps, thermometers, barometers, tachometers and thermostats, and in the mercury-vapour lamps. Mercury is widely used in batteries too. The standard calomel (Hg_2Cl_2) electrode is used as the reference electrode for measurements of potentials in analytical electrochemistry. Mercuric oxide (HgO) has been used in antifouling paint for ships and in mildew-proofing paints and to control fungal infections of seeds, bulb plants, and vegetation. Mercury has been used in medicines, cosmetics and dentistry. W.H.O has warned against the use of alkylmercury compounds in

seed dressing. Methylmercury compounds are still used in laboratory-based research, and so the possibility of occupational exposures remains.

2.7 Bioaccumulation of mercury

Mercury bioaccumulates in aquatic plants, invertebrates, fish, and mammals. Concentrations increase (biomagnify) in higher-trophic-level organisms. Even though the different types of mercury have relatively low K_{ow} values (compared to organic compounds such as PCBs), they are readily accumulated. Inorganic mercury (excluding elemental) and methylmercury's strong reactivity with intracellular ligand is thought to be responsible for their high degree of accumulation (Mason *et al.*, 1996). Uptake and accumulation of mercury are affected by the type of mercury present, with neutral mercury species (e.g., $HgCl_2^0$ and CH_3HgCl^0) absorbed more efficiently than charged mercury species (e.g., $HgCl^-$ and CH_3Hg^+) (Mason *et al.*, 1996). Despite the fact that the neutral inorganic and organic complexes have similar lipid solubilities, methylmercury is selectively accumulated (due to a higher transfer efficiency and lower rate of elimination), resulting in biomagnification in higher trophic levels (Mason *et al.*, 1995). Inorganic mercury species are not biomagnified (Surma-Aho and Paasivirta 1986; Riisgård and Hansen 1990; Hill *et al.*, 1996). Environmental variables also influence the bioavailability and accumulation of inorganic mercury. Although concentrations of mercury in the environment may correlate with concentrations in resident plants and biota, correlation is often difficult. Correlating total mercury in sediment with total mercury in upper-trophic-level organisms is complicated by high methylmercury concentrations in high-trophic-level organisms relative to low methylmercury concentrations in the environment.

2.7.1 The Effect of the Form of Mercury on Bioaccumulation

Both inorganic and methylmercury are taken up directly from water and food (or ingested sediment). However, methylmercury is more efficiently accumulated than inorganic mercury for most aquatic

organisms (Fowler *et al.*, 1978; Julshamn *et al.*, 1982; Riisgård and Hansen 1990; Mason *et al.*, 1995).

The uptake and depuration of mercury depends on the form of mercury, source of mercury (water or food), and the type of receptor tissue, resulting in different patterns of accumulation.

Methylmercury is readily transferred across biological membranes. Within the organism, methylmercury is strongly bound to sulfhydryl groups in proteins of tissues such as muscle, and is much slower to depurate than inorganic mercury. Thus, methylmercury has a much greater potential for bioaccumulation and a longer half-life in organisms than inorganic mercury.

2.7.1.1 Fish

The accumulation of mercury from water occurs via the gill membranes. Gills take up aqueous methylmercury more readily than inorganic mercury (Huckabee *et al.*, 1979; Boudou *et al.*, 1991). Methylmercury is eventually transferred from the gills to muscle and other tissues where it is retained for long periods of time (Julshamn *et al.*, 1982; Riisgård and Hansen 1990). Inorganic mercury taken up with food initially accumulates in the tissues of the posterior intestine of fish (Boudou *et al.*, 1991). Inorganic mercury is not easily transferred through this organ to other parts of the body. After 15 days, 80 % had depurated from the fish intestine. Liver and kidney in fish tend to have higher percentages of inorganic mercury than muscle tissue, although percentages vary by organ and species (Windom and Kendall, 1979; Riisgård and Hansen, 1990).

Methylmercury ingested in food is efficiently transferred from the intestine to other organs (Boudou *et al.*, 1991). Methylmercury has been reported to constitute from 70 to 95 % of the total mercury in skeletal muscle in fish (Huckabee *et al.*, 1979; USEPA, 1985; Riisgård and Famme, 1988; Greib *et al.*, 1990; Spry and Wiener, 1991). Methylmercury accounted for almost all (>99 %) of the mercury in muscle tissue in a wide variety of both freshwater and saltwater fish found in waters not highly contaminated by other organomercurial species (Bloom, 1992).

The ratio of liver to muscle total mercury concentration usually fluctuates around one and can reflect the exposure history of the organisms. For example, the liver: muscle ratio may be less than one in chronically exposed fish, while a recent exposure to mercury may result in a ratio greater than one (Riisgård and Hansen, 1990).

McKim *et al.*, (1976) reported that mercury could be transferred from adult to offspring in brook trout. Exposure of the parent population to aqueous methylmercury concentrations of 0.03 to 2.93 µg/l in the laboratory resulted in mercury concentrations as high as 2 mg/kg in their embryos. Total mercury concentrations in eggs of several species of adult fish from Swedish lakes were much lower than concentrations in other tissues; therefore, spawning did not lower their total mercury body burden (Lindqvist, 1991).

The main depuration pathway is through the kidney and liver in fish. Half-lives for methylmercury in fish range from one to three or more years (McKim *et al.*, 1976; Pentreath 1976a, Pentreath 1976b; Riisgård and Famme, 1986; Riisgård and Hansen, 1990), while estimates of half-lives for inorganic mercury are much lower, ranging from approximately five days to five months (Pentreath, 1976a, Pentreath 1976b; Huckabee *et al.*, 1979).

2.7.2 Factors Affecting Biological Uptake

Mercury appears in aquatic organisms primarily as methylmercury. It is in this form that most mercury is transferred through the food web. Mercury is taken up differentially in the aquatic environment depending on a number of factors. The most conducive environmental conditions for the methylation and uptake of mercury into the aquatic food web include: low pH (<7), low alkalinity (<20 mg/l), low calcium (<15 mg/L), high total organic carbon (TOC), low chlorophyll-a (<10 µg/l), and significant seasonal fluctuations in water level (Rada *et al.*, 1989; Cope *et al.*, 1990; Wiener and Spry, 1996; Lange *et al.*, 1993). Methylmercury is produced initially by microbial activity.

Although nearly insoluble in water, methylmercury forms colloids with humus. Humic material transfers mercury from soils to water, then to the food web via the microbial process.

Wetlands are a ready source of dissolved organic carbon which complexes and transports mercury. Newly impounded lakes, as well as lakes with high organic input, tend to have elevated levels of mercury in the aquatic fauna (Paasivirta, 1991; Wiener and Spry, 1996). Miller and Akagi (1979) suggest that as pH decreases, partitioning of mercury is shifted from sediment to water.

A major pathway for mercury removal from solution is chemical binding with reduced sulphur, which has a high affinity for mercury. Lindberg *et al.*, (1987) was of the view that increased concentrations of some metals such as iron and manganese may affect the nonbiological methylation of mercury. Iron and manganese sulfides, known as acid volatile sulfides (AVS), are a reactive pool of solid phase sulfides that are available to bind with metals, such as mercury (Di Toro *et al.*, 1990). When bound by AVS, mercury is less available for uptake by aquatic organisms.

2.7.3 Biological Factors Affecting Accumulation of Mercury.

The primary biological factors governing the accumulation of mercury in fish include:

- (1). Age
- (2). Weight and (3).

Diet

Differences in accumulation between the sexes have been attributed to differences in diet.

2.8.4 Other Factors Affecting Accumulation

Temperature and season influence the availability and accumulation of mercury in addition to the factors already discussed. Changes in temperature can affect mercury concentrations in organisms either directly by affecting metabolic rate and thereby exposure, or indirectly by influencing the methylation of mercury and therefore enhancing availability. Rates of methyl or inorganic mercury uptake increase with increasing aqueous concentrations and/or increasing temperature in the water for some species (e.g., phytoplankton, gastropods, fish)

(Windom and Kendall, 1979; Rodgers and Beamish, 1981; Tessier *et al.*, 1994). A rise in temperature (and a corresponding rise in respiratory volume) can increase the rate of uptake via the gills (USEPA, 1985).

Total concentrations of mercury in killifish from an estuarine wetland were five times higher in spring and summer than in other seasons (Weis *et al.*, 1986), presumably due to higher methylation rates in summer. Zooplankton mercury concentrations peaked in June in Swedish lakes and fish tissue levels varied by a factor of two, reaching a maximum in spring (Lindqvist, 1991). Mercury content of mussels from the Gulf of St. Lawrence estuary varied seasonally by a factor of two (Cossa and Rondeau, 1985).

The relationship of pH, conductivity, and salinity to mercury accumulation is not well understood. Elevated mercury concentrations have frequently been found in piscivorous fish in poorly buffered (alkalinity < 55 µeq/l and calcium < 2 mg/l), low pH of 6.0-6.5 in lakes and in areas removed from industrial inputs of mercury (Rada *et al.*, 1989; Winfrey and Rudd, 1990; Spry and Wiener, 1991). Total mercury concentrations in yellow perch were inversely correlated with pH in Ten Wisconsin Lakes (Cope *et al.*, 1990). Mercury concentrations in zooplankton in Swedish lakes were correlated with pH but the relative importance of this correlation changed over time (Lindqvist, 1991).

Conductivity was also highly correlated with calcium, magnesium, alkalinity, pH, and sodium. This correlation suggests that the buffering capacity of the lake was an important influence on crayfish accumulation of mercury. Low calcium ion concentrations enhanced the efficiency of methylmercury uptake across the gills of rainbow trout (Rodgers and Beamish, 1981).

2.9 Absorption of Methylmercury

Methylmercury in the diet is almost completely absorbed into the bloodstream (WHO, 1976).

Animal's studies indicate that age, including neonatal stage, has no effect on the efficiency of gastrointestinal absorption, which is usually in excess of 90 % of the oral intake. Data on rats

indicate rapid and virtually complete absorption of inhaled methylmercury vapour into the bloodstream.

2.10 Environmental Levels and Human Exposure

2.10.1 Environmental Levels

There is considerable variation in mercury levels in those media that are the source of human exposure and, consequently, in their contribution to the toxicity risk. Non-occupational groups are primarily exposed through the diet. Concentrations of mercury in most foodstuffs (WHO, 1976, USEPA, 1985; Piotrowski and Inskip, 1981) are often below the reported limit of detection (usually 20 µg/kg fresh weight). Fish and fish products are the dominant source of methylmercury in food. The highest concentrations are found in both freshwater and marine fish at the highest trophic levels.

2.10.2 Exposure Pathways

Aquatic organisms can accumulate mercury from water (including pore water) and food sources (including sediment). Quantity accumulated is a function of the exposure pathway and the physical and environmental factors such as temperature, pH, salinity, total organic carbon, and sulfides. If conditions are favorable for methylation, organisms can accumulate high concentrations of mercury even with low concentrations in the water and sediment.

2.10.2.1 Water

Phytoplankton, invertebrates, fish (including eggs and larvae), and mammals take up inorganic and organic mercury from the water column (McKim *et al.*, 1976; Pentreath, 1976a; Pentreath, 1976b). In phytoplankton, algae, and microorganisms, mercury uptake is primarily a passive process that occurs by adsorption to the cell surface either through interaction with functional groups in the cell wall or through sorptive properties associated with the extracellular matrices

(Darnell *et al.*, 1986; Gadd, 1988). Passive diffusion of lipid-soluble species (uncharged chloride complexes) is responsible for mercury uptake in a marine diatom (Mason *et al.*, 1996). Uptake in phytoplankton and aquatic plants has been correlated with the concentration of mercury in the water (Windom and Kendall, 1979; Lenka *et al.*, 1990). Water is an important exposure pathway for mercury uptake by lower organisms and thus into the food web (Francesconi and Lenanton, 1992). Dissolved mercury concentrations in water are typically very low; the major increase in mercury concentrations occurs between water and phytoplankton of about a factor of 10^5 to 10^6 (Mason *et al.*, 1995). In contrast to microorganisms, uptake is primarily an active process for fish and invertebrates, and is related to respiration rate and metabolic rate (Rodgers and Beamish, 1981).

2.10.2.2 Sediment

Sediment is an important exposure pathway for all forms of mercury to aquatic organisms. High concentrations of organic substances and reduced sulfur that complex free Hg[II] ions in sediment can reduce the availability of mercury to biota (Luoma, 1977; Rubinstein *et al.*, 1983). Correlating mercury concentrations in sediment with concentrations in biota may be difficult, particularly for higher trophic-level species.

The bioavailability of total mercury to benthic invertebrates was reported to be inversely correlated to the organic content of the sediment (Langston, 1982; Langston, 1986). Normalizing sediment mercury concentrations to percent organic matter improved the correlation between total mercury concentrations in sediment and invertebrate species (including gastropods, polychaetes, and deposit and suspension-feeding bivalves) in a marine environment (Bryan and Langston, 1992). Good sediment tissue correlations for mercury have been found in amphipods from a freshwater lake (Becker and Bigham, 1995).

Many investigators report no correlation between sediment and tissue concentrations of mercury for higher-trophic-level species (Nishimura and Kumagai 1983; Jackson, 1988; Rada *et al.*, 1989; Lindqvist, 1991; Duckerschein *et al.*, 1992).

Organic carbon normalization of sediment concentrations did not improve the correlations for pike, a high trophic level species (Lindqvist, 1991). The difficulty in correlating mercury in sediment with mercury in organisms reflects the complexity of variables that affect both the methylation of mercury in surface sediments and the transfer of mercury between trophic levels. Since methylation occurs primarily in surface sediments, the physical factors that affect the rate of methylation (and demethylation) also affect the availability of mercury for uptake by organisms. Sediment total-mercury concentrations alone may not provide information on the exposure potential of resident organisms.

2.10.2.3 Food web

Though sediment may be the ultimate source of mercury for many higher trophic species, the food web is the primary pathway to most organisms (Lindqvist, 1991; Bryan and Langston, 1992). Most of the differentiation between inorganic and methylmercury accumulation occurs during trophic transfer (Mason *et al.*, 1995) because of the differences in assimilation of the different mercury forms and how efficiently the different forms are transferred to predators. Mason *et al.*, (1995) detected assimilation efficiency four times greater for methylmercury compared to inorganic mercury from phytoplankton to zooplankton, and ten times greater between phytoplankton and planktivorous fish.

The transfer efficiency of methylmercury over inorganic mercury in zooplankton was attributed to mercury partitioning in the algal cell. Methylmercury accumulated in the algal cytoplasm, which zooplankton digest, with 62 % of the methylmercury transferred, while inorganic mercury was primarily bound to thiols in the algal cell membrane. Therefore, a smaller percentage (15 %) of inorganic mercury was transferred to zooplankton.

As methylmercury increases in prey items, the transfer efficiency also increases (Windom and Kendall, 1979). Since methylmercury concentrations are highest in fish, piscivorous fish will be exposed to higher concentrations of methylmercury than fish that feed on invertebrates. For example, walleye accumulated mercury at a faster rate and at higher concentrations than

pike from the same freshwater lake (Mathers and Johansen, 1985). The relative importance of dietary versus aqueous mercury uptake pathways is unclear. Probably less than 10 % of the mercury in fish tissue residues is obtained by direct (gill) uptake from water (Francesconi and Lenanton, 1992; Spry and Wiener, 1991). Methylmercury concentrations used in laboratory studies of aqueous uptake are 1,000 to 10,000 times the ambient concentration of methylmercury in natural water (Spry and Wiener, 1991), thereby overestimating the significance of direct aqueous uptake. The proportion of mercury taken up from dietary sources versus water in invertebrates has not been estimated. Suspension-feeding bivalves may principally accumulate mercury by consuming algal cells (Riisgård and Hansen, 1990). Although mercury correlations are complicated by the importance of the food chain exposure pathway, mercury concentrations in predators and prey have been correlated (e.g., Allard and Stokes, 1989; Lindqvist, 1991; Spry and Wiener, 1991). For example, mercury concentrations in smallmouth bass from Ontario lakes were directly correlated with mercury in crayfish, which comprised 60 % of their diet.

2.11 Interactions of Mercury with Other Metals

The effects on aquatic organisms due to interactions of mercury with cadmium, copper, selenium, and zinc were found to be dependent on exposure concentrations (Birge *et al.*, 1979). In general, effects were less than additive at lower exposure concentrations and greater than additive (synergistic) at higher concentrations. Zinc and cadmium were reported to reduce the teratogenic effects of methylmercury to killifish (Weis *et al.*, 1986)

The percentage of embryos affected and degree of malformation observed due to exposure of killifish eggs to 20-50 µg/l methylmercury was reduced when cadmium or zinc was added. Selenium was reported to reduce the developmental effects of inorganic mercury to embryos of the medaka (Japanese rice fish), but only after the formation of the embryonic liver (Bowers *et al.*, 1980). Interactions between inorganic mercury and zinc, PCBs, and a PAH (fluoranthene) were observed to be generally additive in sediment exposure to a marine

amphipod (Swartz et al., 1992). A mixture of an inorganic form of mercury (mercuric chloride) and the chlorides of zinc and lead had a synergistic toxic effect on the water exposure of a marine ciliate *Uronema marinum* (Parker, 1979).

2.12 Organic Mercury compounds

In addition to simple salts, such as chloride, nitrate and sulphate, mercury (II) forms an important class of organometallic compounds. These are characterized by mercury bonded to either one or two carbon atoms to form compounds of the type RHgX and RHgR' where R and R' represent the organic moiety. The most numerous of the organometallic compounds are those of RHgX where X may be one of a variety of anions. The carbon mercury bond is chemically stable and it is not split in water or by weak acids and bases.

The stability is not due to the high strength of the carbon-mercury bond but due to the very low affinity of mercury for oxygen bonded to carbon. The organic moiety, R, takes a variety of forms with the most common being the alkyl (mainly methyl), the aryl (mainly phenyl) and the alkoxyalkyl (mainly methoxyethyl) radicals. If the anion X is nitrate or sulphate, the compound tends to be salt like having appreciable solubility in water. If the anion is chloride the compound is a covalent non-polar compound that is more soluble in organic solvents than in water. The behaviour of organic mercury in the environment and the organism differs from that of inorganic mercury. The toxicological characteristics of organic mercury are also different. However, from the toxicological perspective, the most important of these organic mercury compounds are the ones in which mercury is attached to the carbon atom of a methyl (CH_3), ethyl (C_2H_5) or propyl (C_3H_7) group forming compounds of the type CH_3HgX , $\text{C}_2\text{H}_5\text{HgX}$ or $\text{C}_3\text{H}_7\text{HgX}$. Methylmercury is generated naturally primarily by microorganisms in addition to synthetic processes and it is the most important from the standpoint of environmental pollution and toxicology.

Most of the mercury in the environment, including waters, soils, sediments and biota, occurs in the forms of inorganic mercuric salts and organomercurials. The mercury compounds most likely to be found in the environment (atmospheric mercury being the exception) include the following: mercuric salts; for example HgCl_2 , $\text{Hg}(\text{OH})_2$ and HgS and methylmercury compounds; for example methylmercuric chloride (CH_3HgCl), methylmercuric hydroxide (CH_3HgOH) and to a lesser extent dimethylmercury and phenylmercury (USEPA, 1997). Though organomercurials are not readily soluble and do not react with weak acids or bases, methylmercuric hydroxide (CH_3HgOH) is however highly soluble due to the strong hydrogen bonding capability of the hydroxide group.

Although the carbon-mercury bond is chemically stable, in the living animal, the bond is subject to cleavage. The nature of the R radical is all-important. If R is a phenyl or methoxyalkyl group, rapid breakdown occurs in animal tissues so that most of the organic compound has disappeared within a few days. Enzymes that break the carbon-mercury bond have been discovered and isolated (WHO, 1976). The short-chain alkylmercurials undergo the slowest breakdown *in vivo* with methylmercury being the most stable. Differences in the stability of the carbon-mercury bond play an important role in determining the toxicity and mode of action in man. The rapid breakdown of phenyl- and methoxy-mercury results in toxic effects similar to those of inorganic mercury salts. The relative stability of the alkylmercurials is one important factor in their unique position with regard to toxicity and risks to human health.

The organic and inorganic cations of mercury, in common with other heavy metal cations, will react reversibly with a variety of organic ligands (e.g. carboxyl, sulfhydryl) found in biologically important molecules. The chemical affinity of mercury(II) and of its monovalent alkylmercury cations for a variety of biologically occurring ligands is so great that free mercury would be present *in vivo* at concentrations so low as to be undetectable by present methods (WHO, 1976).

2.13 Toxicity of Mercury Compounds

2.13.1 Toxicity of Metallic Mercury

Metallic mercury poisoning occurs through inhalation due to the easy vaporization and high vapour saturation concentration of metallic mercury. The vapour has a high absorption rate in the airway (80 % or more in humans). After being absorbed into the body; mercury is oxidized into the divalent mercury ion. However, since a certain amount of time is required before oxidation, some of the unoxidized mercury vapour exists in the blood stream. Mercury vapour has no charge and easily passes through the blood-brain barrier. Therefore, even though metallic mercury is classified as inorganic, mercury poisoning with primarily central nervous system symptoms occurs. With high concentration exposures chemical pneumonitis occurs. At lower concentrations mercury poisoning with primarily central nervous system symptoms occurs. Biological effects still occur when the concentration of the exposure is even lower. In addition to inhalation, mercury can be absorbed through ingestion and contact with the skin. However, the quantities absorbed through these routes are small. Insomnia, lack of appetite, restlessness and diarrhea has been reported after human inhalation of $150 \mu\text{g}/\text{m}^3$ /46 days) Tremors and jaundice have also been reported after oral ingestion of 43 mg/kg by man (Japan Public Health Association, 2001).

2.13.2 Toxicity of Inorganic Mercury Compounds

The problematic poisonous characteristic of inorganic mercury compounds is corrosion. When solutions with high concentration of these compounds are ingested, corrosion occurs inside the oral cavity and in the upper digestive tract. Pain is felt in the oral cavity and pharynx and is accompanied by continuous vomiting, chest pain, abdominal pain, and bloody diarrhea. When the corrosion is severe, dehydration and shock occur. The absorption rate in the digestive tract is approximately 10 % at most. In contrast to metallic mercury, distribution to

the central nervous system is low and kidney damage is the primary result. Renal insufficiency occurs due to the degeneration of renal tubules (Japan Public Health Association, 2001).

Inorganic mercury compounds include mercury (I) chloride and mercury (II) chloride. A poisonous characteristic of mercury (I) chloride is acrodynia (pink disease). This was seen in children exposed to tooth pastes, lotions, and ointments containing the compound. Other poisonous characteristics are derived from decomposition into metallic mercury and divalent mercury.

Some poisonous characteristics of mercury (II) chloride have been reported. For example, human oral ingestion of $50 \mu\text{g Kg}^{-1}$ led to miscarriage at 10 weeks of pregnancy. Human oral ingestion of 57 mg kg^{-1} resulted in gastritis and lung function damage. Human oral ingestion of 86 mg Kg^{-1} results in blood plasma volume changes and bleeding from the stomach (Japan Public Health Association, 2001).

2.13.3 Toxicity of Methylmercury Compounds

In contrast to inorganic mercury compounds (excluding mercury vapour), these compounds are distributed in greater quantities in the central nervous system. The toxicity of the compounds is based on this characteristic.

Alkyl mercury compounds also include ethylmercury ($\text{C}_2\text{H}_5\text{HgX}$) and propylmercury ($\text{C}_3\text{H}_7\text{HgX}$). Although these other compounds are thought to behave within and have the same effects on living organisms as methylmercury, these other compounds are more easily decomposed. Phenylmercury and methoxy-ethylmercury decompose quickly in the body. Their toxicity is therefore nearly identical to that of inorganic mercury. Dimethyl mercury becomes monomethyl mercury within the body and then becomes toxic. The toxicity is therefore the same as that of methylmercury. However, dimethyl mercury is volatile and therefore easily inhaled. The compound is also easily absorbed through the skin.

Organic mercury compounds have been used as pesticides, particularly fungicides. Mercury compounds used in this capacity include arylmercurics such as phenyl mercuric dimethyldithiocarbamate, which was used as a slimicides and mould retardant in the paper/pulp industry, and alkylmercurics such as ethylmercuric chloride, C_2H_5HgCl that is used as a seed fungicide. Organic mercury compounds are also used for their germicidal properties, for example phenylmercuric acetate.

2.13.4 Toxicity Associated with Mercury in Tissues

Few studies report both tissue residues and effects in either short- or long-term exposure to low concentrations of mercury. Both the tissue concentration and the exposure time and route (i.e. water, food, and maternal transfer) are critical factors in producing toxic symptoms in aquatic receptors.

According to Wiener and Spry (1996), mercury transferred from the female to the eggs during oogenesis may pose a greater risk to embryos than exposure to mercury in the water column.

For rainbow trout, mercury residues in ovaries of 0.5 mg/kg were associated with a significant reduction in larval survival and abnormal development (Birge *et al.*, 1979). Whitney (1991) reported that hatching success and embryonic survival in walleye were inversely correlated with mercury concentrations in the egg (range 0.002 to 0.058 mg/kg). However, only one of 12 samples had hatching success or embryonic survival less than 90%, and there was no apparent dose-response relationship.

Mercury concentrations in brain tissue associated with lethal effects appear to show less variation than that of other tissues (e.g., muscle, whole body). For example, mercury concentrations in most types of tissues of brook trout killed by exposure to 2.9 µg/l of mercury in the water column varied among individuals, whereas concentrations in the brain showed little variation (McKim *et al.*, 1976). These results are consistent with the hypothesis that the central nervous system, rather than muscle tissue or other organs, is the site of the most harmful toxic action in fish exposed to mercury (Wiener and Spry, 1996). In their review of

the literature, Wiener and Spry (1996) concluded that mercury concentrations of 7 mg/kg or greater in fish brain probably cause severe, potentially lethal effects. In sensitive species such as the walleye, brain tissue concentrations of 3 mg/kg or greater probably indicate significant toxic effects.

Based on a review of the literature, Niimi and Kisson (1994) suggest that a total mercury body burden of 1-5 mg/kg represents a threshold concentration for chronic adverse effects in aquatic organisms. Wiener and Spry (1996) reviewed the literature and provided guidance for interpreting mercury residues in the axial muscle tissue in adult fish associated with toxicity; both field and laboratory studies indicate that residues of 6 to 20 mg/kg are toxic. Whole body mercury concentrations of about 5 mg/kg in brook trout and 10 mg/kg in rainbow trout were associated with sublethal and lethal effects. Both of these papers are recent examples of attempts to identify a threshold of mercury in tissue that is associated with adverse effects. The "thresholds" presented in these papers are based on effects in adult fish and probably do not represent a truly protective level for all species and life stages, including maternal transfer. We begin to become concerned about reproductive or early life stage effects when total Hg in whole bodies of fish are between 0.5 and 1.0 ppm.

2.14 Analytical Methods for the Determination of Total Mercury in Fish

Methods of analysis are usually classified according to the type of instrument used in the final measurement. Measurement of the very low levels of mercury found in the non-contaminated environment makes special demands both on the skills of the analyst and the resources of the method employed. Several research papers exist concerning methods of determining mercury. Several recent reviews have appeared (Swedish Expert Group, 1971, Wallace *et al.*, 1971, D'Itri, 1972, NIOSH, 1973, CEC Working Group of Experts, 1974). The most frequently used methods for measurements of total mercury are colorimetric (dithizone), flameless atomic absorption, and neutron activation. The flameless atomic absorption method has become the

"work-horse" for measurement of environmental samples. Difficulties might arise in the measurement of mercury owing to the fact that it is strongly bound to the organic materials in most samples. Many procedures require the destruction of organic materials by wet oxidation or by high temperatures. Loss of mercury by volatilization may occur. If the wet oxidation is too mild the result will be inadequate recovery. A high reagent blank may be introduced by the chemicals used for oxidation. In certain procedures involving atomic absorption or neutron activation the digestion of the sample or heating of the sample is not necessary. These procedures have the advantage of having a low blank but problems of variable recovery or interference may arise.

The determination of mercury by colorimetric measurement of a mercury dithizonate complex has been the basis of most of the methods in the 1950s and in the 1960s. The above procedures all make use of wet oxidation of the sample followed by extraction of mercury in an organic solvent as a dithizonate complex and finally the colorimetric determination of the complex itself. Selectivity for mercury is obtained by adjusting the conditions of extraction. Copper is the metal most likely to interfere with mercury measurement by dithizone.

The dithizone procedure has an absolute sensitivity of about 0.5 μg of mercury. A sample size of 10 g is suitable for most digestion procedures so that mercury can be determined at the 0.05 mg/kg level in most foodstuffs and tissues. The quoted recovery rates for the dithizone procedure from foodstuffs and tissues are in the range of 85-99 % and the reproducibility can yield a coefficient of variation of as low as 2 %. On account of its long history of use, the dithizone procedure has been used to measure mercury in virtually all types of environmental samples including air, water, food, tissues, and soils. It suffers from the disadvantage that it is time consuming and its sensitivity is not high when compared with atomic absorption procedures. Magos (1971) has described a reduction technique that selectively determines total and inorganic mercury in biological samples without digestion of the material. This technique has been modified by Magos and Clarkson (1972) to permit determination of

mercury in blood samples at the low levels found in unexposed populations (0.1-1.0 $\mu\text{g}/100$ ml). The technique has a sensitivity of approximately 0.5 ng of mercury. The relative standard deviation was 2% and the recovery rates were quoted as being close to 100 %. The technique has the advantage of high speed- each determination taking less than 2 minutes- high sensitivity, and the apparatus involved is light, portable, and suitable for field applications. The atomic absorption technique is subject to interference. The most common interfering substances are benzene and other aromatic hydrocarbons that absorb strongly in the 253.7 nm region. The combustion-amalgamation method has undergone a series of developments to avoid difficulties due to interfering substances. All these methods have sensitivities down to the 1 $\mu\text{g}/\text{l}$ level and avoid the risk of interference from other substances. However, as pointed out by Burrows (1975), care must be taken in the design and operation of the combustion tube to avoid losses of volatile mercury derivatives.

Procedures for neutron activation analysis of total mercury have been reviewed by Wallace *et al.*, (1971), Swedish Expert Group (1971) and Burrows, (1975). The method is based on the principle that when natural mercury (a mixture of stable isotopes) is exposed to a high flux of thermal (slow) neutrons, it is converted to a mixture of radioactive isotopes, principally Hg^{197} and Hg^{203} , which have decay half-lives of 65 hours and 47 days, respectively. After the sample has been irradiated with neutrons, a precise weight of carrier mercury is added and the sample subjected to digestion and organic destruction. On completion of digestion, mercury is isolated by electro deposition on a gold foil and the radioactivity is determined with a gamma counter. The use of carrier mercury corrects for any losses of mercury during the digestion, extraction, and isolation procedures. The limit of detection is 0.1-0.3 ng of mercury. The sample size is 0.3 g, giving a concentration limit of 0.3-1 $\mu\text{g}/\text{kg}$ in most biological samples.

In general, the analyst is faced with three major options in the use of neutron activation procedures; (a) destruction or non-destruction of the sample, (destruction and isolation of the

mercury is usually required in samples containing less than 1 μg of mercury); (b) the choice of isotope Hg-197 (if the longer-lived isotope, Hg-203, is used the sample may be allowed to stand to avoid interference from short-lived elements activated along with the mercury, however, Hg-203 requires a more intense neutron flux or a longer irradiation time to achieve the same activity as the Hg-197); (c) the choice of detector (the sodium iodide (thallium) detector does not have as high a resolution as the germanium (lithium) detector, although its sensitivity is significantly higher).

Interference may come from the following elements, produced at the same time as the radioactive mercury isotopes, Na-24, Br-82, P-32, and Se-75. Interference from these isotopes may be avoided by chemical isolation of the radioactive isotope. However, Se-75 may not be completely removed by the isolation procedures and might interfere if the sodium iodide (thallium) detector is used. The better resolution of the germanium (lithium) detector allows correction for Se-75 interference through use of other lines in the Se-75 spectrum. For samples containing more than 1 μg of mercury, the required selectivity can be achieved without destruction of the sample, i.e., by instrumental analysis only. One procedure is to measure the Hg-203 isotope, after allowing the sample to stand for approximately one month to eliminate interference due to sodium, phosphorous and bromine. Another procedure is to make use of the discriminating germanium (lithium) detector when the gamma irradiation from the radioactive isotope may be determined to the exclusion of most of the interfering radioactivity.

Compared with other methods reviewed here, the neutron activation procedure has the following advantages; (1) high sensitivity (approximately 0.5 $\mu\text{g/kg}$); (2) no reagent blank; (3) independence from the chemical form of the element; and (4) non-destructive instrumental methods applicable to samples containing 1 μg of mercury or more. It has the disadvantages that it cannot be adapted to field use and, that if there are large numbers of samples, special radiation facilities and data processing are required. It is generally agreed that the neutron

activation procedure finds its most important use as a reference method against which other procedures can be checked.

A variety of other instrumental techniques, such as X-ray fluorescence, mass spectrometry, and atomic fluorescence, for the measurement of total mercury have been reviewed. In general, some of these methods may have a potentially higher sensitivity or selectivity for mercury.

2.14.1 Sample Preparation /Digestion

Most matrices require digestion of some type to free mercury from the inorganic and organic forms contained in the sample. Preparation/digestion methods for the determination of mercury are still an active area of research (Suckle and Povondra, 1989; Adeloju *et al.*, 1995). Biological matrices require decomposition, typically by wet oxidation mineralization. The organic and inorganic matrix must be decomposed (digested) and all mercury present must be converted (oxidized) to mercury (II) prior to the cold vapour process. The oxidized mercury then is reduced to elemental metallic mercury vapour by a strong reducing agent, such as tin (II) or sodium borohydride. The digestion methods (decomposition) may be generalized in that each approach uses a strong acid or combination of acids, such as nitric acid, hydrochloric acid, perchloric acid and/or sulfuric acid generally in combination with an oxidant such as hydrogen peroxide, Potassium permanganate, potassium dichromate, potassium persulfate, or vanadium oxide. The function of the acid(s) is to decompose the inorganic and organic matter of the biological matrix. There are a host of processes/mechanisms, which are needed to represent the whole decomposition of solids and/or organic matter (Suckle and Povondra, 1989). Briefly, most wet oxidation processes involving organic matter are oxidative hydrolytic processes. The samples are typically treated with an oxidant in excess. The

purpose of the oxidant is to oxidize any organic bound mercury along with any unoxidized organic matter.

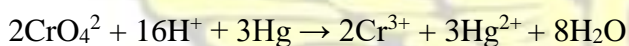
In this way mercury is ensured to be in the soluble (non-volatile) free form of Hg (II). An example of such an oxidant is potassium permanganate whose reaction is described in

Equation 1: Equation

1:

$\text{MnO}_4^- + (\text{inorganic or organic})\text{-Hg} \rightarrow \text{MnO}_2 + \text{Hg}^{2+} + \text{CO}_2\uparrow + (\text{inorganic salts})$. In similar fashion potassium chromate may be used; samples may often be preserved with an oxidant such as chromate to ensure that mercury remains as Hg^{2+} . Chromium (IV) can prevent the formation of Hg^0 and thereby prevent the loss of mercury through volatilization as shown in Equation 2:

Equation 2:

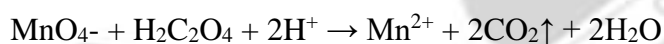


Though potassium permanganate oxidizes many of these compounds some studies have indicated that a number of organic mercurials, including phenylmercuric acetate and methylmercuric chloride, are only partially oxidized by this reagent (United States Environmental Protection Agency, 1997). However, other studies have reported good recoveries of phenylmercuric acetate using potassium permanganate (Anderson *et al.*, 1995). Potassium persulfate has also been reported to give good recoveries when used as the oxidant with these compounds (United States Environmental Protection Agency, 1997).

The digestions are routinely done at modest temperatures: 95°C, due to the possible loss of mercury at elevated temperatures (e.g. dimethylmercury bp 96 °C). However, if closed vessel digestions are done such as, microwave closed vessel techniques or other sealed vessels such as teflon or quartz lined bombs, losses of mercury are minimized. It must be

noted here that excess oxidant will interfere by consuming some of the reducing reagent(s). The cold vapour chemistry recess potentially leaving insufficient reagent for the mercury chemistry. There is therefore the need to remove excess oxidant (e.g. hydrogen peroxide, potassium permanganate, potassium dichromate, potassium persulfate, or vanadium oxide) prior to the cold vapour chemistry. This involves the addition of reagents such as oxalic acid (Anderson *et al.*, 1995), or hydroxylamine hydrochloride (Pineau *et al.*, 1990) to reduce the excess oxidants without reducing the mercury ions. For example, excess oxidizing agent such as permanganate is pre-reduced with oxalic acid as shown in Equation 3. Oxalic acid is added to reach the final end point, which is a colorless solution (i.e. Mn^{2+} is colourless, MnO_4^- is purple, and MnO_2 is a brown precipitate).

Equation 3:



Oxalic acid does not react with the mercury and thus leaves the mercury as non-volatile mercury (II). This mild reduction should be performed immediately prior to the cold vapour process.

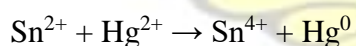
2.14.2 The Cold Vapour Technique

Mercury is the only metal that exists as a liquid at ambient conditions and at the same time exhibits a considerable vapour pressure. These unique properties allow for unique methods of detection to be exploited. In addition, mercury determination by traditional techniques; such as flame AAS, AFS or ICPAES, exhibit poor sensitivity; therefore alternative methods of detection are necessary. Since mercury has a high vapour pressure (0.16 Pa at 20 °C), mercury may be determined by AAS without the use of an atomizer. Mercury must be simply reduced to metallic mercury from its compounds and transferred as the vapour phase. This is accomplished by a chemical reduction reaction used to generate the gaseous mercury species - Cold Vapour (CV). There are two primary advantages of the cold vapour process. First

mercury, the analyte, is removed from the sample matrix, which reduces the potential for matrix interferences. Second, the detection limits are improved because the entire mercury sample is introduced into the atomizer (nebulizer in the case of ICP AES) within a few seconds. Therefore, the density of mercury in the cell during data collection (absorption, fluorescence or emission depending on the detection technique) is greatly enhanced as compared to typical sample introduction.

Two reducing agents used exclusively for cold vapour analysis are, tin (II) chloride and sodium borohydride. Tin (II) chloride (SnCl_2) was used in most of the early applications for mercury cold vapour analysis, and more recently sodium borohydride, NaBH_4 , (also called sodium tetrahydroborate in the IUPAC nomenclature) has gained some favour. Tin (II) chloride reacts with mercury as described by Equation 4:

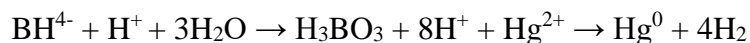
Equation 4:



The tin chloride technique requires that the metallic mercury be transported by an inert gas stream (for example argon or nitrogen) bubbled through the solution to drive out the mercury vapour to the absorption cell. Later applications use tin sulfate (stannous sulfate) instead of tin (II) chloride (Sturman, 1985; Delft and Vos, 1988)

Sodium borohydride in an alkaline solution is becoming the preferred reagent because it requires no other reagents for the reduction. Sodium borohydride is also a stronger reducing agent than tin (II) chloride. In addition, sodium borohydride produces hydrogen (H_2) as part of the reduction reaction. The hydrogen produced can then aid to transport the metallic mercury from the solution into the absorption cell. The sample digest is acidic from the mineralization process and the reduction reaction is as shown in Equation 5:

Equation 5:



Sodium boron hydride is prepared in base (e.g. NaOH) for stabilization purposes. The boron hydride technique is limited to open systems because of the production of hydrogen.

2.14.2.1 Principle of cold vapor atomic absorption spectrometry (CVAAS) (circulationopen air flow system).

The present method involving reduction and cold vapor atomic absorption spectrometry (CVAAS) (circulation-open air flow system) is, in principle, similar to the conventional circulation system in that the method includes the following: reduction of Hg^{2+} ions in the sample test solution with stannous chloride to generate elemental mercury vapor (Hg^0); and the introduction of mercury vapor into the photo-absorption cell for the measurement of absorbance at 253.7 nm. However, unlike the conventional closed system in which the elemental mercury vapor generated is continuously circulated with a diaphragm pump through a reaction vessel, a U-shaped tube packed with a drying agent, and the photo-absorption cell, the present method uses a circulation-open air flow system as shown in Figure 1. The apparatus constitutes a closed system and comprises a diaphragm pump, reaction vessel, acid gas trap, moisture trap (ice bath), and a 4-way cock. During its operation, the elemental vapor generated by the addition of tin (II) chloride is circulated via 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 then 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.

Additionally, in the method for preparing the sample test solution for the present method, the conventional wet digestion method is improved by the use of a 50 ml flask with a long neck (at least 10 cm), such as a thick-walled volumetric flask¹ with a ground glass stopper, as well as a mixed acid system with an increased rate of sulfuric acid, $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$ (1+1+5),

that already contains perchloric acid, for the sample digestion. This is innovative in that sample digestion can be completed in a relatively short time without loss of mercury. It is a simple method where the sample is subjected to wet digestion on a hot plate at 200-230 °C for 30 minutes and cooled followed by topping up to a fixed volume with water. This method can be applied directly to the digestion of biological samples including hair, blood, and fish as well as various solid samples such as sediment and soil. A reflux condenser is not required during heating.

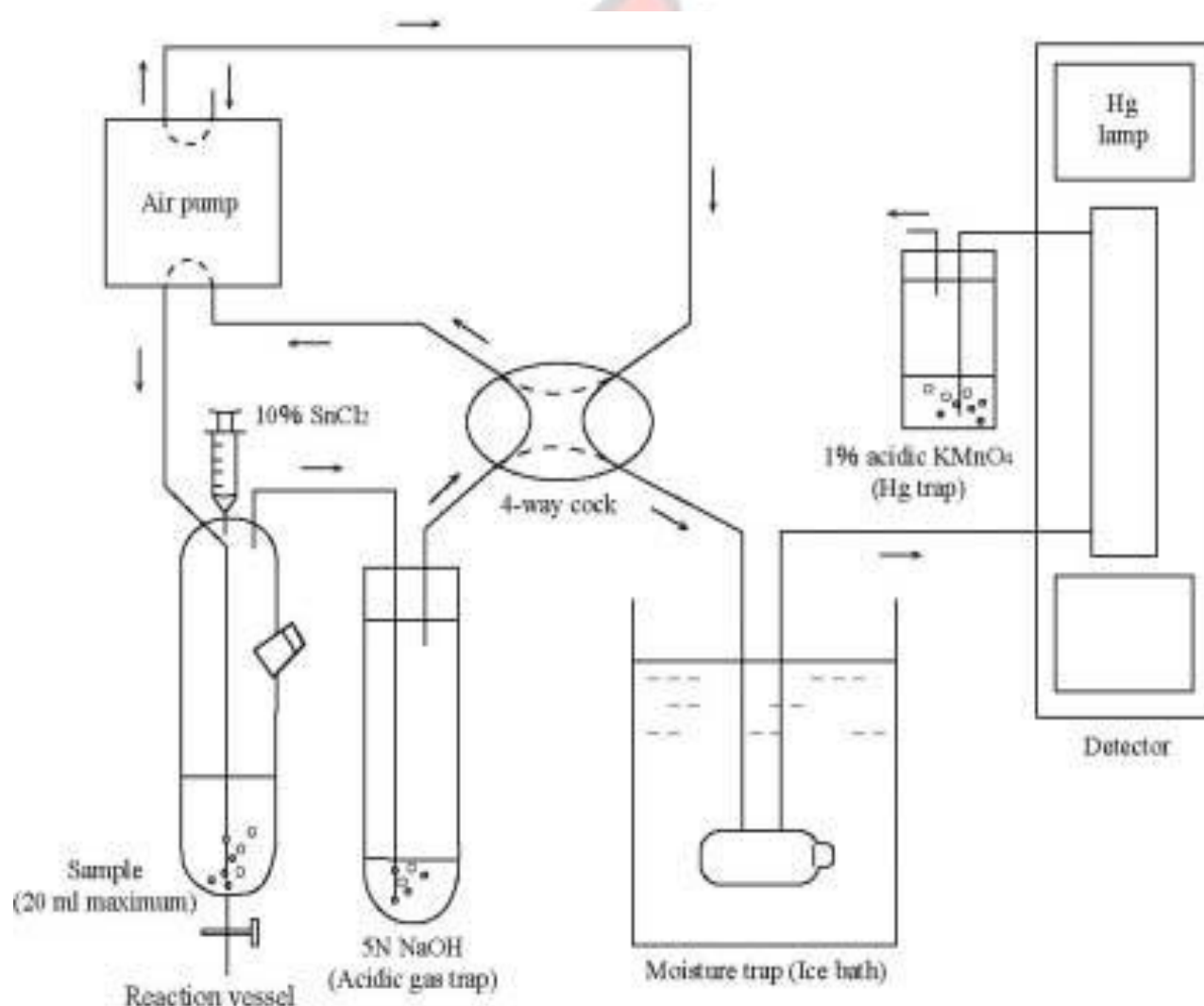


Figure 2.3: Schematic Diagram of Reduction/Cold Vapor Atomic Absorption Spectrometry

Source: (Akagi, 1985)

Chapter 3

3. MATERIALS AND METHODS

3.1 Apparatus

All glassware used were soaked in detergent solution overnight; rinsed and soaked in 10% (v/v) HNO_3 overnight. They were rinsed with distilled water followed by 0.5% (w/v) Potassium permanganate (KMnO_4) solution, rinsed with distilled water again and dried before use.

Automatic Mercury Analyzer Model HG-5000 (Sanzo Seisakusho Co., Ltd, Japan), equipped with a mercury lamp operated at a wavelength of 253.7 nm was used for mercury determinations. The signals were obtained on a computer and results printed out using a printer. Digestion apparatus were a thick walled long neck 50 ml volumetric flasks and a hot plate Model 67891(Clifton Hotplate, Nickel Electro LTD) with a temperature range of 150-350 °C.

3.2 Reagents

All reagents used were of analytical reagent grade (BDH Chemicals Ltd, Poole, England) unless otherwise stated. Double distilled water was used for the preparation of all solutions.

Mercury stock standard solution (1000 mg L^{-1}) was prepared by dissolving 0.0677 g of mercury (II) chloride (HgCl_2) in the acid mixture $\text{HNO}_3 - \text{H}_2\text{SO}_4 - \text{HClO}_4$ (1 + 5 + 1) ml in a 50 ml digestion flask with heating on a hot plate at a temperature of 200 °C for 30 min. The solution was then diluted to 50 mL with distilled water. Blank solutions were also prepared alongside and bulked together for use as a diluent. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solution through intermediate

solutions using blank solution. Stannous chloride solution (10% w/v) was prepared by dissolving 10 g of the salt in 100 mL 1M HCl.

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3.3 Sampling and Sample Preparation

The fish species, tilapia (*Oreochromis niloticus*) and mudfish (*Clarias agboyensis*) were obtained from random commercial catches from the Kpong Hydroelectric Reservoir in the Eastern Region, Tono irrigation dam in Navrongo and White Volta at Yapei in Northern Region between February 2010 and June 2010 in three batches from each sampling site, depending on the species available for sale. Samples collected were therefore reflective of species meant for consumption. A total of fifteen (15) mud fish and fifteen (15) tilapia species were obtained from each sampling site. Therefore a total of Ninety (90) fish samples were obtained from all three sampling sites. The samples were sorted according to the two species, bagged in clean plastic bags and iced. They were then transported to the laboratory, identified and kept in a freezer at -20 °C prior to preparation for chemical analysis. The samples were defrosted, washed with distilled water and dried on tissue paper. The total length and body weight of each was taken. A portion of the edible muscle tissue was removed from the dorsal part of each fish. The dorsal fin, pelvic fin, pectoral fin, caudal fin and anal fin of each sample were obtained after careful cutting with the aid of stainless steel knife and a pair of scissors which has been cleaned thoroughly prior to use. The tissues obtained were then homogenized and placed in separate plastic bags and kept frozen ready for analysis.

3.4 Digestion procedure for Muscle and Fin tissues

The fish tissues were digested for total mercury determination by an open flask procedure as shown in Chart 3.1 developed at the National Institute for Minamata Disease (NIMD) in Japan by Akagi and Nishimura (1991). The accuracy of this method has been verified at NIMD through interlaboratory comparison exercises and by participating in the analyses of Certified Reference Materials (CRMs) supplied by the International Atomic Energy Agency (IAEA). In the procedure, 0.5 g of homogenized tissues was weighed into 50 ml volumetric digestion flask and a mixture of 1 ml H₂O, 2 ml HNO₃ – HClO₄ (1:1) and 5 ml H₂SO₄ were added in turns. The mixture was then heated at a temperature of 200°C ± 5 for 30 min. The sample solution was then cooled and diluted to 50 mL with double distilled water. A blank and standard solution digests with concentrations 0.5, 1.0 and 2.0 µg Hg mL⁻¹ from the 1 µg mL⁻¹ standard Hg solution were subjected to the same treatment.

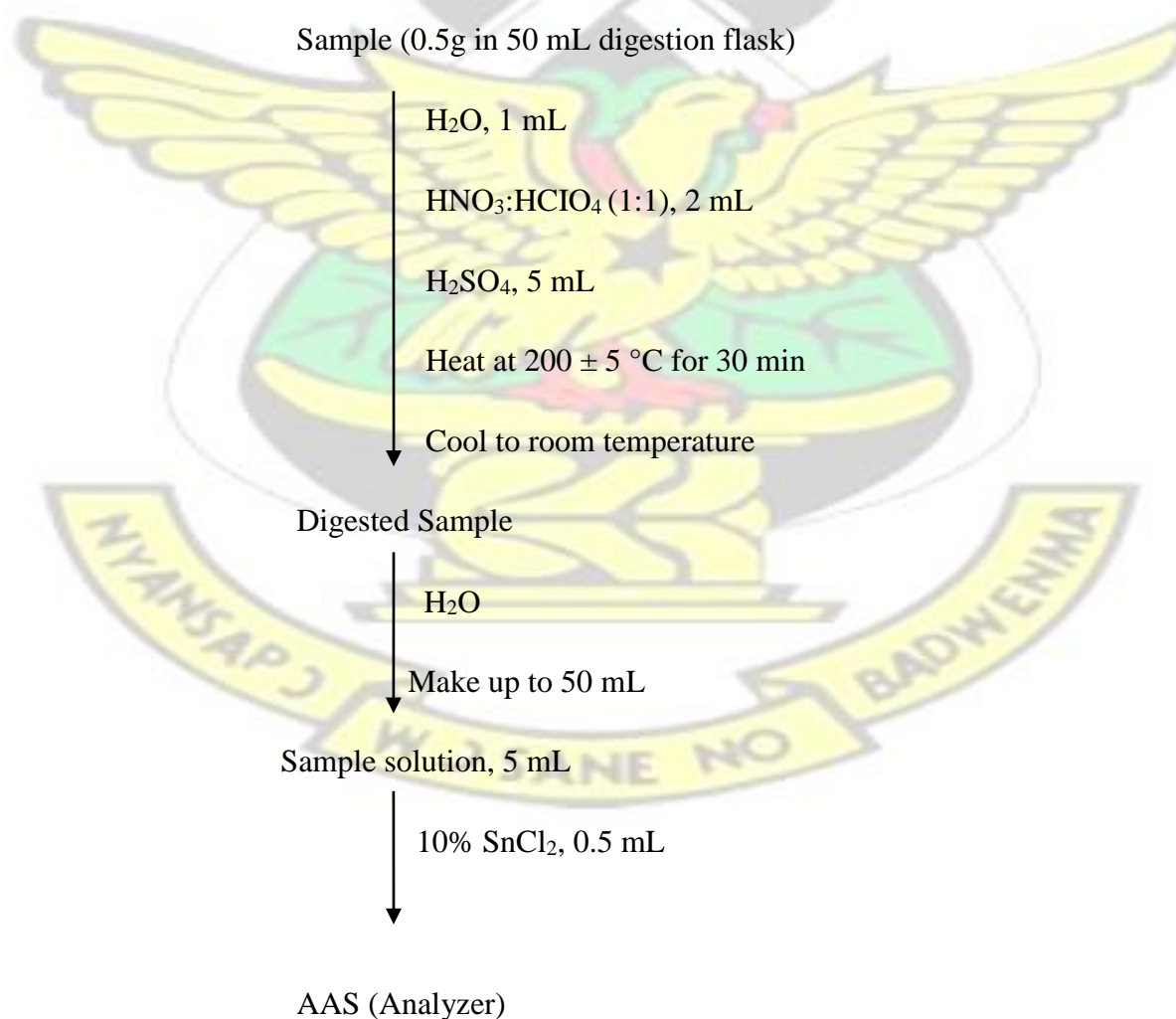
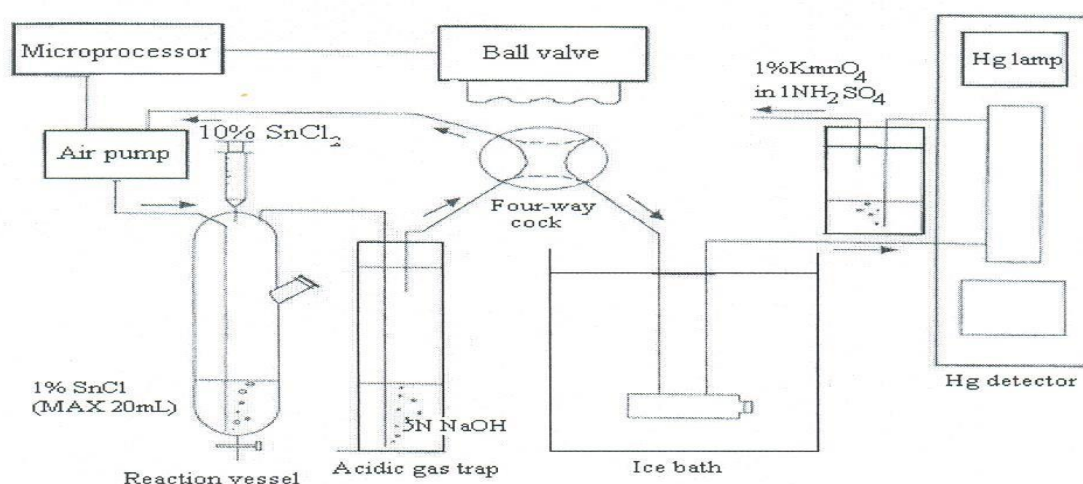


Chart 3.1 Analytical procedure for total mercury determination in fish tissues

3.5 Determination of mercury

Determination of mercury in all the digests was carried out by cold vapour atomic absorption Spectrophotometry using an Automatic Mercury Analyzer Model HG-5000 (Sanzo Seisakusho Co., Ltd, Japan) developed at NIMD. The analyzer is an instrument designed specifically for the measurement of mercury using the cold vapour technique. It makes use of the batch mercury cold vapour generation system. The analyzer consists of an air circulation pump, a reaction vessel, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ dispenser, an acidic gas trap and a four-way stop-cock with tygon tubes to which is attached a ball valve. The operations of the ball valve and the air circulation pump are controlled by a microprocessor. A schematic diagram of the system is shown in Fig 3.1. During the determination, a known volume of the sample solution normally 5 ml is introduced into the reaction vessel using a micropipette (1-5 mL). The reaction vessel is immediately stoppered tightly and 0.5 ml of 10 % (w/v) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 1M HCl is added from a dispenser for the reduction reaction. During this time, air is circulated through the fourway stopcock to allow the mercury vapour to come to equilibrium and the acidic gases produced by the reaction also swept into the sodium hydroxide solution. After 30 seconds the four-way stopcock is rotated through 90° and the mercury vapour is swept into the absorption cell. Peak heights were used for computations. Standards used for calibration of the analyzer included solutions containing 0.0, 0.5, 1.0 and 2.0 ng Hg mL⁻¹. Calibration graph was obtained from which concentration of the sample digests was extrapolated. Quality assurance samples



analysed included method (digestion) blanks, replicate samples, pre-digestion spikes, post digestion spikes.

Figure 3.1: Schematic Diagram of the Apparatus for Mercury Determination by Cold Vapour Atomic Absorption Spectrophotometry (CVAAS)

3.6 Quality Assurance

Recovery of mercury was determined by adding increasing amounts of mercury to tissues of two different fish species which were taken through the digestion procedure. The resulting solutions were analysed for mercury concentration. The results are reported in Table 4.1. The instrument was calibrated based on a linear four-point calibration curve (0.0, 0.5 ngHgml⁻¹, 1.0 ngHgml⁻¹, and 2.0 ngHgml⁻¹). Standard calibration curves with an $r^2=0.9999$ were run during measurements. To monitor the calibration curve, a continuing calibration verification standard and blank sample was analyzed at a 10 percent frequency and at the end of each analytical batch. Certified reference material (CRM), samples and reagent blank were analysed to validate the methodology used in this study

3.7 Statistical Analysis

The data obtained in this study were subjected to statistical analyses using SPSS for Windows Statistical Package Version 10.0. Two basic statistical approaches were used to assess the differences between mercury concentrations among species. Linear regression and correlation analysis were conducted using the following variables: mercury concentration in fish muscle, fins, fish length and fish weight. A regression of mercury concentration against fish length and/or weight of the fish were made for the two species.

Chapter 4

4. Results and Discussion

In this study a total of ninety fish samples covering two species were obtained from Kpong Hydroelectric Reservoir, Tono Irrigation dam and the White Volta at Yapei. Six tissues of fish namely muscle, dorsal fin, caudal fin, pectoral fin, pelvic fin and anal fin were analysed for total Hg using CVAAS.

Quality assurance was performed by analysing reagents blanks, tissues samples in replicates, certified reference materials (CRM), and by carrying out recovery studies. The results for the recoveries in this study ranged from 96.00 to 102.00 %. The results for the recovery studies are presented in Table 4.1. Precision was carried out by repeated analysis of samples. The validity of the method has been proved by agreement between values obtained for the measured (0.215 mg/kg) and certified (0.216-0.228 mg/kg) for fish Homogenate (IAEA407).

4.0 Mercury in Tissues of Fish

Table 4.1: Recovery of mercury from fish samples

Sample code	Hg added (μg)	Hg found (μg)	Hg recovered (ng)	% Recovery
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MFN12 <i>Mudfish</i> (0.5g)	- 0.025 0.050	0.287 0.310 0.338	- 0.023 0.051	- 92% 102.00
MFN13 <i>Mudfish</i> (0.5g)	- 0.025 0.050	0.202 0.226 0.253	- 0.024 0.051	- 96.00 102.14

From the results for total mercury concentration in Table 4.2, most of the muscle tissues analysed showed mercury concentrations below the World Health Organization (WHO/FAO) limit of 0.5 µg/g wet weight. The low levels of mercury in all these muscle tissues may be due to the fact that Hg contamination in the Kpong hydroelectric reservoir, White Volta and the Tono irrigation dam may be very low. However few of the samples recorded levels in excess of the WHO limit, and these include samples from all the sampling sites. There are no artisanal mining activities around these river bodies. Farming and fishing are the main activities along these water bodies and the contribution of these agricultural activities to mercury pollution is very low. And this may account for the reason why only few of the fish samples recorded levels a little in excess of the WHO guide line. The rate of atmospheric deposition could also be very low since concentrations are also low. The low mercury levels could also be ascribed to the unfavourable conditions such as low organic matter and pH that control methylation. The evasion of elemental mercury as a result of microbial activities could also represent a significant pathway for reducing the level of the metal in aquatic ecosystem. Total mercury concentration in fish depends on the fish species and factors such as total length of fish and fresh weight of fish. There was a significant variation

between mercury concentrations, fish length and fish weight in this study. Although growth rate data of fish from the studied areas are not available, variations suggest that all the fish species are not growing at the same rate and may not also be of the same age.

A comparison of the muscle mercury concentration for the tilapia fish from all the three sampling sites indicate that tilapia fish from Yapei ($0.680 \mu\text{g/g}$) recorded the highest concentration of mercury, followed by tilapia from Navrongo ($0.314 \mu\text{g/g}$) and the least being the tilapia fish from Kpong ($0.227 \mu\text{g/g}$).



Table 4.2: Results of T Hg concentration in muscle tissue, wet weight and length of fish species

Sampling Site	FISH SPECIES	Sample Size (n)	Fresh weight Range (g)	Mean Weight (g)	Fish length range(cm)	Mean fish length(cm)	Muscle Hg concentration Range (µg/g)	Mean Muscle Hg Concentration (µg/g)
NAVRONGO	Tilapia	15	115.61-182.52	149.871	18.0-21.5	19.913	0.048-0.975	0.314
	Mudfish	15	97.98-1246.20	392.005	25.1-56.9	38.073	0.186-1.074	0.4604
YAPEI	Tilapia	15	157.44-245.21	194.674	19.0-23.5	21.660	0.181-2.745	0.680
	Mudfish	15	183.77-962.50	431.681	31.7-55.2	40.620	0.14-1.033	0.431
KPONG	Tilapia	15	153.51-594.63	312.612	19.5-31.2	25.120	0.028-0.751	0.227
	Mudfish	15	129.401556.87	606.824	27.0-61.5	42.080	0.087-0.445	0.236

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It can also be seen from these results that the average mercury concentration in the muscle of tilapia fish from Yapei (0.680 $\mu\text{g/g}$) is slightly higher than the WHO limit of 0.5 $\mu\text{g/g}$. With regards to mudfish samples, samples from Navrongo recorded the highest mercury concentration of 0.46 $\mu\text{g/g}$, followed by mudfish samples from Yapei (0.43 $\mu\text{g/g}$) and the least was 0.24 $\mu\text{g/g}$ for mudfish from Kpong. All the average mercury concentrations in the muscle of mudfish from the three sampling sites were all below the WHO standard limit of 0.5 $\mu\text{g/g}$.

4.0 MERCURY IN TISSUES OF FISH

4.1 Mercury in Fish Tissues from Navrongo (Tono Irrigation Dam) Sampling Site

4.1.1 Mercury in Tilapia Tissues from Navrongo

Fifteen (15) tilapia fish samples were collected from the Tono dam in Navrongo and the muscle and various fin tissues were then digested and mercury levels determined using CVAAS and the results presented in Table 4.3. The fins analyzed are caudal fin, pelvic fin, pectoral fin, dorsal fin and anal fin.

Table 4.3: Mercury concentrations in the Muscle and fin tissues of Tilapia from Tono Irrigation Dam (Navrongo)

Tissues	Mean Hg \pm Std.Dev($\mu\text{g/g}$)	Number of samples	Range ($\mu\text{g/g}$)
Muscle	0.33 \pm 0.28	15	0.048-1.149
Anal Fin	0.21 \pm 0.18	15	0.039-0.498
Caudal Fin	0.22 \pm 0.24	15	0.065-0.975
Dorsal Fin	0.20 \pm 0.18	15	0.036-0.570
Pectoral Fin	0.20 \pm 0.15	15	0.036-0.450
Pelvic Fin	0.24 \pm 0.19	15	0.037-0.591

The mean weight and length of the tilapia fish samples collected are 149.87 ± 20.59 g and 19.91 ± 1.16 mm respectively and the weight range from 115.61 g to 182.52 g. The length of the fish ranged from 18.0 mm to 21.5 mm.

The mercury level in the muscle for the 15 samples ranged from 0.05-1.15 $\mu\text{g/g}$ and recorded a mean \pm standard deviation (SD) mercury concentration of 0.33 ± 0.28 $\mu\text{g/g}$. A mean mercury concentration of 0.21 ± 0.18 $\mu\text{g/g}$ was recorded for anal fin and the concentration ranged from 0.04-0.50 $\mu\text{g/g}$. The caudal fins mercury concentration ranged from 0.07-0.98 $\mu\text{g/g}$ with a mean mercury concentration of 0.22 ± 0.24 $\mu\text{g/g}$ was also recorded. The mean \pm SD mercury concentration in the pectoral fin, pelvic fins and dorsal fin are 0.20 ± 0.15 $\mu\text{g/g}$, 0.24 ± 0.19 $\mu\text{g/g}$ and 0.20 ± 0.18 $\mu\text{g/g}$ respectively, and their concentration range are 0.036-0.45 $\mu\text{g/g}$, 0.037-0.591 $\mu\text{g/g}$ and 0.036-0.570 $\mu\text{g/g}$ respectively. The mean concentration of Mercury in the muscle tissues and the various fins are represented in Figure 4.1 below.

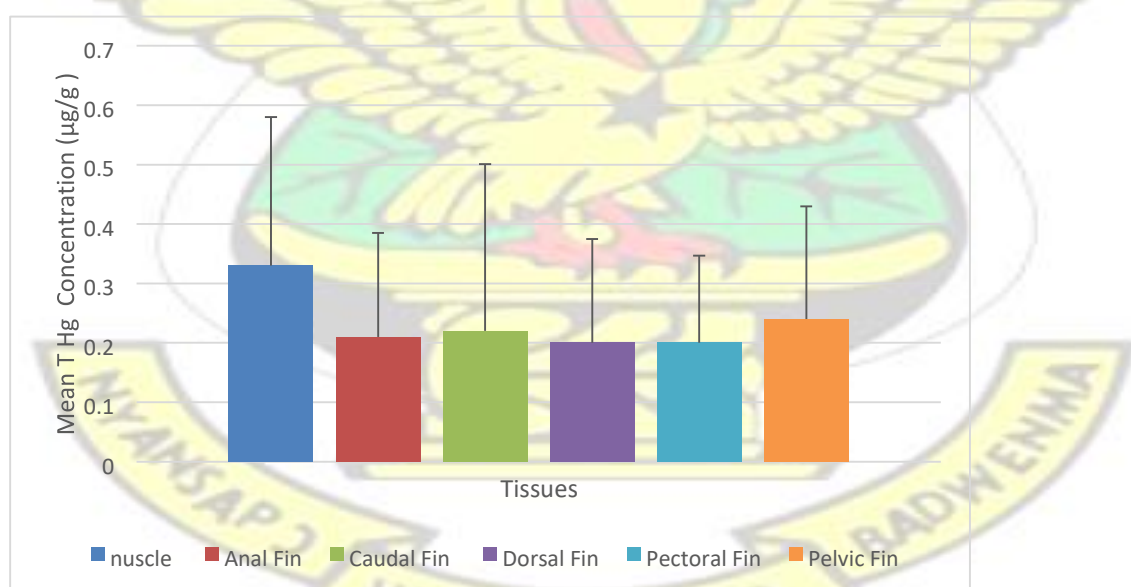


Figure 4.1: Results for Mean Mercury in Muscle tissue and various fins for Tilapia Sample

from Tono dam, Navrongo.

In order to determine any relationship between mercury levels in fish muscle and fin tissues, the levels were subjected to correlation using Person's correlation analysis method. The results are presented as coefficients in a correlation matrix in Table 4.4.

Table 4.4: Correlation Matrix for Navrongo Tilapia Samples

	Muscle	Dorsal Fin	Caudal Fin	Pectoral Fin	Pelvic Fin	Anal Fin	Weight	Length
Muscle		0.669	0.946	0.685	0.604	0.591	0.870	0.778
		0.006	0.000	0.005	0.017	0.020	0.000	0.001
Dorsal Fin	0.669		0.666	0.708	0.688	0.695	0.742	0.692
	0.006		0.007	0.003	0.005	0.004	0.002	0.004
Caudal Fin	0.946	0.666		0.677	0.601	0.604	0.747	0.638
	0.000	0.007		0.006	0.018	0.017	0.001	0.010
Pectoral Fin	0.685	0.708	0.677		0.800	0.373	0.642	0.780
	0.005	0.003	0.006		0.000	0.172	0.010	0.001
Pelvic Fin	0.604	0.688	0.601	0.800		0.516	0.573	0.646
	0.017	0.005	0.018	0.000		0.049	0.026	0.01
Anal Fin	0.591	0.695	0.604	0.373	0.516		0.525	0.482
	0.020	0.004	0.017	0.172	0.049		0.045	0.069
Weight	0.869	0.742	0.747	0.642	0.573	0.525		0.920
	0.000	0.002	0.001	0.010	0.026	0.045		0.000
Length	0.778	0.692	0.638	0.780	0.646	0.482	0.920	
	0.001	0.004	0.010	0.001	0.010	0.069	0.000	

Observation of the correlation data in Table 4.4 showed that all the fins recorded positive significant correlation coefficient. These correlation coefficients ranged from a minimum of 0.60 to a maximum of 0.95 with the p-value ranging from 0 to 0.02. The correlation coefficients recorded are 0.67, 0.95, 0.69, 0.60 and 0.59 for dorsal fins, caudal fin, pectoral fins, pelvic fins and anal fins respectively with respective p-value of 0.010, 0, 0.010, 0.017 and 0.020. Since the P-values for the above mentioned pair of variables as can be seen in the correlation matrix Table 4.4 are less than 0.05, there is a statistically significant relationship between muscle mercury concentration and the various fin mercury concentration at 95.0% confidence level. The results indicate a strong positive correlation between the muscle

mercury concentration and the mercury concentration of the various fins. The concentrations were also subjected to regression analysis and the results presented in Table 4.5.

Table 4.5: Regression Analysis Results for Muscles Tissues against Various fins

Source	Slope	Intercept	F-Ratio	P-Value	R ²
T Hg in Muscle vrs T Hg in Caudal fin	1.114	0.082	110.34	0.000	0.895
T Hg in Muscle vrs T Hg in Dorsal fin	1.086	0.109	10.52	0.006	0.447
T Hg in Muscle vrs T Hg in Pectoral fin	1.326	0.066	11.51	0.005	0.470
T Hg in Muscle vrs T Hg in Pelvic fin	0.906	0.105	7.46	0.017	0.365
T Hg in Muscle vrs T Hg in Anal fin	0.959	0.136	6.97	0.020	0.349
T Hg in Muscle vrs Weight	0.012	-1.474	40.22	0.000	0.756
T Hg in Muscle vrs Length	0.191	-3.474	19.88	0.001	0.605

Where *T* = total and *Hg* = Mercury

The results of regression analysis shown in Table 4.5 above reports that the regression coefficient (r^2) for the linear regression between the total muscle mercury concentration and the total mercury in the various fins are, 0.895, 0.447, 0.470, 0.365, and 0.349, for caudal fins, dorsal fins, , pectoral fins, pelvic fins and anal fins respectively.

The linear regression graphs shown in Figure 4.2, Figure 4.3 and the rest of the graphs in Appendix 1 sub-section 1.1 indicate that the regression of total mercury concentrations in muscle against those in anal fins yielded a slope of 0.959 ($p = 0.001$), whereas the regression of concentrations in muscle against those in pelvic fins yielded a slope of 0.906 ($p = 0.017$).

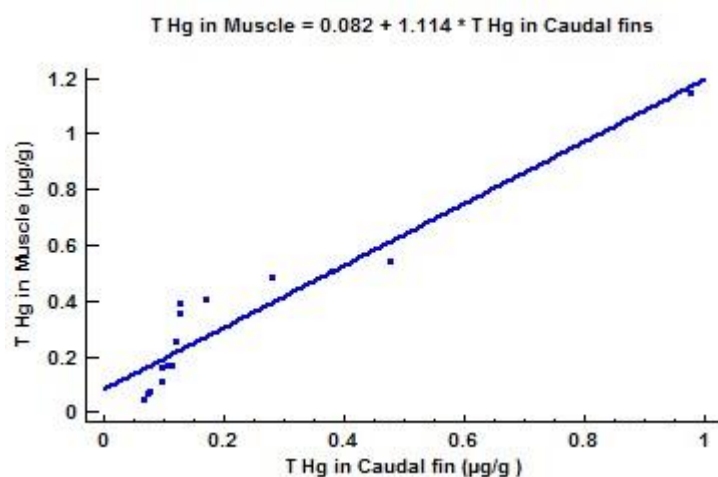


Figure 4.2 T Hg in Muscle against T Hg in Caudal fins

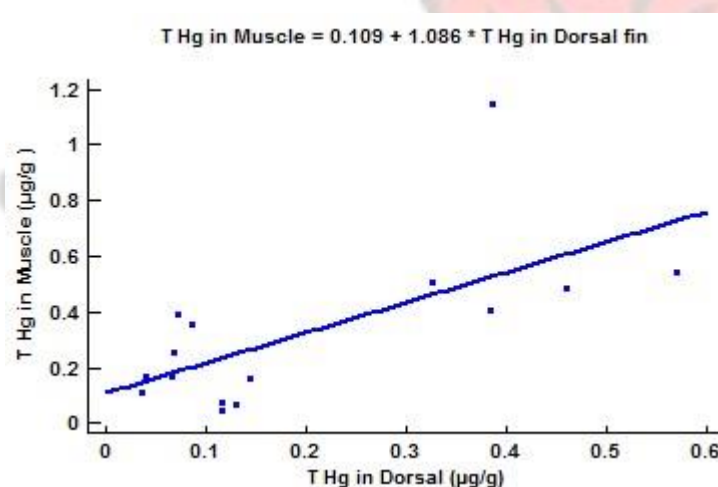


Figure 4.3: T Hg in Muscle against T Hg in Dorsal fin

Furthermore, regression analysis of concentration of mercury in muscle against those in caudal fin yielded a slope of 1.114 ($p=0.000$), slope of 1.086 ($p = 0.006$, $F = 10.52$) being the regression of concentration in muscle against dorsal fin. Regression analysis of mercury concentration in muscle against concentration in pectoral fin yielded a slope of 1.326 ($p = 0.005$). Since the P-values are less than 0.05 at 95% confidence level, there is a statistically significant relationship between total mercury in muscle against fins such as caudal fins and dorsal with good correlation coefficients.. From the results caudal fins recorded the highest regression coefficient (0.895) followed by pectoral fins (0.470), dorsal fins (0.447), pelvic fin

(0.365), and anal fin (0.349). From the regression plots the relationship between the concentration of Hg in the muscle and the fins can be established by an equation. The equations which can be useful in predicting Hg concentration in muscle using concentrations in fins are shown in Table 4.6.

Table 4.6: Equations for Prediction of Mercury in Muscle of Fish

TISSUES	EQUATION	r^2
Dorsal fins	T Hg in Muscle = $0.109 + 1.086 * \text{T Hg in Dorsal Fins}$	0.895
Caudal fins	T Hg in Muscle = $0.082 + 1.114 * \text{T Hg in Caudal Fins}$	0.447
Pectoral Fins	T Hg in Muscle = $0.066 + 1.326 * \text{T Hg in Pectoral Fins}$	0.470
Pelvic Fins	T Hg in Muscle = $0.105 + 0.906 * \text{T Hg in Pelvic Fins}$	0.365
Anal Fins	T Hg in Muscle = $0.123 + 0.959 * \text{T Hg in Anal Fins}$	0.349

The equation most appropriate for estimation of the total mercury in the muscle of Tilapia fish samples from Navrongo, Tono dam as indicated in Table 4.6 is:

$$\text{T Hg in Muscle} = 0.082 + 1.114 * \text{T Hg in Caudal fins} \quad (r^2 = 0.895)$$

Hence caudal fins mercury concentration better predicts the mercury concentration in the muscle than the rest of the other fins since it recorded the highest regression coefficient (r^2). The result for caudal fins is consistent with Gremillion *et al.*, (2005), who reported significant relationship between muscle mercury concentration and the caudal fin mercury concentration in walleye and northern pike from three rivers in northern Arizona. The other fins recorded

significant correlation since their recorded p-values were less than 0.05 however their regression coefficients were lower than 0.5 making the relationships weaker.

Mercury concentration in muscle also correlated very well with fish weight and length with regression coefficients of 0.756 and 0.605 respectively. It indicated that there is significant relationship between muscle mercury concentration and fish weight and length. This is consistent with what has been reported elsewhere (Voegborlo and Akagi, 2007; Gremillion *et al.*, 2005; Kristofer *et al.*, 2008)

A comparison of the total mercury concentration in the fins to that in the muscle showed that in all cases the mercury in the muscle were all higher than that recorded for the individual fins. Results for tilapia samples in this study agrees with those observed for Arctic grayling a salmonid (brown trout, *Salmo trutta*) by Jewett *et al.*, 2003 and Skurdal *et al.*, 1986 who reported a strong correlation between total mercury in the axial muscle and adipose fin ($r^2 = 0.86$).

4.1.2 Mudfish Samples from Tono Irrigation Dam in Navrongo

A total of 15 mudfish samples were collected from the Tono dam in Navrongo, and the various tissues samples were then taken through the necessary experimental procedure and the total mercury determined using CVAAS and the results are presented in Table 4.7 and plot of the mean mercury concentrations in muscle tissue and the various fins are shown in Figure 4.9

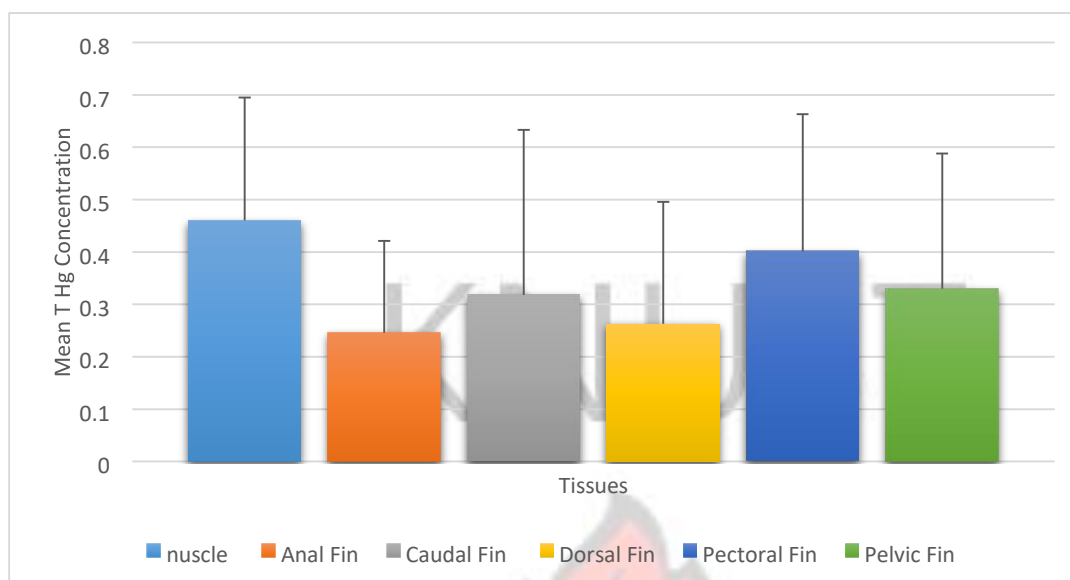


Figure 4.9: Plot of mean Hg concentration in Muscle Tissue and various fins for Mudfish samples from Tono Irrigation dam Navrongo

Table 4.7: Mercury Concentrations in the muscle and fin tissues of Mudfish from Tono Irrigation Dam, Navrongo

Tissues	Mean Hg \pm Stn.Dev($\mu\text{g/g}$)	Number of samples	Range
Muscle	0.460 ± 0.235	15	0.186-1.074
Anal Fin	0.246 ± 0.175	15	0.063-0.743
Caudal Fin	0.318 ± 0.315	15	0.018-1.039
Dorsal Fin	0.263 ± 0.233	15	0.05-1.007
Pectoral Fin	0.401 ± 0.262	15	0.04-0.968
Pelvic Fin	0.330 ± 0.258	15	0.113-1.062
Weight(g)	392.005 ± 275.654	15	97.98-1246.2
Length(cm)	38.073 ± 7.618	15	25.1-56.9

The total mercury in the muscle of the 15 samples from the Tono dam ranged from 0.186-1.074 $\mu\text{g/g}$ with a mean \pm SD of 0.460 ± 0.235 $\mu\text{g/g}$. A mean total mercury concentration of 0.246 ± 0.175 $\mu\text{g/g}$ was recorded for anal fin and the concentration ranged from 0.063-0.743 $\mu\text{g/g}$. The caudal fin mercury concentration ranged from 0.018-1.039 $\mu\text{g/g}$ with a mean of

0.294±0.232 µg/g. The mean±SD mercury concentration in the pectoral fin, pelvic fin and dorsal fin were 0.398±0.260 µg/g, 0.323±0.232 µg/g and 0.263±0.233 µg/g respectively, and their respective concentration ranges were 0.04- 0.968 µg/g, 0.113-1.062 µg/g and 0.05-1.007 µg/g.

Correlation data presented in Table 4.8 shows that all the fins recorded positive significant correlation coefficient. These correlation coefficients ranged from a minimum of 0.527 to a maximum of 0.867 and the p-value also ranged from 0.000 to 0.043.

Correlation between mercury level in the muscle tissue and weight and length of the fish yielded significant correlations. A correlation coefficient of 0.658 and p-value of 0.008 was recorded for mercury level in the muscle and the weight of the fish. A correlation coefficient of 0.579 and a p-value of 0.024 was also recorded for the relationship between the mercury in the muscle and the length of the fish. Since the p-value for muscle with weight and muscle with length were below 0.05, there is a strong positive correlation between total mercury in the muscle and the various fins and also between muscle and the weight and length of the samples.

Table 4.8: Correlation Matrix for Mudfish Samples from Navrongo, Tono Dam

	Muscle	Dorsal Fin	Caudal Fin	Pectoral Fin	Pelvic Fin	Anal Fin	Weight	Length
Muscle		0.662	0.730	0.527	0.867	0.666	0.658	0.579
		0.007	0.002	0.043	0.000	0.007	0.008	0.024
Dorsal Fin	0.662		0.863	0.519	0.800	0.666	0.748	0.626
	0.007		0.000	0.0473	0.0003	0.007	0.001	0.013
Caudal Fin	0.730	0.863		0.467	0.810	0.612	0.710	0.589
	0.002	0.000		0.079	0.0003	0.015	0.0030	0.021
Pectoral Fin	0.527	0.519	0.467		0.565	0.941	0.920	0.972
	0.043	0.047	0.079		0.028	0.000	0.000	0.000
Pelvic Fin	0.867	0.800	0.810	0.565		0.765	0.784	0.628
	0.000	0.0003	0.0003	0.028		0.001	0.001	0.012
Anal Fin	0.666	0.666	0.612	0.941	0.765		0.968	0.925
	0.007	0.007	0.015	0.000	0.001		0.000	0.000
Weight	0.658	0.748	0.710	0.920	0.784	0.9683		0.950
	0.008	0.001	0.003	0.000	0.001	0.0000		0.000
Length	0.579	0.626	0.589	0.972	0.628	0.925	0.950	
	0.024	0.013	0.021	0.000	0.012	0.000	0.000	

From the results all the fins recorded moderate to strong positive correlation between the mercury in the fins against the muscle mercury concentration. A strong positive correlation between the weight and length of the mudfish samples against the muscle mercury concentration was also recorded.

Table 4.9 shows the results of the regression analysis of the relationship between the total mercury in muscle and the various fins (dorsal fins, caudal fins, pectoral fin, pelvic fin and anal fin) and weight and length of mudfish samples from Tono dam, Navrongo. The regression coefficients (r^2) recorded for the various fins are 0.438, 0.532, 0.278, 0.752, 0.444, 0.433 and 0.336 respectively. From the results in Table 4.8, it can be observed that there is a statistically significant relationship between Total Hg in muscle against Total Hg in the fins at the 95.0% confidence level since the P-values in the Table 4.8 above are less than 0.05. Results from Table 4.8 above also indicates the slopes for the regression lines between muscle mercury concentration and mercury concentration in the various fins namely Dorsal fin (0.665, $p = 0.0017$), caudal fin (0.738, $p = 0.002$), Pectoral fin (0.476, $p = 0.043$), pelvic fin (0.875, $p = 0.000$) and Anal fin (0.894, $p = 0.0067$).

Table 4.9: Regression Results for Mudfish Samples from Tono Dam, Navrongo

Source	Slope	Intercept	F-Ratio	P-Value	r^2
T Hg in Muscle vrs Dorsal fin	0.665	0.285	10.14	0.007	0.438
T Hg in Muscle vrs Caudal fin	0.738	0.243	14.80	0.002	0.532
T Hg in Muscle vrs Pectoral fin	0.476	0.271	5.00	0.043	0.278
T Hg in Muscle vrs Pelvic fin	0.875	0.178	39.51	0.000	0.752
T Hg in Muscle vrs Anal fin	0.894	0.240	10.37	0.007	0.444
Source	Slope	Intercept	F-Ratio	P-Value	r^2
T Hg in Muscle vrs Weight	0.0006	0.241	9.92	0.008	0.433
T Hg in Muscle vrs length	0.018	-0.219	6.57	0.024	0.336

The various regression graphs for the relationship between the total mercury in the muscle and the total mercury in the selected fins with significant regression coefficients are shown in the Figure 4.10 and Figure 4.11.

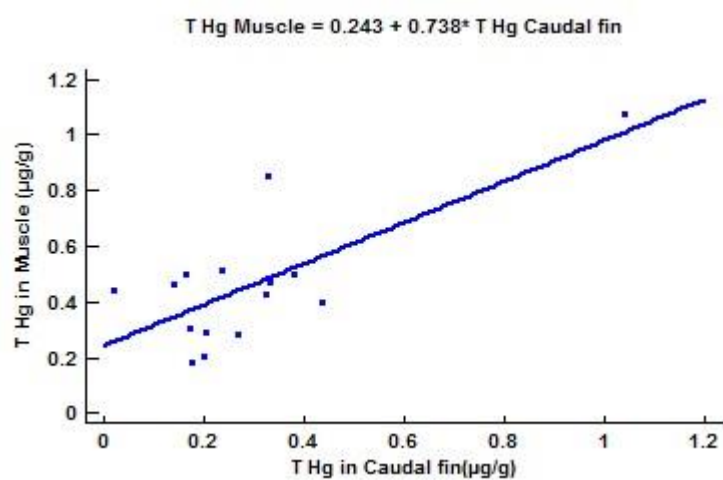


Figure 4.10: T Hg in Muscles Verses T Hg in Caudal fins

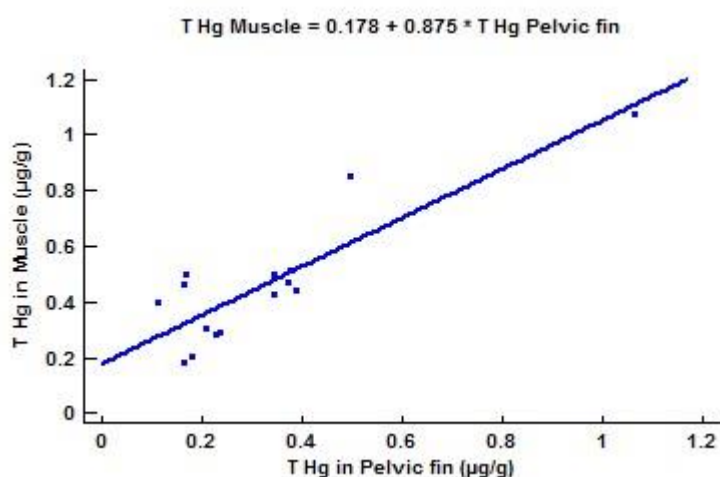


Figure 4.11: T Hg in muscles verses T Hg in pelvic fins

Examination of the coefficients of determination from regression data in Table 4.5 for Tilapia fish samples from the same location indicated that caudal fin ($r^2=0.823$) was a better predictor of total mercury in muscle tissue of samples collected from Tono dam, followed by dorsal fin ($r^2=0.501$), pectoral fin ($r^2 = 0.482$), pelvic fin ($r^2 = 0.373$), and anal fin ($r^2 = 0.349$). However,

in the case of mudfish samples caudal fin was not a good predictor of muscle tissue mercury concentration even though it recorded a significant regression coefficient of 0.532. The results in Table 4.8 indicates that pelvic fin ($r^2 = 0.752$) was a better predictor of muscle tissue mercury concentration, followed by caudal fin ($r^2 = 0.532$), Dorsal fin ($r^2 = 0.438$), anal fin ($r^2 = 0.444$) and Pectoral fin ($r^2 = 0.278$) respectively. In order to predict the mercury concentration in the muscle of mudfish samples from Tono dam, Navrongo, the following equations as can be seen in the Table 4.9 can be used.

From Table 4.10 the equation which can be used to predict mercury concentration in the muscle of Mudfish samples from Tono dam, Navrongo can be shown in the equation below:

$$\text{T Hg Muscle} = 0.178 + 0.875 * \text{T Hg in Pelvic fins} \quad (r^2 = 0.752)$$

Table 4.10: Equations for Prediction of Mercury in Muscle of Mudfish Samples from Tono dam, Navrongo

TISSUES	EQUATION	r^2
Dorsal fins	T Hg in Muscle = $0.285 + 0.665 * \text{T Hg in Dorsal Fins}$	0.438
Caudal fins	T Hg in Muscle = $0.243 + 0.738 * \text{T Hg in Caudal Fins}$	0.532
Pectoral Fins	T Hg in Muscle = $0.271 + 0.476 * \text{T Hg in Pectoral Fins}$	0.278
Pelvic Fins	T Hg in Muscle = $0.178 + 0.875 * \text{T Hg in Pelvic Fins}$	0.752
Anal Fins	T Hg in Muscle = $0.240 + 0.894 * \text{T Hg in Anal Fins}$	0.444

4.2 KPONG SAMPLING SITE (KPONG HYDRO DAM)

4.2.1 Tilapia Fish Samples

The results of mercury concentrations in Tilapia collected from Kpong are presented in Table 4.11 and the mean mercury concentrations for muscle and the various fin in Figure 4.17. The average mercury concentration \pm standard deviation for muscle tissue was $0.227 \pm 0.184 \mu\text{g/g}$, $0.139 \pm 0.073 \mu\text{g/g}$ for anal fins, $0.176 \pm 0.212 \mu\text{g/g}$ caudal fin, $0.035 \pm 0.276 \mu\text{g/g}$ dorsal fin, $0.139 \pm 0.099 \mu\text{g/g}$ for pectoral fin and $0.145 \pm 0.142 \mu\text{g/g}$ for pelvic fin.

Table 4.11: Mercury concentrations in the muscle and fin tissues of Tilapia from Kpong Hydro Dam

Tissues	Mean Hg \pm Std.Dev ($\mu\text{g/g}$)	Number of samples	Range
Muscle	0.228 ± 0.183	15	0.035-0.914
Anal Fin	0.139 ± 0.073	15	0.027-0.301
Caudal Fin	0.176 ± 0.212	15	0.027-0.751
Dorsal Fin	0.124 ± 0.068	15	0.028-0.276
Pectoral Fin	0.139 ± 0.099	15	0.014-0.421
Pelvic Fin	0.145 ± 0.142	15	0.022-0.598
Weight(g)	312.612 ± 134.239	15	153.51-594.63
Length(cm)	25.12 ± 3.563	15	19.5-31.2

The mercury concentration ranged from 0.035 to 0.914 $\mu\text{g/g}$ for muscle tissue mercury concentration, 0.027-0.301 $\mu\text{g/g}$ for anal fin mercury concentration, 0.027-0.751 $\mu\text{g/g}$ for caudal fin, 0.0285-0.276 $\mu\text{g/g}$ for dorsal fin, 0.014-0.421 $\mu\text{g/g}$ for pectoral fin and 0.022-0.598 $\mu\text{g/g}$ for pelvic fin.

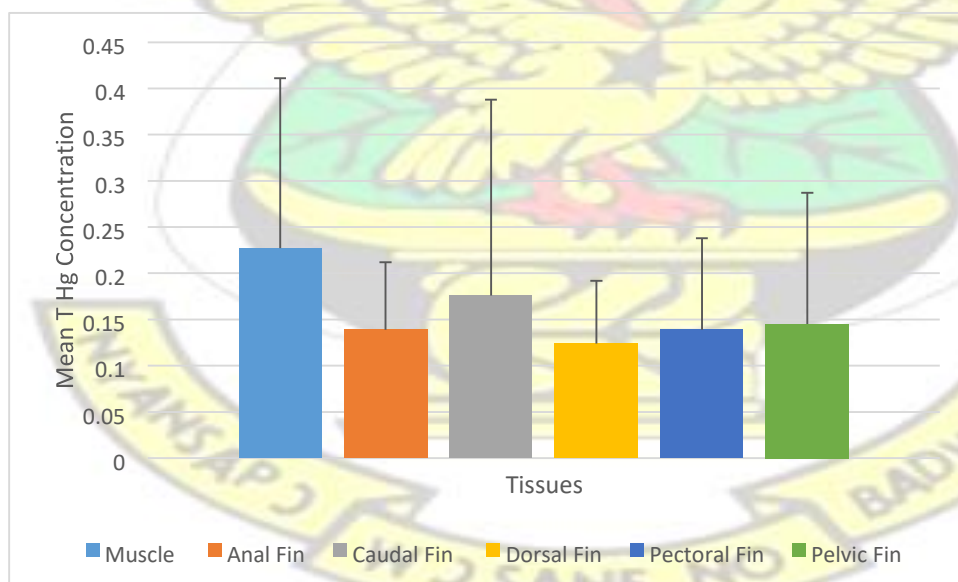


Figure 4.17: Plot of Mean Mercury Concentration in Muscle Tissue and Various Fins for Tilapia fish Samples from Kpong Hydro Dam

From Table 4.12, all the fins recorded correlation coefficients values above 0.5 indicating significant correlation between the mercury concentration in the muscle and the various fins.

The relationship between the mercury concentration in the muscle and the various fins is indicated by Pearson correlation coefficients of 0.708 ($p = 0.003$) for dorsal fin, 0.820 ($p = 0.000$) for caudal fin, 0.763 ($p = 0.001$) for pectoral fin, 0.659 ($p = 0.008$) for pelvic fin and 0.681 ($p = 0.005$) for anal fin.

Similarly, correlation coefficients in Table 4.12 also indicated that there is statistically significant correlation between mercury concentration in the muscle and the length and weight of the fish, since the recorded Pearson correlation coefficients of 0.729 ($p = 0.002$) and 0.659 ($p = 0.004$) respectively are above 0.5 and p -values lower than 0.05.

Table 4.12: Correlation Matix results for Tilapia Fish Samples from Kpong Hydro Dam

	Muscle	Dorsal fin	Caudal fin	Pectoral fin	Pelvic fin	Anal fin	Weight	Length
Muscle		0.708	0.820	0.763	0.659	0.681	0.729	0.695
		0.003	0.000	0.001	0.008	0.005	0.002	0.004
Dorsal fin	0.708		0.741	0.683	0.4961	0.489	0.487	0.504
	0.003		0.002	0.005	0.060	0.064	0.066	0.055
Caudal fin	0.820	0.741		0.877	0.282	0.402	0.482	0.400
	0.000	0.002		0.000	0.310	0.138	0.069	0.140
Pectoral fin	0.763	0.683	0.877		0.265	0.511	0.460	0.439
	0.001	0.005	0.000		0.340	0.052	0.085	0.101
Pelvic fin	0.659	0.496	0.282	0.265		0.804	0.449	0.501
	0.008	0.060	0.310	0.340		0.000	0.093	0.057
Anal fin	0.681	0.489	0.402	0.511	0.804		0.435	0.553
	0.005	0.064	0.138	0.052	0.000		0.105	0.033
Weigth	0.729	0.487	0.482	0.460	0.449	0.435		0.969
	0.002	0.066	0.069	0.085	0.093	0.105		0.000
Length	0.695	0.504	0.400	0.439	0.501	0.553	0.969	
	0.004	0.055	0.140	0.101	0.057	0.033	0.000	

Results of linear regression analysis of data in Table 4.13 revealed strong positive relationship between total mercury in muscle and the fins. The regression data of total-mercury concentration in muscle against that of anal fin yielded a slope of 2.045 ($p = 0.005$) and r^2 of

0.463, whereas that of pelvic fin yielded a slope of 1.014 ($p = 0.008$) and r^2 of 0.434. Similarly regression of concentration of mercury in muscle against that in caudal fin yielded a slope of 1.033 ($p=0.0002$) and r^2 of 0.672. A slope of 2.263 ($p = 0.003$) and r^2 of 0.501 was recorded for the regression of concentration in muscle against dorsal fin.

Table 4.13: Regression Analysis Results for Tilapia fish Samples from Kpong Hydro Dam

Source	Slope	Intercept	F-Ratio	P-Value	r^2
T Hg in Muscle vrs T Hg in Dorsal fin	2.263	-0.041	13.05	0.003	0.501
T Hg in Muscle vrs T Hg in Caudal fin	1.034	0.076	26.61	0.0002	0.672
T Hg in Muscle vrs T Hg in Pectoral fin	1.671	0.006	18.07	0.001	0.582
T Hg in Muscle vrs T Hg in Pelvic fin	1.014	0.092	9.98	0.008	0.434
T Hg in Muscle vrs T Hg in Anal fin	2.045	-0.046	11.21	0.005	0.463
	Slope	Intercept	F-Ratio	P-Value	r^2
T Hg in Muscle vrs Weight	0.001	-0.131	14.71	0.002	0.531
T Hg in Muscle vrs Length	0.043	-0.829	12.14	0.004	0.483

Regression of mercury concentration in muscle against concentration in pectoral fin yielded a slope of 1.671 ($p = 0.001$) and r^2 of 0.582. The results thus indicate that the r^2 values for caudal fin > pectoral fin > dorsal fin > anal fin > pelvic fin. This implies that for tilapia fish samples from Kpong hydro dam, mercury concentration in the caudal fin is a better predictor of the mercury concentration in the muscle since it provides the highest variability of the muscle mercury concentration, according to the prediction equations in Table 4.14. Graphical representation of the regression graphs for the fins with significant regression coefficients can be seen in the Figures 4.18, 4.19 and 4.20:

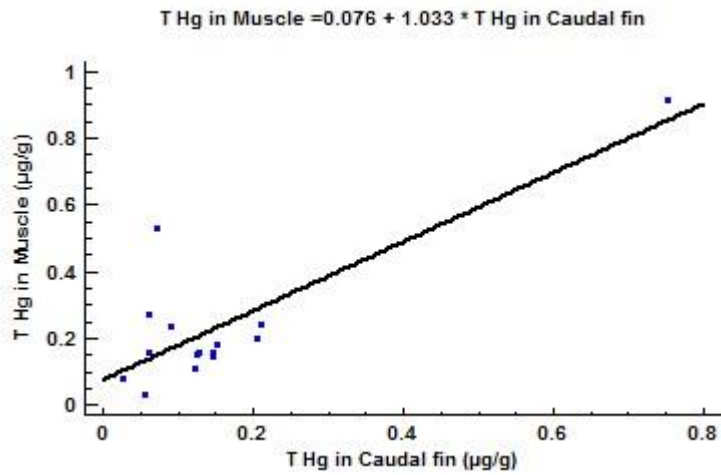


Figure 4.18: T Hg in Muscles against T Hg in Caudal fin

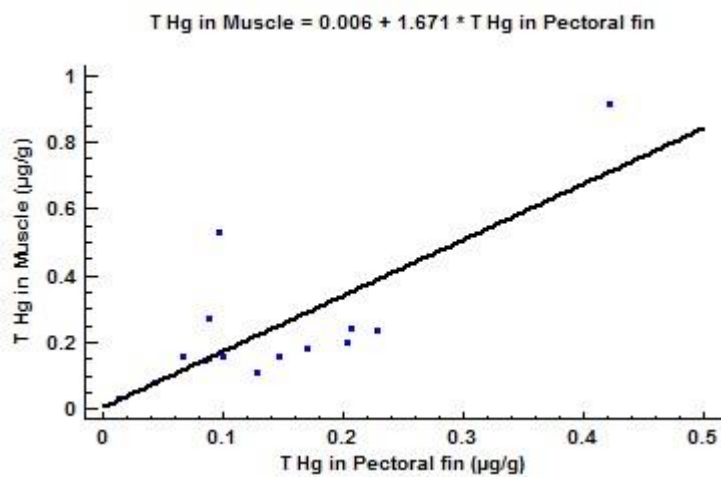


Figure 4.19: T Hg in Muscles verses T Hg in Pectoral fins

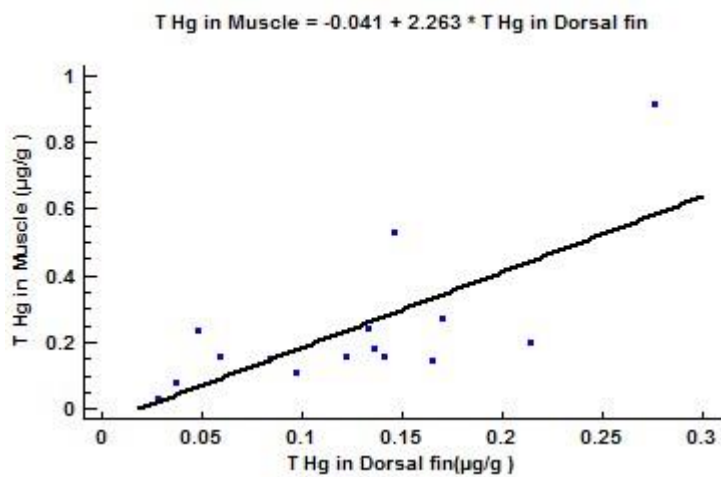


Figure 4.20: T Hg in Muscles verses T Hg in Dorsal fins.

The regression of the concentration of mercury in the muscle against the weight and length of fish yielded slopes of 0.001 ($p=0.002$) and 0.037 ($p = 0.002$) respectively and their respective r^2 values are 0.962 and 0.951. Examination of the results (p-values) in the regression Table 4.13 indicate that the p-values are less than 0.05 indicating a statistically significant relationship between total mercury in the muscle and weight and length of the fish samples at the 95.0% confidence level.

The equations for predicting the mercury concentration in the muscles of fish using the mercury concentration in the various fins is shown in Table 4.14.

Table 4.14: Equations for Prediction of Mercury in Muscle of Tilapia fish from Kpong Hydro dam.

TISSUES	EQUATION	r^2
Dorsal fins	T Hg in Muscle = $-0.041 + 2.263 * \text{T Hg in Dorsal Fins}$	0.501
Caudal fins	T Hg in Muscle = $0.076 + 1.033 * \text{T Hg in Caudal Fins}$	0.672
Pectoral Fins	T Hg in Muscle = $0.066 + 1.671 * \text{T Hg in Pectoral Fins}$	0.582
Pelvic Fins	T Hg in Muscle = $0.092 + 1.014 * \text{T Hg in Pelvic Fins}$	0.434
Anal Fins	T Hg in Muscle = $-0.046 + 2.045 * \text{T Hg in Anal Fins}$	0.463

From the regression data, the most appropriate equation based on the fins with the highest regression coefficient for predicting mercury concentration in muscle is shown in the equation below:

$$\text{T Hg in Muscle} = 0.076 + 1.033 * \text{T Hg in Caudal Fins} \quad (r^2 = 0.672)$$

4.2.2 MUDFISH SAMPLES FROM KPONG

Fifteen (15) mud fish samples were obtained from the Kpong hydro dam and the mercury content in the fish muscle and the various fins determined using the CVAAS method and the results are presented in Table 4.15 and graphically represented in Figure 4.25.

The mercury level in the muscle of the 15 samples ranged from 0.087 to 0.445 $\mu\text{g/g}$ with a mean \pm SD of $0.235 \pm 0.121 \mu\text{g/g}$. A mean mercury concentration of $0.108 \pm 0.095 \mu\text{g/g}$ was recorded for anal fin and the concentration ranged from 0.006 to 0.383 $\mu\text{g/g}$.

The caudal fin mercury concentration ranged from 0.018 to 0.383 $\mu\text{g/g}$ with a mean of $0.126 \pm 0.104 \mu\text{g/g}$. The mean \pm SD mercury concentration in the pectoral fin, pelvic fin and dorsal fin are $0.156 \pm 0.111 \mu\text{g/g}$, $0.185 \pm 0.141 \mu\text{g/g}$ and $0.101 \pm 0.077 \mu\text{g/g}$ respectively, and their concentration ranged from 0.032 to 0.323 $\mu\text{g/g}$, 0.025 to 0.39 $\mu\text{g/g}$ and 0.015 to 0.265 $\mu\text{g/g}$ respectively for the pectoral fin, pelvic fin and the dorsal fin.

Table 4.15: Concentration of Mercury in Mudfish Tissues from Kpong, Hydro Dam

Tissues	Mean Hg \pm Stn.Dev($\mu\text{g/g}$)	Number of samples	Range
Muscle	0.236 ± 0.121	15	0.087-0.445 $\mu\text{g/g}$
Dorsal Fin	0.101 ± 0.077	15	0.015-0.265 $\mu\text{g/g}$
Caudal Fin	0.202 ± 0.149	15	0.018-0.383 $\mu\text{g/g}$
Pectoral Fin	0.198 ± 0.168	15	0.032- 0.361 $\mu\text{g/g}$
Pelvic Fin	0.185 ± 0.141	15	0.025-0.39 $\mu\text{g/g}$
Anal Fin	0.108 ± 0.095	15	0.006-0.383 $\mu\text{g/g}$
	Mean \pm Std.Dev	Number of samples	Range
Weight(g)	$606.824 \pm 525.265\text{g}$	15	129.4-1556.87g
Length(cm)	$42.08 \pm 13.238\text{cm}$	15	27.0-61.5cm

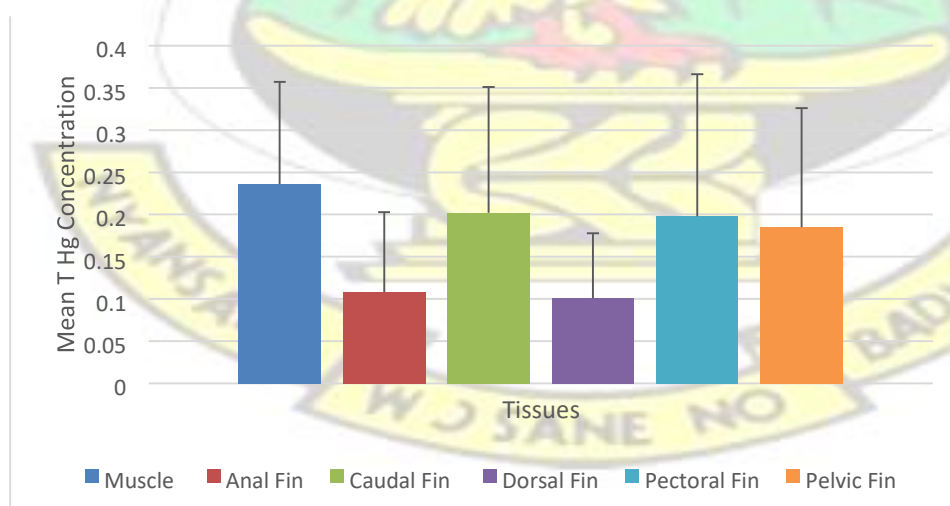


Figure 4.25: Plot of Mean Mercury Concentration in Muscle Tissue And Various Fins for Mud fish Samples from Kpong Hydro Dam

The mean weight and length of mudfish samples collected from Kpong were 312.612 ± 134.239 g and 25.12 ± 3.563 cm respectively with a range of 153.51 g to 594.63 g for weight. The length of the mudfish samples also ranged from 27.0 cm to 61.5 cm.

Pearson correlation analysis was undertaken to determine which fin mercury concentration correlated with mercury concentration in the muscle tissue and the results are presented in Table 4.16. The Pearson correlation coefficients ranged from 0.738 to 0.965 with p-values of 0.000 to 0.002.

The highest correlation coefficient (0.965, p-value = 0.000) for the relationship between muscle tissue mercury concentration and mercury concentration in the various fins was recorded by muscle against dorsal fin and the lowest (0.738, p-value = 0.002) by the muscle against anal fin. The results in Table 4.16 also indicate that all the pairs of variables recorded positive significant Pearson correlation coefficients at the 95% confidence interval. The correlation between the mercury concentration in the muscle and the weight and length of the fish also recorded positive strong correlation coefficients and P-value of 0.967 and 0.000 and 0.951 and 0.000 respectively.

Table 4.16: Correlation Matrix for Tissues of Mudfish from Kpong Hydro Dam

	Muscle	Dorsal fin	Caudal fin	Pectoral fin	Pelvic fin	Anal fin	Weight	Length
Muscle		0.965	0.925	0.738	0.765	0.882	0.967	0.951
		0.000	0.000	0.002	0.001	0.000	0.000	0.000
Dorsal fin	0.965		0.940	0.769	0.758	0.903	0.929	0.867
	0.000		0.000	0.001	0.001	0.000	0.000	0.000
Caudal fin	0.925	0.940		0.637	0.696	0.945	0.895	0.853
	0.000	0.000		0.011	0.004	0.000	0.000	0.0001
Pectoral fin	0.738	0.769	0.637		0.781	0.648	0.731	0.681
	0.002	0.001	0.011		0.001	0.009	0.002	0.005
Pelvic fin	0.765	0.758	0.696	0.781		0.712	0.762	0.747
	0.001	0.001	0.004	0.001		0.003	0.001	0.001
Anal fin	0.882	0.903	0.945	0.648	0.712		0.862	0.799
	0.000	0.000	0.000	0.009	0.003		0.000	0.0003
Weight	0.967	0.929	0.895	0.731	0.762	0.862		0.976
	0.000	0.000	0.000	0.002	0.001	0.000		0.000
Length	0.951	0.867	0.853	0.681	0.747	0.799	0.976	
	0.000	0.000	0.0001	0.005	0.001	0.0003	0.000	

Linear regression analysis results in Table 4.17 for mudfish samples from Kpong revealed strong positive relationship between total mercury in muscle and the various fins. The regression results for total mercury concentrations in muscle against those in pelvic fins yielded a slope of 0.655 ($p = 0.001$) and r^2 of 0.585, whereas the regression of muscle tissue mercury concentration against that in anal fin yielded a slope of 1.122 ($p = 0.001$) and r^2 of 0.778.

Table 4.17: Regression Analysis for Mudfish from Kpong hydro Dam

Source	Slope	Intercept	F-Ratio	P-Value	R ²
Muscle vrs dorsal fin	1.516	0.083	178.03	0.000	0.932
Muscle vrs Anal fin	1.122	0.114	45.54	0.000	0.778
Muscle vrs caudal fin	1.078	0.099	77.54	0.000	0.856
Muscle vrs Pelvic fin	0.655	0.114	18.36	0.001	0.585
Muscle vrs Pectoral fin	0.803	0.108	15.50	0.002	0.544
	<i>Slope</i>	<i>Intercept</i>	<i>F-Ratio</i>	<i>P-Value</i>	<i>R²</i>
Muscle vrs Weight	0.0002	0.100	186.15	0.000	0.935
Muscle vrs Length	0.009	-0.130	122.38	0.000	0.904

Regression of concentration of mercury in muscle against that in caudal fin yielded a slope of 1.078 ($p=0.000$) and r^2 of 0.856, slope of 1.516 ($p = 0.0001$) and r^2 of 0.932 being the regression of concentration in muscle against dorsal fin. Regression of concentration in muscle against concentration in pectoral fin yielded a slope of 0.803 and r^2 of 0.544. The regression analysis of the concentration of mercury in the muscle against the weight and length of fish yielded slopes of 0.0002 ($p=0.000$) and 0.009 ($p=0.000$) respectively and respective r^2 values of 0.935 and 0.904. Furthermore, there is also a strong positive relationship between the total mercury level in the muscle and the weight and length of the fish and these recorded Pearson correlation coefficient of 0.95 and 0.92 with corresponding pvalue of 0.00 for both the relation between the mercury level in the muscle and the length and the weight of the fish. Since the P-values corresponding to total mercury in muscle tissue and weight and total

mercury in muscle tissues and length of fish in Table 4.17 are less than 0.05 and r^2 values greater than 0.5, it can be concluded that there is statistically significant relationship between total mercury concentration in muscle and weight and length of fish samples at the 95.0% confidence level.

The r^2 results for the regression analysis between the muscle tissue mercury concentration and the individual fins indicates that the relationship between pectoral fin mercury concentration and muscle mercury concentration recorded the lowest regression coefficient of 0.544 and the highest of 0.932 was recorded by the relationship between the mercury concentrations in the muscle and dorsal fin.

A graphical representation of the regression for the fins with significant regression coefficients are shown in the graphs in Figure 4.25 to Figure 4.32:

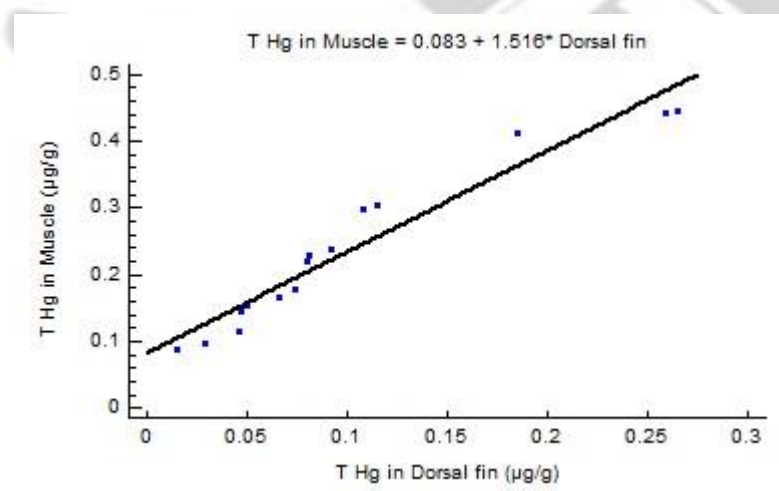


Figure 4.26: T Hg in Muscles Verses Dorsal fins from Kpong Hydro Dam

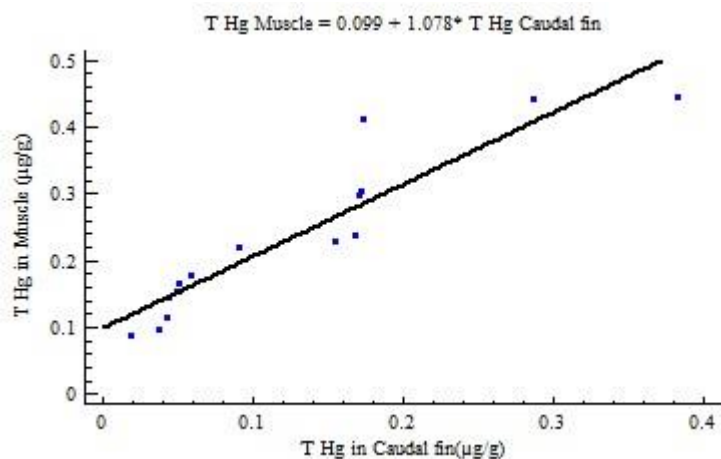


Figure 4.27: T Hg in Muscles Verses Caudal fins from Kpong Hydro Dam

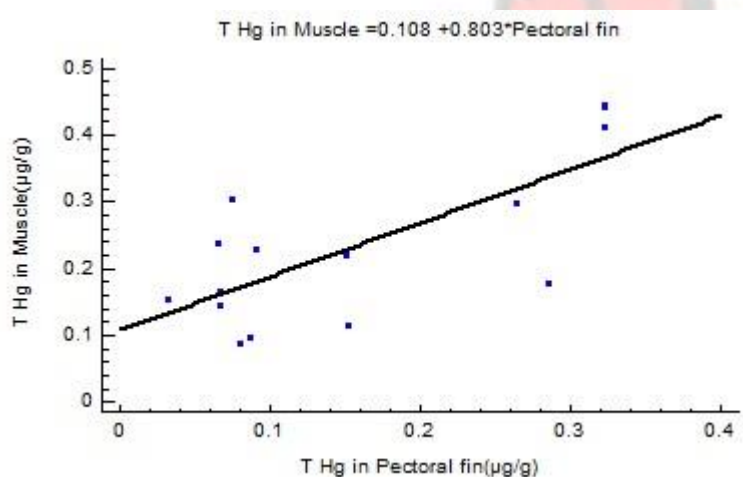


Figure 4.28: T Hg in Muscles Verses T Hg in Pectoral fins from Kpong Hydro Dam

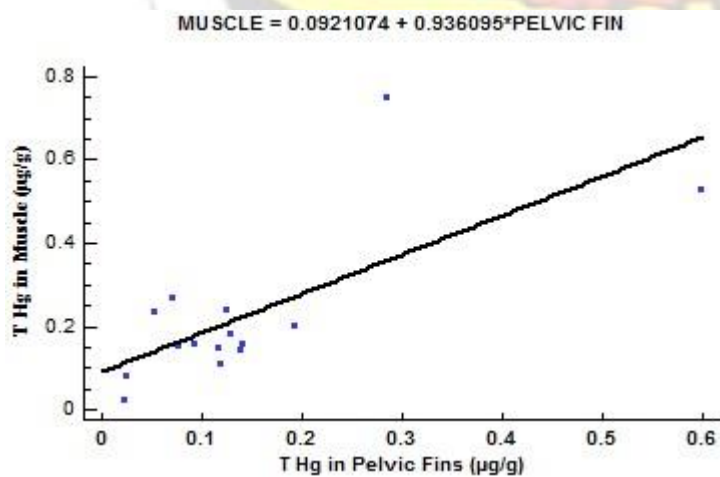


Figure 4.29: T Hg in Muscles Verses T Hg in Pelvic fins from Kpong Hydro Dam

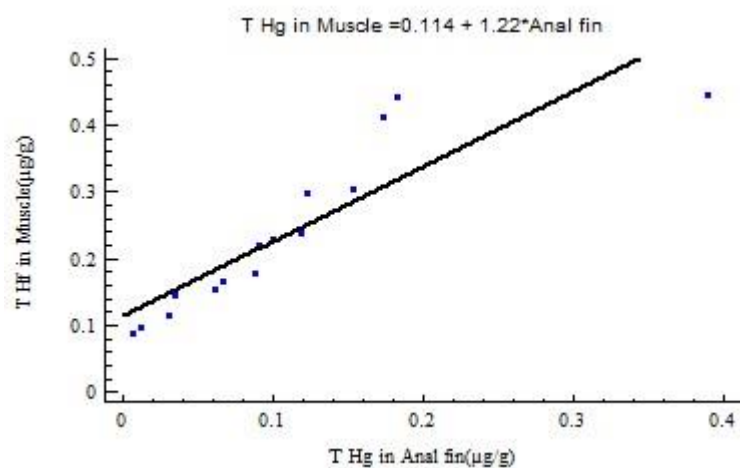


Figure 4.30: T Hg Muscles Verses T Hg in Anal fins from Kpong Hydro Dam

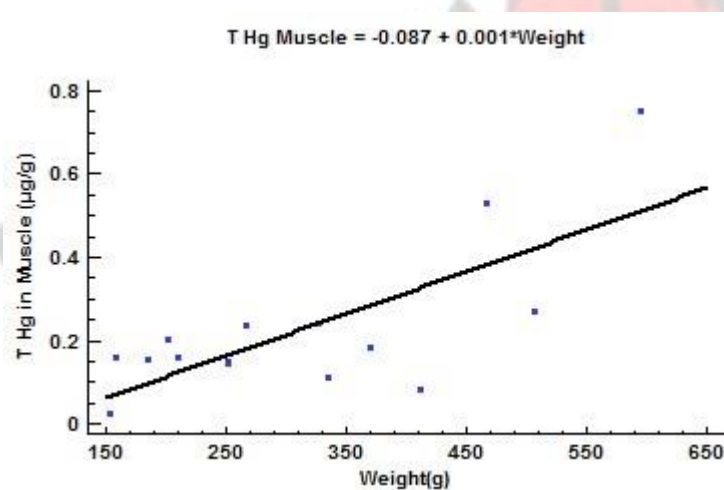


Figure 4.31: T Hg in Muscle verse Weight of Mudfish samples from Kpong hydro dam

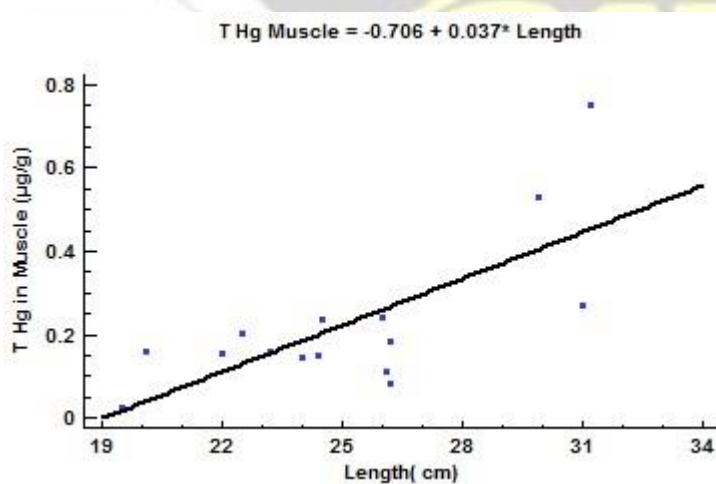


Figure 4.32: T Hg in Muscle verses Length of Mudfish samples form Kpong hydro dam

In the case of tilapia fish samples from Kpong hydro dam, caudal fin ($r^2 = 0.715$) was a better predictor of muscle mercury concentration than the other fins namely ($r^2 = 0.531$ for Pectoral fins; $r^2 = 0.521$ for pelvic; $r^2 = 0.492$ for dorsal fins and $r^2 = 0.532$ for anal fins) as can be seen in Table 4.14. However for mudfish samples from Kpong hydro dam, dorsal fin ($r^2 = 0.932$) emerged as the better predictor of muscle mercury concentration, even though caudal fin also recorded a very significant regression coefficient ($r^2 = 0.856$). In summary regression coefficients can be arranged in descending order as follows, dorsal fin ($r^2 = 0.932$) > caudal fin ($r^2 = 0.856$) > anal fin ($r^2 = 0.778$) > pelvic fin ($r^2 = 0.585$) > pectoral fin ($r^2 = 0.544$). The equations for predicting muscle mercury concentration in mudfish samples from Kpong are shown in

Table 4.18.

Table 4.18: Equations for Prediction of Muscle Mercury Concentration in Mudfish Samples from Kpong, Hydro dam.

TISSUES	EQUATION	r^2
Dorsal fins	T Hg in Muscle = $0.083 + 1.516 * \text{T Hg in Dorsal Fins}$	0.932
Caudal fins	T Hg in Muscle = $0.099 + 1.078 * \text{T Hg in Caudal Fins}$	0.856
Pectoral Fins	T Hg in Muscle = $0.108 + 0.803 * \text{T Hg in Pectoral Fins}$	0.544
Pelvic Fins	T Hg in Muscle = $0.114 + 0.655 * \text{T Hg in Pelvic Fins}$	0.585

Anal Fins	T Hg in Muscle = 0.114 + 1.122 * T Hg in Anal Fins	0.778
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The equation most appropriate for predicting the mercury concentration in mudfish samples based on the regression data is shown below:

$$\text{T Hg in Muscle} = 0.083 + 1.908 * \text{T Hg in Dorsal Fins} (r^2 = 0.932).$$

4.3 YAPEI (White Volta LAKE) SAMPLING SITE

4.3. 1. Concentration of Hg in Tilapia Fish Samples from White Volta in Yapei

Fifteen (15) tilapia fish samples were bought from landing sites at the White Volta in Yapei and the mercury content in the fish muscle tissue and the various fins determined using the CVAAS method.

The mean weight and length of the tilapia fish samples collected are 194.67 ± 26.01 g and 21.66 ± 1.29 cm respectively and the weight of the fish collected ranged from a minimum of 157.44 g to a maximum of 245.21 g. The length of the fish also ranged from a minimum of 19.0 cm to a maximum of 23.5 cm. The mercury level in the muscle tissue for the 15 samples ranged from 0.181 $\mu\text{g/g}$ to 2.745 $\mu\text{g/g}$ and recorded a mean mercury concentration of 0.680 ± 0.679 $\mu\text{g/g}$. A representation of the mean mercury concentrations in muscle tissues and the various fins is shown below in Figure 4.33.

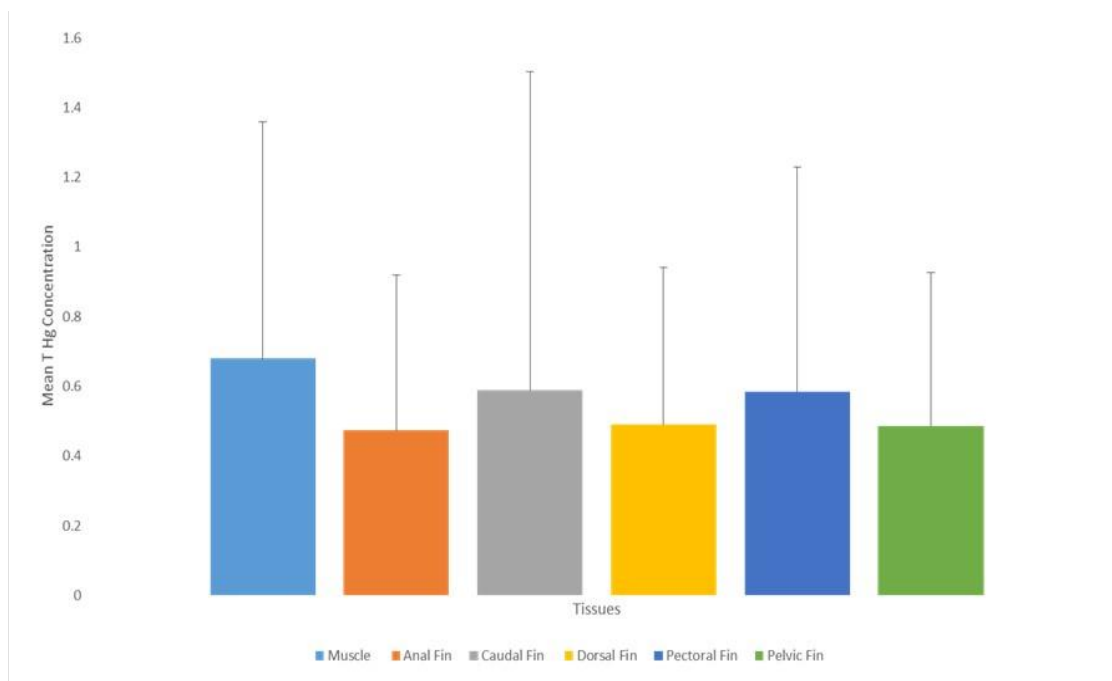


Figure 4.33: Mean Mercury Concentration In Tilapia fish from Yapei, White Volta

A mean mercury concentration of 0.473 ± 0.447 $\mu\text{g/g}$ was recorded for anal fin and the concentration ranged from 0.154-1.698 $\mu\text{g/g}$. The caudal fin mercury concentration range from 0.100-2.027 $\mu\text{g/g}$ and a mean mercury concentration of 0.588 ± 0.915 $\mu\text{g/g}$. The mean mercury concentration in the pectoral fin, pelvic fins and dorsal fin are 0.584 ± 0.646 $\mu\text{g/g}$, 0.752 ± 1.212 $\mu\text{g/g}$ and 0.490 ± 0.452 $\mu\text{g/g}$ respectively, and their range were 0.147-2.624 $\mu\text{g/g}$, 0.137-4.848 $\mu\text{g/g}$ and 0.132-1.839 $\mu\text{g/g}$ respectively for the pectoral fin, pelvic fin and the dorsal fin as can be seen in Table 4.19.

Table 4.19: Mean Mercury Concentration for Tilapia fish from Yapei, White Volta

Tissues	Mean Hg \pm Std.Dev ($\mu\text{g/g}$)	Number of samples	Range ($\mu\text{g/g}$)
Muscle	0.680 ± 0.679	15	0.181-2.745
Dorsal fin	0.490 ± 0.452	15	0.132-1.839
Caudal fin	0.580 ± 0.915	15	0.100-2.027
Pectoral fin	0.584 ± 0.646	15	0.147-2.624
Pelvic fin	0.486 ± 0.441	15	0.137-1.589

Anal fin	0.473±0.447	15	0.154-1.698
Weight(g)	194.674±26.011	15	157.44-245.21
Length(cm)	21.66±1.29494	15	19.0-23.5

The results of the correlation analysis is (Table 4.20) which shows the Pearson correlation coefficient and the corresponding p-values for the mercury level in the muscle against the various fins. The results in Table 4.20 indicate that the relationship between the total mercury in the muscle against dorsal fin, caudal fin, pectoral fin, pelvic fin and anal fin recorded correlation coefficients with (P-value) of 0.886 (0.000), 0.643 (0.010), 0.913 (0.000), 0.900 (0.000) and 0.945 (0.000) respectively.

From the results, the highest correlation coefficient of 0.945 was for muscle total mercury concentration and anal fin while the lowest value of 0.643 was for total mercury in muscle and caudal fin mercury concentration. Since the P-values recorded are below 0.05 and the correlation coefficients recorded are greater than 0.5, there is statistically significant positive correlation at the 95.0% confidence level.

Further examination of the correlation Table 4.20 indicates that there is a strong positive correlation between the mercury level in the muscle and the weight and length of the fish since Pearson correlation coefficients of 0.808 (p-value = 0.0003) and 0.556 (p-value = 0.032) are greater than 0.5 and have p-values lower than 0.05.

Table 4.20: Correlation Matrix for Tilapia fish from Yapei, White Volta

	Muscle	Dorsal fin	Caudal fin	Pectoral fin	Pelvic fin	Anal fin	Weight	Length
Muscle		0.886	0.643	0.913	0.900	0.945	0.808	0.556
		0.000	0.010	0.000	0.000	0.000	0.0003	0.032
Dorsal fin	0.886		0.664	0.773	0.838	0.836	0.678	0.354
	0.000		0.007	0.001	0.0001	0.0001	0.006	0.196
Caudal fin	0.643	0.664		0.692	0.752	0.488	0.383	0.155
	0.001	0.007		0.004	0.001	0.065	0.159	0.582
Pectoral fin	0.913	0.773	0.692		0.875	0.879	0.728	0.504

	0.000	0.001	0.004		0.000	0.000	0.002	0.055
Pelvic fin	0.900	0.838	0.752	0.875		0.871	0.713	0.408
	0.000	0.0001	0.001	0.000		0.000	0.003	0.131
Anal fin	0.945	0.836	0.488	0.879	0.871		0.911	0.634
	0.000	0.0001	0.065	0.000	0.000		0.000	0.011
Weight	0.808	0.678	0.383	0.728	0.713	0.911		0.823
	0.0003	0.006	0.159	0.002	0.003	0.000		0.0002
Length	0.556	0.354	0.155	0.504	0.408	0.634	0.823	
	0.032	0.196	0.582	0.055	0.131	0.011	0.0002	

A linear regression analysis of the results revealed strong positive relations between total mercury in muscle and some of the fins. From the linear regression analysis results, anal fin recorded the highest regression coefficient ($r^2 = 0.893$) while caudal fin recorded the lowest regression coefficient (r^2) of 0.413 as can be seen in Table 4.21.

Table 4.21: Regression Analysis Results for Tilapia fish from Yapei, White Volta

Source	Slope	Intercept	F-Ratio	P-Value	R ²
T Hg in Muscle vrs T Hg in Anal fin	1.435	0.002	109.03	0.000	0.893
T Hg in Muscle vrs T Hg in Pectoral fin	0.959	0.024	64.85	0.000	0.833
T Hg in Muscle vrs T Hg in Pelvic fin	1.386	0.007	55.20	0.000	0.809
T Hg in Muscle vrs T Hg in Dorsal	1.330	0.028	47.67	0.000	0.786
T Hg in Muscle vrs T Hg in caudal	0.477	0.352	9.14	0.010	0.413
T Hg in Muscle vrs Weight	0.021	-3.426	24.43	0.000	0.653
T Hg in Muscle vrs Length	0.291	-5.631	5.81	0.032	0.308

The regression data of total-mercury concentrations in muscle against those in anal fin yielded a slope of 1.435 ($p = 0.002$) and r^2 of 0.893, whereas the regression of concentrations in muscle against those in pelvic fin yielded a slope of 1.386 ($p = 0.007$) and r^2 of 0.809. Similarly regression analysis of concentration of mercury in muscle against those in caudal fin yielded a slope of 0.477 ($p = 0.010$) and r^2 of 0.413, slope of 1.330 ($p = 0.000$) and r^2 of 0.786 being the regression of concentration in muscle against dorsal fin. Regression analysis of mercury concentration in muscle against concentration in pectoral fin yielded a slope of 0.959 ($p =$

0.000) and r^2 of 0.833. The graphical relationship between Muscle mercury concentration and various fins that recorded significant r^2 values are shown in Figure 4.34 to Figure 4.37.

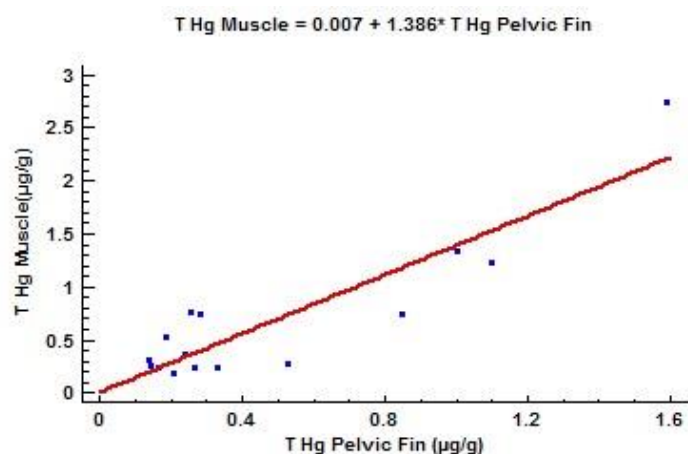


Figure 4.34: T Hg in Muscle Verses T Hg in Pelvic fins of Tilapia fish from Yapei

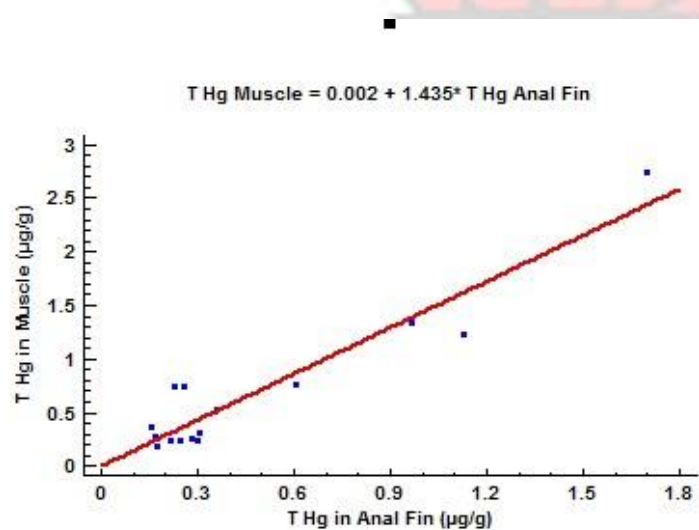


Figure 4.35: T Hg in Muscles Verses T Hg in Anal fins of Tilapia fish from Yapei

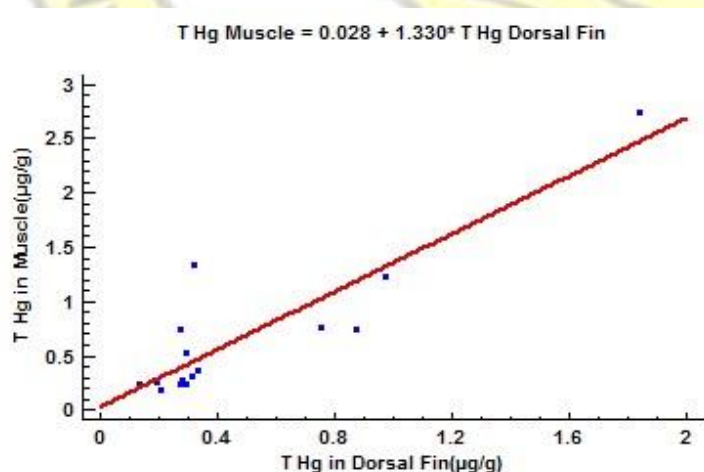


Figure 4.36: T Hg in Muscles Verses T Hg in Dorsal fins of Tilapia fish from Yapei

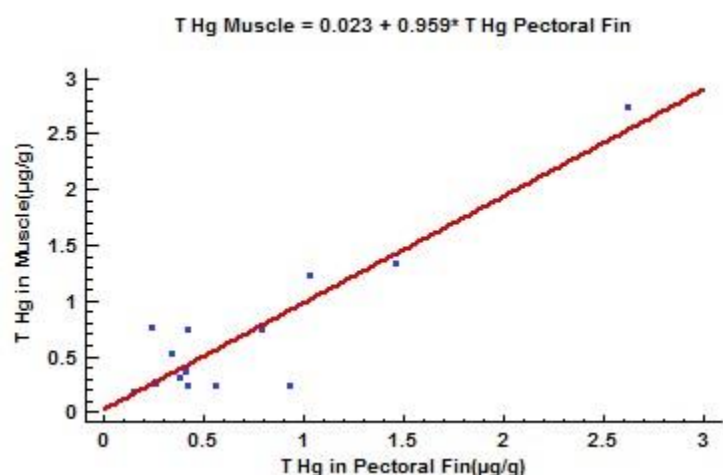


Figure 4.37: T Hg in Muscles Verses T Hg in Pectoral fins of Tilapia fish from Yapei

The regression analysis of the concentration of mercury in the muscle against the weight and length of fish yielded slopes of 0.021 ($p=0.000$) and 0.291 ($p = 0.032$) respectively and their respective r^2 values are 0.653 and 0.308.

In summary, the mercury concentration in the muscle of tilapia fish can be estimated using the equations in Table 4.22.

Table 4.22: Equations for Prediction of Muscle Mercury Concentration of Tilapia Samples from White Volta, Yapei.

TISSUES	EQUATION	r^2
Dorsal fins	$T\ Hg\ in\ Muscle = 0.028 + 1.330 * T\ Hg\ in\ Dorsal\ Fins$	0.786
Caudal fins	$T\ Hg\ in\ Muscle = 0.351 + 0.476 * T\ Hg\ in\ Caudal\ Fins$	0.413
Pectoral Fins	$T\ Hg\ in\ Muscle = 0.024 + 0.959 * T\ Hg\ in\ Pectoral\ Fins$	0.833
Pelvic Fins	$T\ Hg\ in\ Muscle = 0.007 + 1.386 * T\ Hg\ in\ Pelvic\ Fins$	0.809
Anal Fins	$T\ Hg\ in\ Muscle = 0.002 + 1.435 * T\ Hg\ in\ Anal\ Fins$	0.893

The equations in Table 4.22 show that a prediction of the total mercury concentration in muscle tissues can be undertaken with the knowledge of the mercury concentration in the fins of the fish samples. Hence, in predicting mercury concentration in the muscle of Tilapia fish from Yapei, the most appropriate equation for estimation is shown below:

$$\text{T Hg in Muscle} = 0.002 + 1.435 * \text{T Hg in Anal Fins} (r^2 = 0.893)$$

4.3.2 YAPEI MUDFISH SAMPLES

Fifteen (15) samples were collected from the White Volta River at Yapei and analyzed for mercury concentration. The total mercury concentration in the muscle ranged from 0.14µg/g to 1.033µg/g with a mean concentration of 0.439 µg/g ±0.263 µg/g. The anal fin, caudal fin, dorsal fin, pectoral and pelvic fin also recorded mean±SD mercury concentration of 0.353±0.303 µg/g, 0.238 ± 0.134 µg/g, 0.216±0.154 µg/g, 0.384±0.693 µg/g and 0.250±0.187 µg/g respectively.

The mercury concentration in these fins also ranged from 0.083 µg/g to 0.823 µg/g for anal fin, 0.091 µg/g to 0.472 µg/g for caudal fin, 0.077 µg/g to 0.536 µg/g for dorsal fin, 0.084 µg/g to 2.133 µg/g for pectoral fin and 0.089 µg/g to 0.669 µg/g for pelvic fin. From the above results it is evident that pectoral fin recorded the highest mercury concentration (0.906 µg/g) as can be seen in Table 4.23 and Figure 4.41.

Table 4.23: Mean Mercury Concentration in Mudfish from White Volta River at Yapei

Tissues	Mean Hg ± Std.Dev(µg/g)	Number of samples	Range (µg/g)
Muscle	0.431±0.261	15	0.14-1.033
Anal fin	0.343±0.301	15	0.083-0.823
Caudal fin	0.222±0.121	15	0.091-0.472
Dorsal fin	0.194±0.126	15	0.077-0.417
Pectoral fin	0.384±0.693	15	0.084-0.906
Pelvic fin	0.229±0.170	15	0.089-0.669
WEIGHT(g)	431.681±231.323g	15	962.5-183.77g
LENGTH(g)	40.62±6.37cm	15	31.7-55.2cm

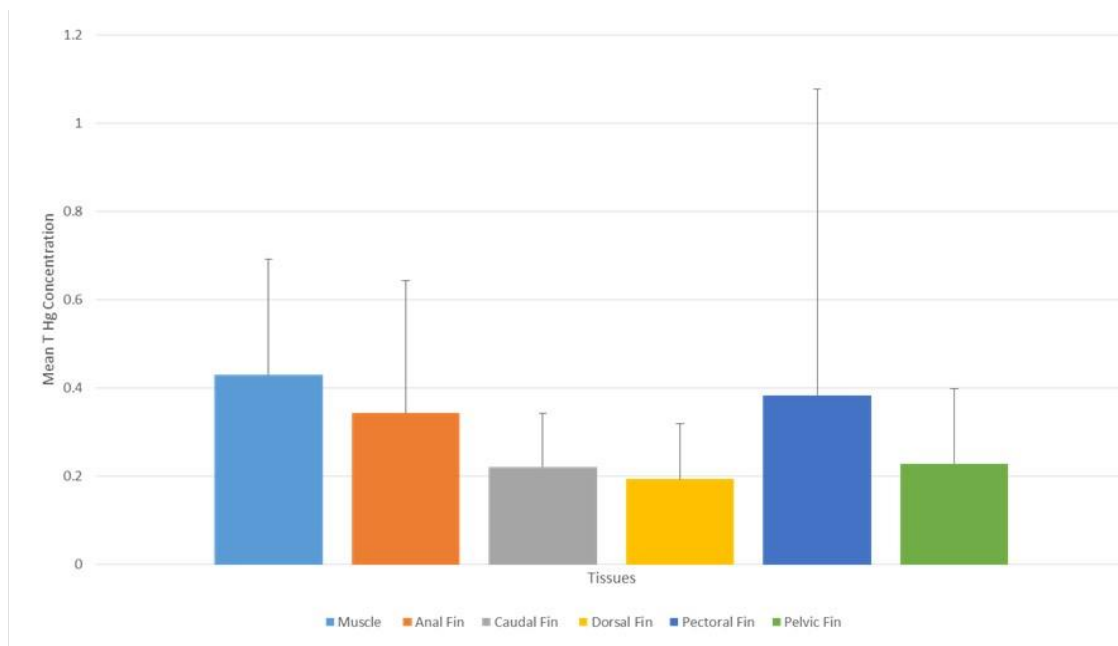


Figure 4.41: Mean Mercury Concentration for Mudfish from White Volta River at Yapei

The results of the Pearson correlation coefficient and the P-values as can be seen in Table 4.24 for the correlation between the muscle mercury concentration and the various fins mercury concentration, ranged from 0.546 to 0.981 and the corresponding P-values also ranged from 0.00 to 0.035.

The correlation between the total mercury concentrations in the muscle with Pectoral fin recorded the highest coefficient of 0.980 while the lowest value of 0.546, was recorded for muscle with anal fin. From the results all the pairs of variables recorded positive significant Pearson correlation coefficients at the 95% confidence interval.

The correlation between the mercury concentration in the muscle and the weight and length of the fish also recorded positive strong correlation with coefficients of 0.981 (p-value = 0.000) for weight and 0.975 (p-value = 0.000) for length.

Table 4.24: Correlation Matrix for Mudfish from White Volta River at Yapei.

	Muscle	Dorsal fin	Caudal fin	Pectoral fin	Pelvic fin	Anal fin	Weight	Length
Muscle		0.966	0.976	0.980	0.945	0.546	0.981	0.975
		0.000	0.000	0.000	0.000	0.035	0.000	0.000
Dorsal fin	0.966		0.931	0.949	0.870	0.402	0.965	0.954
	0.000		0.000	0.000	0.000	0.138	0.000	0.000
Caudal fin	0.976	0.931		0.936	0.955	0.587	0.950	0.937
	0.000	0.000		0.000	0.000	0.021	0.000	0.000
Pectoral fin	0.980	0.949	0.936		0.941	0.588	0.983	0.970
	0.000	0.000	0.000		0.000	0.021	0.000	0.000
Pelvic fin	0.945	0.870	0.955	0.941		0.699	0.949	0.887
	0.000	0.000	0.000	0.000		0.004	0.000	0.000
Anal fin	0.546	0.402	0.587	0.588	0.699		0.556	0.517
	0.035	0.138	0.021	0.021	0.004		0.032	0.049
Weight	0.981	0.965	0.950	0.983	0.949	0.556		0.964
	0.000	0.000	0.000	0.000	0.000	0.032		0.000
Length	0.975	0.954	0.937	0.970	0.887	0.517	0.964	
	0.000	0.000	0.000	0.000	0.000	0.049	0.000	

The regression data for total mercury concentrations in muscle against anal fin yielded a slope of 2.005 ($p < 0.001$) and r^2 of 0.931, whereas the regression of concentrations in muscle against those in pelvic fins yielded a slope of 1.450 ($p < 0.001$) and r^2 of 0.892. Similarly regression of concentration of mercury in muscle against those in caudal fin yielded a slope of 2.116 ($p = 0.001$) and r^2 of 0.949, slope of 2.005 ($p = 0.0001$) and r^2 of 0.933 being the regression data for concentration in muscle against dorsal fin.

Furthermore, regression of concentration in muscle against concentration in pectoral fin yielded a slope of 1.103 and r^2 of 0.960. The regression analysis of the concentration of mercury in the muscle against the weight and length of fish yielded slopes of 0.001 ($p = 0.0001$) and 0.040 ($p = 0.0001$) respectively while r^2 values of 0.962 and 0.951 were obtained.

Regression coefficients results from Table 4.25 indicates that Pectoral fin is better than pelvic fins, caudal fins, dorsal fins and anal fins as predictors of muscle total mercury concentration

for mudfish samples from Yapei ($r^2 = 0.960$ for Pectoral fins; $r^2 = 0.952$ for caudal fin; $r^2 = 0.933$ for Dorsal fin; $r^2 = 0.892$ for Pelvic fins and $r^2 = 0.546$ for Anal fins).

That is pectoral fin > pelvic fin > caudal fin > dorsal fin > anal fin. For Tilapia fish samples from Yapei White Volta, pectoral fin is better than the rest of the other fins as predictor of muscle mercury concentration in the river ($r^2 = 0.833$ for Pectoral fin; $r^2 = 0.809$ for pelvic fin; $r^2 = 0.786$ for Dorsal fin and $r^2 = 0.413$ for caudal fin).

Table 4.25: Regression Analysis Results for Mudfish from White Volta River at Yapei

Source	Intercept	Slope	Df	F-Ratio	P-Value	R ²
T Hg in Muscle vrs Dorsal fin	0.043	2.005	1	181.19	0.000	0.933
T Hg in Muscle vrs Caudal Fin	-0.039	2.116	1	259.97	0.000	0.952
T Hg in Muscle vrs Pectoral Fin	0.106	1.103	1	313.51	0.000	0.960
T Hg in Muscle vrs Pelvic Fin	0.098	1.454	1	107.40	0.000	0.892
T Hg in Muscle vrs Anal Fin	0.244	0.682	1	5.53	0.035	0.546
T Hg in Muscle Vrs Weight	-0.047	0.001	1	18.179	0.000	0.962
T Hg in Muscle Vrs Length	-1.193	0.040	1	252.11	0.000	0.951

There was variation in slopes and r^2 values for all the samples and this is in agreement with Rolfhus *et al.*, (2008) who reported variations were in fish from selected fresh water bodies in the USA and further stated that these variations are unrelated to mean total mercury in muscle of fish from the different lakes, lake area, or geographic position. The mean concentrations of total mercury in fins were positively correlated with those in muscles among all fish species and water bodies examined in this study.

The graphical relationship between mercury concentration in the muscle and the various fins that recorded significant regression coefficients and correlation coefficients are shown in Figure 4.42 to Figure 4.45. The rest of the graphs showing the relationship between the muscle and the less significant fins mercury concentration for mudfish fish samples from Yapei are shown in the Appendix 3.

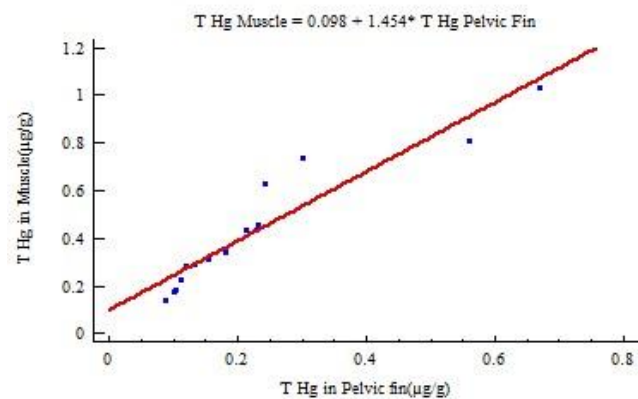


Figure 4.42: T Hg in Muscle Verses T Hg in Pelvic fins of Tilapia fish from White Volta River at Yapei.

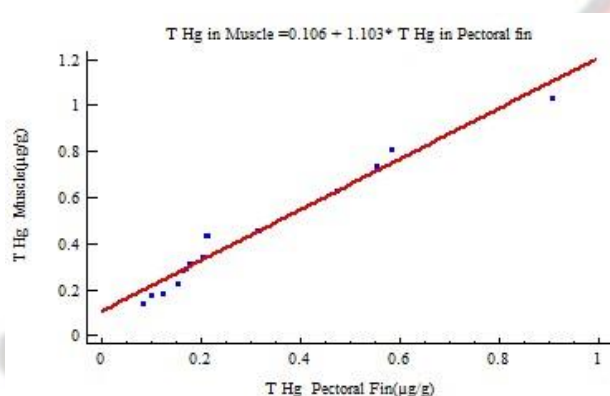


Figure 4.43: T Hg in Muscles Verses T Hg in Pectoral fins of Tilapia fish from White Volta River at Yapei.

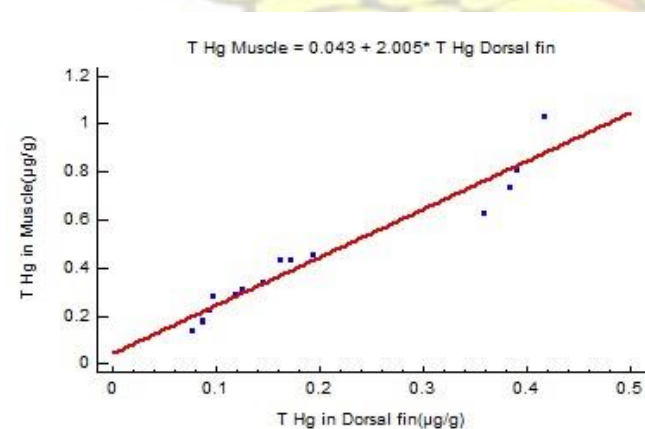


Figure 4.44: T Hg in Muscles Verses T Hg in Dorsal fins of Tilapia fish from White Volta River at Yapei

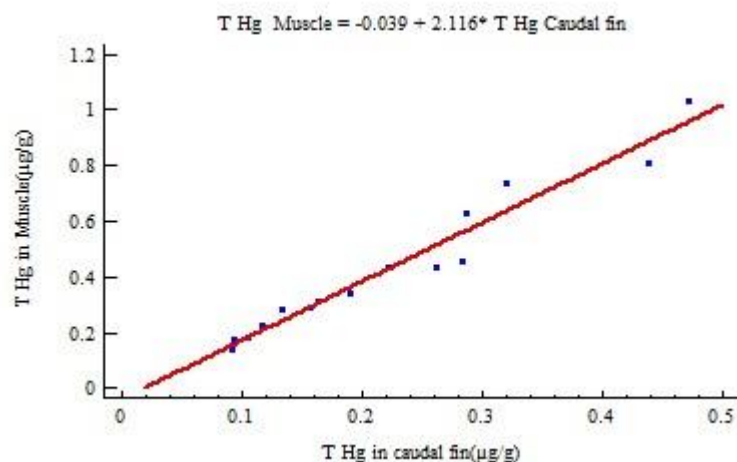


Figure 4.45: T Hg in Muscles verses T Hg in Caudal fins of Tilapia fish from White Volta River at Yapei

In order to predict the mercury concentration in muscle of the Mudfish samples from Yapei, the equations as can be seen Table 4.26 are used to estimate the mercury concentration in the muscle of the fish.

Table 4.26: Equations for Prediction of Muscle Mercury Concentration of Mudfish Samples from White Volta, Yapei.

TISSUES	EQUATION	r ²
Dorsal fins	$T\ Hg\ in\ Muscle = 0.028 + 1.330 * T\ Hg\ in\ Dorsal\ Fins$	0.933
Caudal fins	$T\ Hg\ in\ Muscle = 0.351 + 0.476 * T\ Hg\ in\ Caudal\ Fins$	0.952
Pectoral Fins	$T\ Hg\ in\ Muscle = 0.024 + 0.959 * T\ Hg\ in\ Pectoral\ Fins$	0.960
Pelvic Fins	$T\ Hg\ in\ Muscle = 0.007 + 1.386 * T\ Hg\ in\ Pelvic\ Fins$	0.892
Anal Fins	$T\ Hg\ in\ Muscle = 0.002 + 1.435 * T\ Hg\ in\ Anal\ Fins$	0.546

From the study, for Tilapia fish samples caudal fin was a better predictor of muscle mercury concentration in two (Navrongo and Kpong) out of the three locations. However anal fins

recorded the highest regression coefficient for tilapia fish samples from Yapei. Similar results have been reported by Gremillion *et al.*, (2005); Kristopher *et al.*, (2008) and Maria and David, (2013).

Furthermore examinations of the regression data for mudfish samples indicate pectoral fin was better predictor of muscle mercury concentration in fish from Yapei whiles pelvic fins and dorsal fins recorded the highest regression coefficient for mudfish samples from Kpong Navrongo respectively.



Chapter 5

CONCLUSION

The following conclusions are drawn from this study:

There was positive correlations ($P \leq 0.05$) between length and weight of fish and mercury concentration in muscle tissues for all the fish samples from the three locations.

Caudal fins recorded the highest r^2 value of 0.895 and because of that it better explains the variability of Hg concentration in muscle than the rest of the other fins for Tilapia fish samples

from Tono dam, Navrongo. For mudfish samples from Navrongo however, pelvic fins emerged as the better predictor of the muscle mercury concentration since it recorded the highest r^2 of 0.752.

A significant regression coefficient of 0.672 (p-value = 0.0002) was recorded for caudal fin indicating that caudal fins was a better predictor of Hg concentration than the rest of the other fins for tilapia fish sample from Kpong hydro dam. Furthermore regression data analysis for mudfish samples from Kpong shows that even though caudal fins recorded significant regression coefficient of 0.856 (p-value = 0.000), dorsal fins was a better predictor of Hg concentration in the muscle than the rest of the other fins since it recorded the highest regression coefficient ($r^2 = 0.932$).

In the case of tilapia fish samples from Yapei, White Volta, anal fins were better predictor of Hg concentration in muscle tissues than the rest of the other fins recording a regression coefficient (r^2) of 0.893. Furthermore for Mudfish samples from Yapei, pectoral fins emerged the better predictor of mercury concentration in muscle than pelvic fins, caudal fins, dorsal fins and anal fins.

The variability in muscle Hg explained by the various fin Hg differed significantly among fish, as no one fin was a better predictor of muscle Hg concentration for the two different species of fish from the three different location and thus, the efficacy of this nonlethal approach is highly species and location specific.

From the results of the three different water bodies, it can be realized that no one single fin could be used for predicting mercury concentration in the muscle tissues of two or more different species of fish from the three locations.

Given the level of uncertainty in all regression models, the examination of fin clips as predictors of muscle Hg content in freshwater fishes should be limited to a cursory screening tool and should not be the foundation for developing human consumption advisories as reported by Maria and David, (2013).

In addition the mercury concentration in the muscle tissue for both species of fish from all the sampling sites recorded values below the WHO limit 0.5µg/g and thereby does not pose any health risk to consumers.

KNUST

The logo of KNUST (Kenya National University of Science and Technology) is centered in the background. It features a yellow eagle with spread wings perched on a green shield. Above the eagle is a black mortar and pestle with a red flame. The entire emblem is set against a white background with a yellow border.

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APPENDIXS 1

1.1 Mercury Concentration in Tilapia fish from Tono Dam, Navrongo

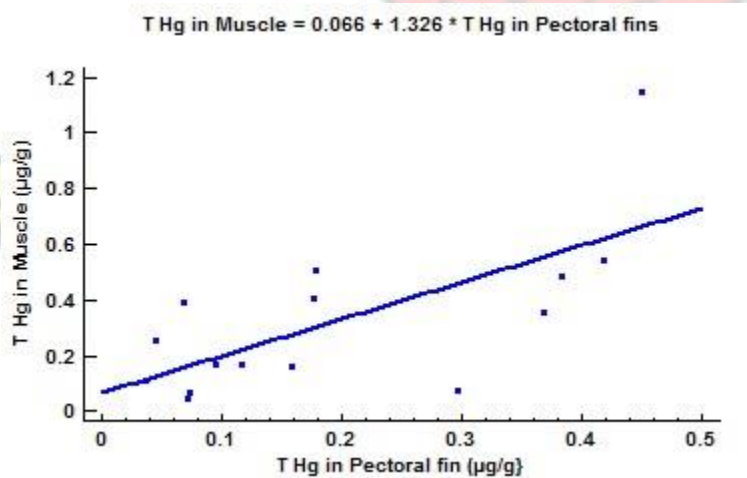


Figure 5.4: T Hg in Muscle verse T Hg in Pectoral fins

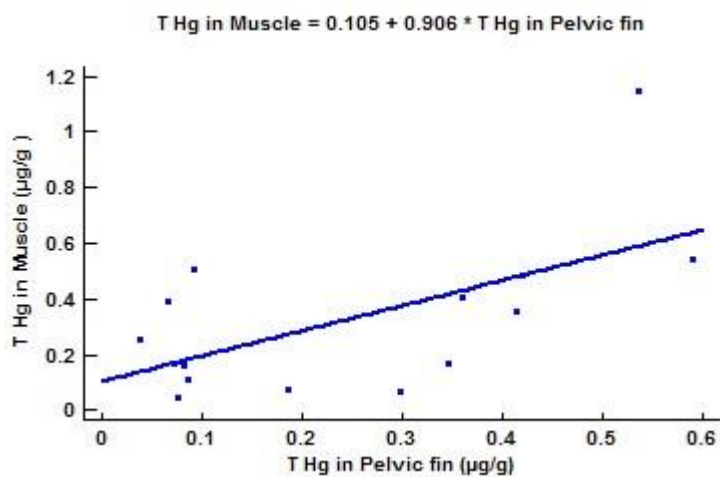


Figure 5.5: *T Hg in Muscle verses T Hg in Pelvic fins*

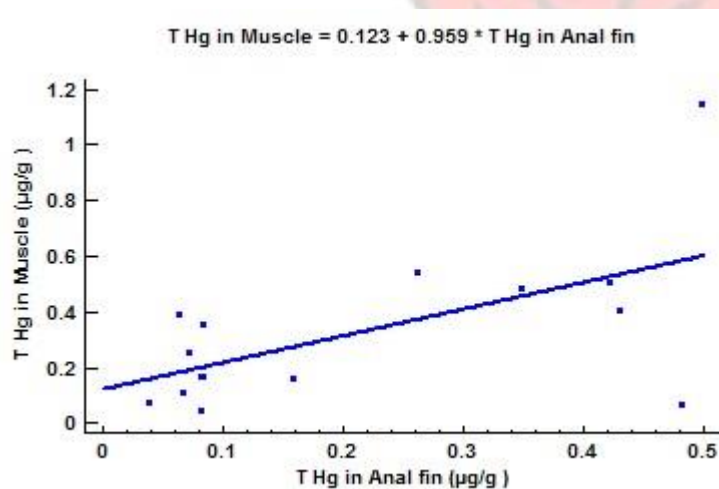


Figure 5.6: *T Hg in Muscle Verses T Hg in Anal fins*

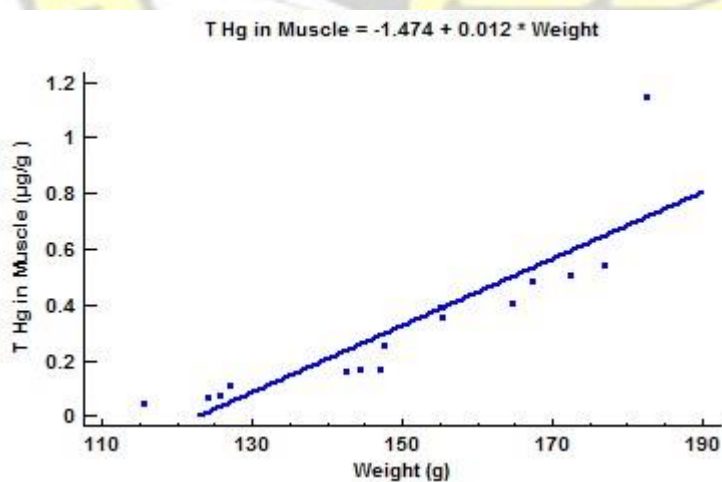


Figure 5.7: T Hg in Muscle versus Weight

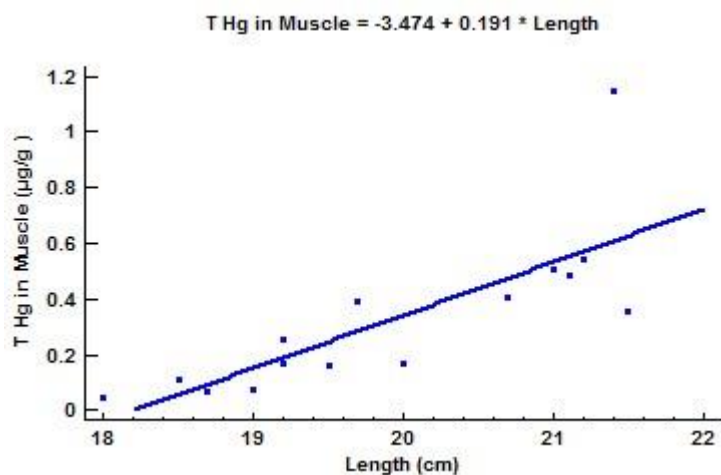


Figure 5.8: T Hg in Muscle versus Length

1.2 Mercury Concentration in Mudfish from Tono Dam, Navrongo

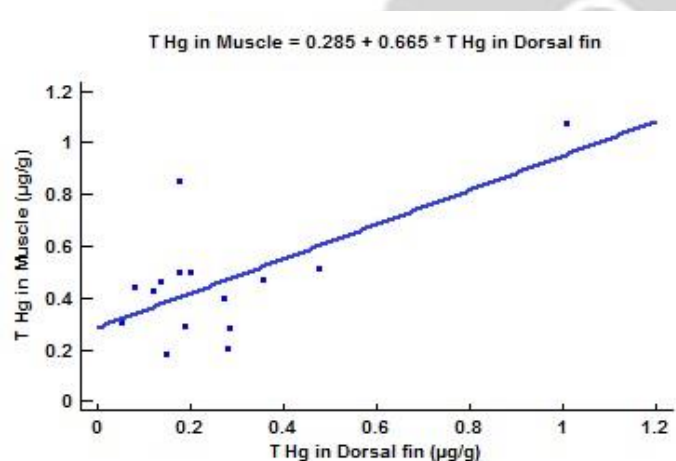


Figure 4.12: T Hg in Muscle versus T Hg in Dorsal fin

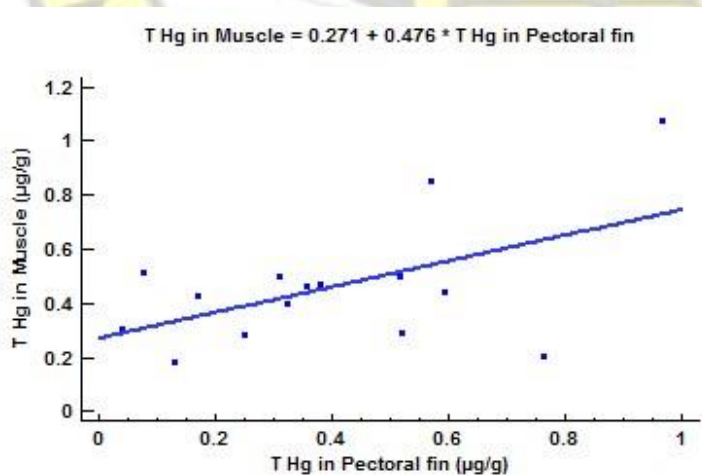


Figure 4.13: T Hg in Muscle verses T Hg in Pectoral fin

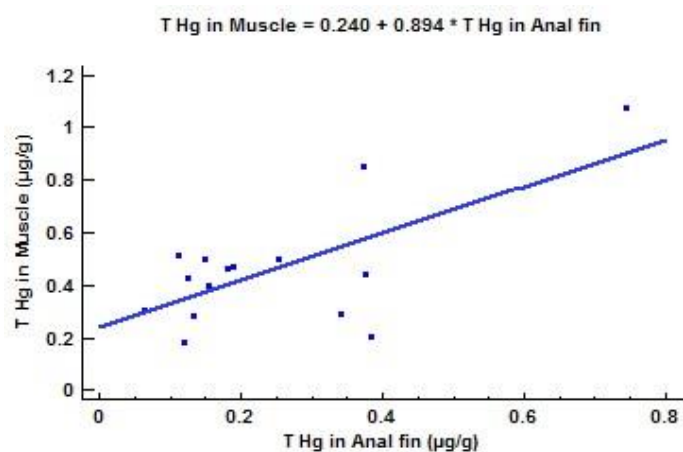


Figure 4.14: T Hg in Muscle verses T Hg in Anal fin

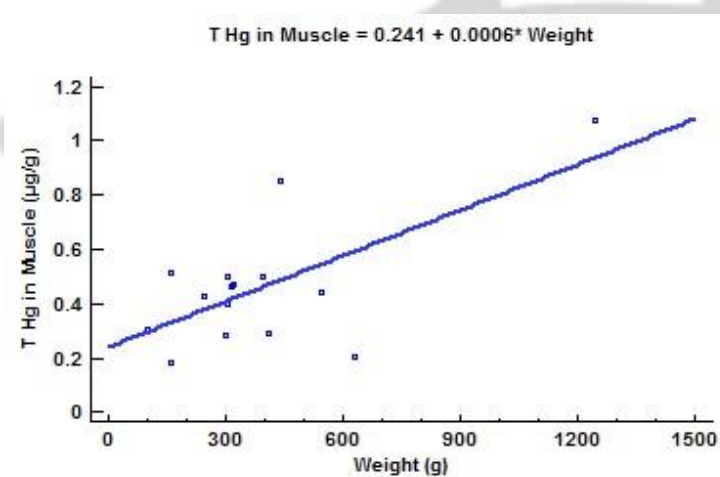


Figure 4.15: T Hg in Muscle verses Weight of Mudfish Samples from Tono dam, Navrongo

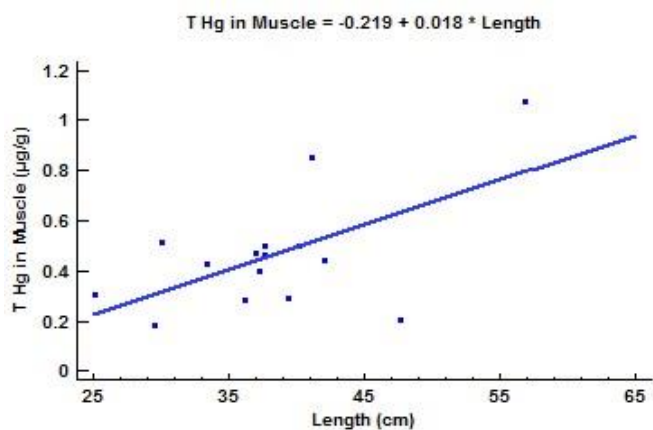


Figure 4.16: T Hg in Muscle verses Length of Mudfish Samples from Tono dam, Navrongo

APPENDIX 2

2.1 Mercury Concentration in Tilapia fish from Kpong, Hydro Dam

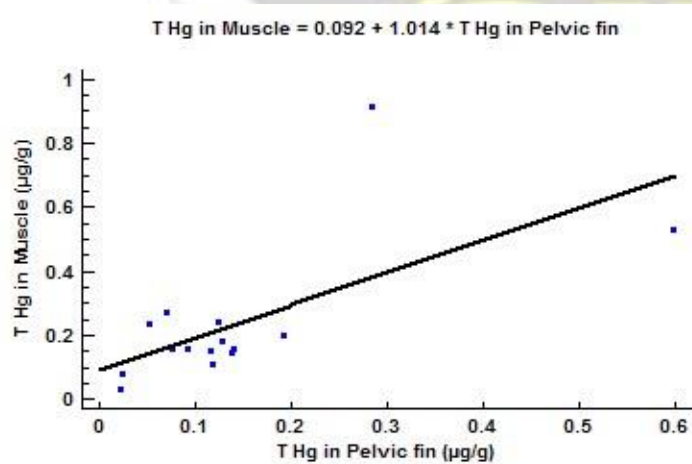


Figure 4.21: T Hg in Muscle verses T Hg in Pelvic fin

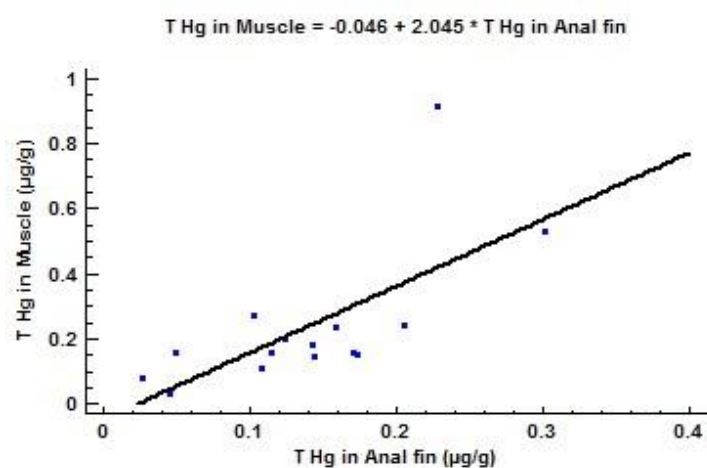


Figure 4.22: T Hg in Muscle verses T Hg in Anal fin

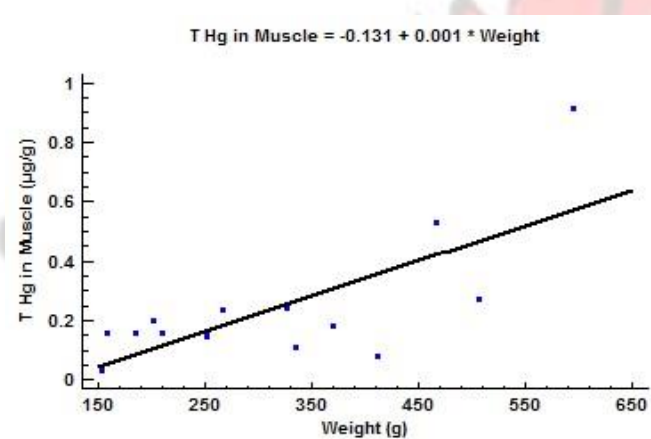


Figure 4.23: T Hg in Muscle verses Weight of fish

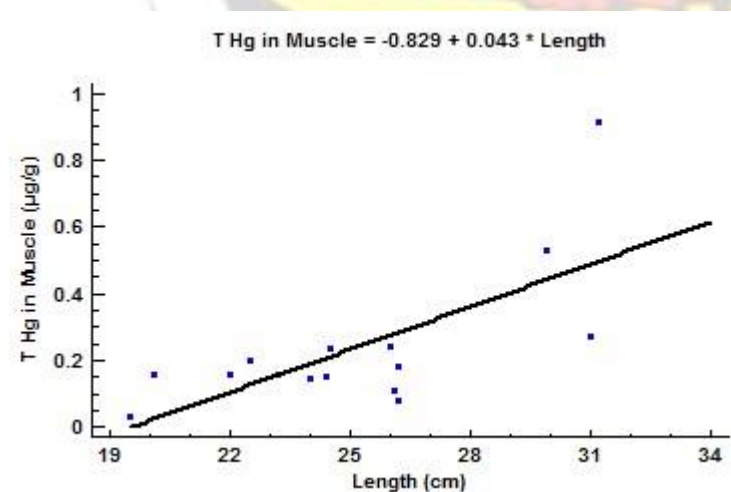


Figure 4.24: T Hg in Muscle verses Length of fish

APPENDIX 3

3.1 Mercury Concentration in Tilapia fish from Yapei

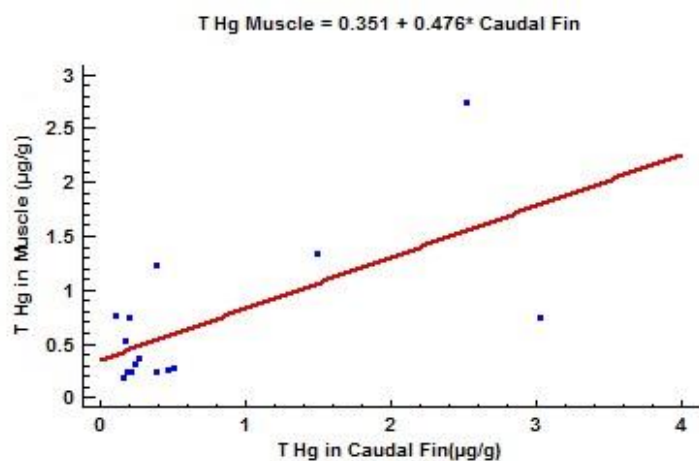


Figure 4.38: T Hg in Muscle verses caudal fin

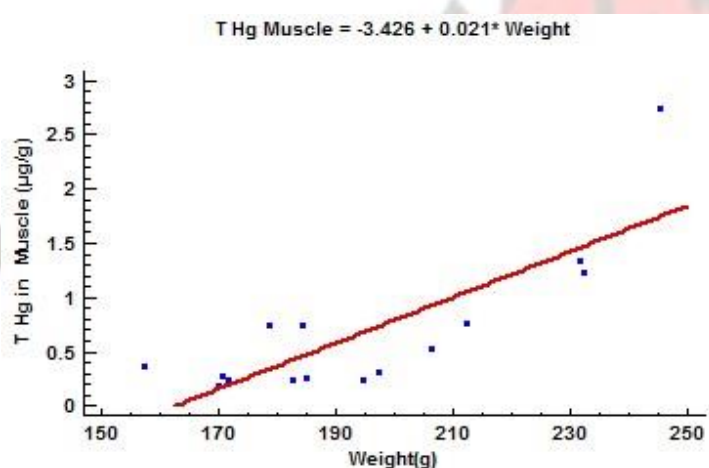


Figure 4.39: T Hg in Muscles Verses Weight of Tilapia fish

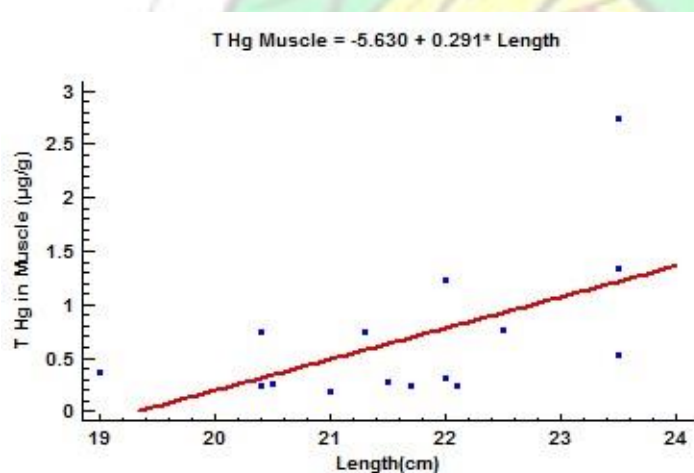


Figure 4.40: T Hg in Muscles verses Length of Tilapia fish

3.2 Mercury Concentration in Mudfish from Yapei

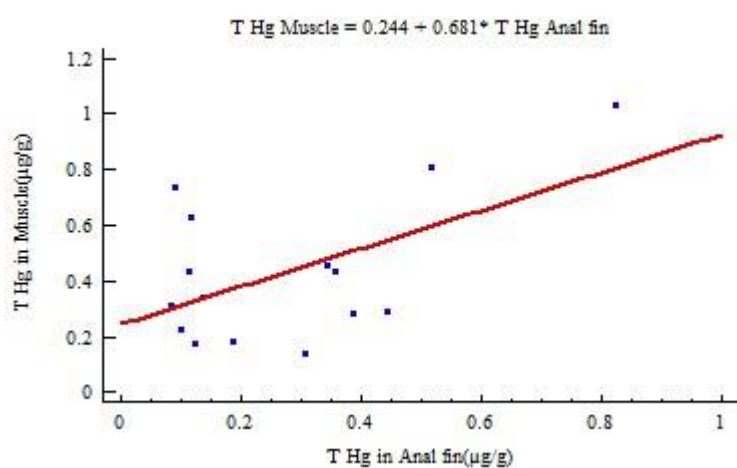


Figure 4.46: T Hg in Muscles Verses T Hg in Anal fins

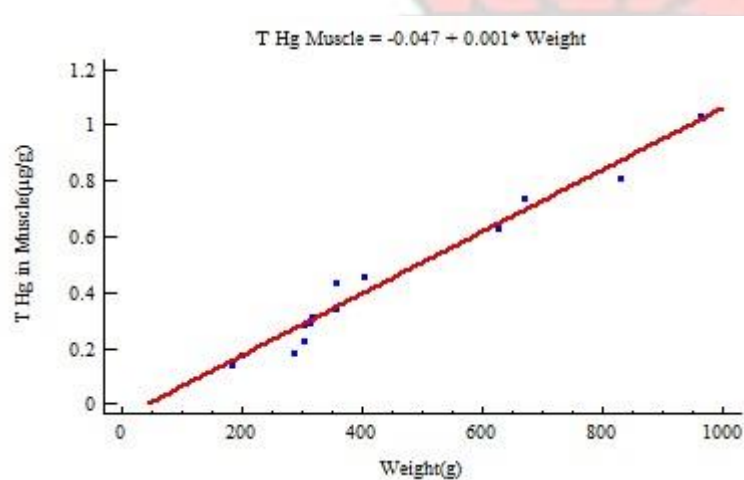


Figure 4.47: T Hg in Muscle Verses Weight

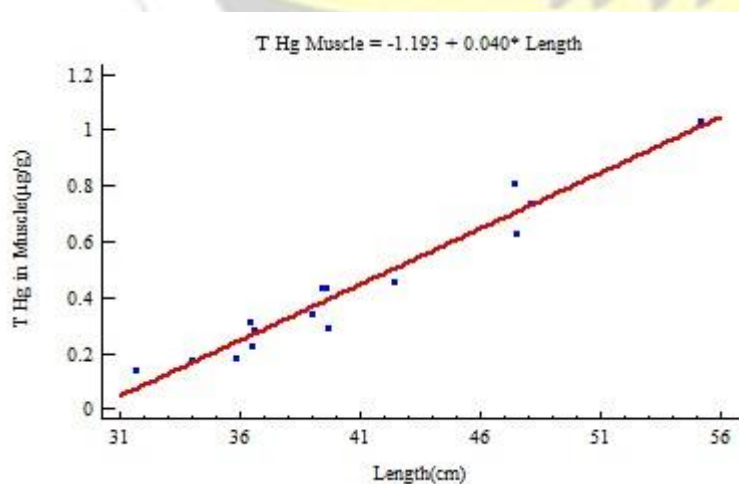


Figure 4.48: T Hg in Muscles Verses Length

anal fin	Weight	Length
µg/g	g	cm
0.262	176.96	21.2

Muscle µg/g	Dorsal fin µg/g	caudal fin µg/g	Pectoral fin µg/g	Pelvic fin µg/g	anal fin µg/g	Weight g	Length cm
0.474	0.358	0.33	0.381	0.372	0.19	322.35	37
0.424	0.118	0.325	0.171	0.344	0.125	247.35	33.4
0.853	0.176	0.327	0.57	0.496	0.373	441.12	41.1
0.398	0.27	0.436	0.323	0.113	0.154	306.26	37.2
0.498	0.199	0.164	0.517	0.167	0.254	394.32	40.2
0.497	0.177	0.38	0.311	0.346	0.15	304.16	37.7

0.186	0.149	0.174	0.131	0.162	0.119	159.29	29.5
0.517	0.477	0.236	0.075	0.376	0.112	158.57	30
0.283	0.282	0.269	0.251	0.229	0.134	299.64	36.2
0.445	0.081	0.018	0.592	0.39	0.375	546	42
0.464	0.134	0.14	0.358	0.165	0.182	316.5	37.7
0.287	0.188	0.205	0.52	0.236	0.34	408.45	39.4
0.202	0.279	0.199	0.762	0.178	0.385	631.86	47.7
0.304	0.05	0.172	0.04	0.206	0.063	97.98	25.1
1.074	1.007	1.039	0.968	1.062	0.743	1246.2	56.9
						2	

KPONG MUDFISH SAMPLES DATA

Muscle µg/g	Dorsal fin µg/g	caudal fin µg/g	Pectoral fin µg/g	Pelvic fin µg/g	anal fin µg/g	Weight g	Length cm
1.237	0.972	0.392	1.031	1.096	1.126	232.35	22
2.745	1.839	2.517	2.624	1.589	1.698	245.21	23.5
0.311	0.312	0.238	0.38	0.137	0.305	197.21	22
0.743	0.872	3.027	0.791	0.848	0.26	184.17	20.4
0.755	0.755	0.1	0.244	0.257	0.603	212.21	22.5
0.363	0.331	0.261	0.409	0.241	0.154	157.44	19
0.253	0.195	0.461	0.258	0.142	0.282	185.06	20.5
0.246	0.132	0.386	0.926	0.167	0.247		
182.79	21.7	0.242	0.293	0.215	0.56	0.268	0.298
0.968	231.56	23.5	0.274	0.278	0.501	0.258	0.53
0.189	0.36	206.25	23.5				
0.232	0.27	0.193	0.416	0.329	0.216	171.67	20.4
0.181	0.204	0.158	0.147	0.207	0.176	170.1	21

0.751 0.276 0.205 0.421 0.284 0.228 178.75

21.3 Mercury

concentration tilapia fish from YAPEI

Muscle µg/g	Dorsal fin µg/g	caudal fin µg/g	Pectoral fin µg/g	Pelvic fin µg/g	anal fin µg/g	Weighg	Length cm
0.808	0.39	0.439	0.584	0.56	0.517	830.1	47.4
0.172	0.086	0.094	0.1	0.101	0.123	201.38	34
0.228	0.093	0.117	0.153	0.111	0.101	301.93	36.5
1.033	0.417	0.472	0.906	0.669	0.823	962.5	55.2
0.343	0.145	0.19	0.204	0.182	0.135	355.7	39
0.14	0.077	0.091	0.084	0.089	0.305	183.77	31.7
0.433	0.171	0.262	0.213	0.229	0.355	358.01	39.4
0.626	0.359	0.286	0.472	0.243	0.117	628.04	47.5
0.282	0.097	0.134	0.163	0.119	0.386	304.2	36.6
0.458	0.194	0.284	0.314	0.232	0.343	403.65	42.4
0.432	0.162	0.222	0.21	0.212	0.114	357.76	39.6
0.737	0.383	0.32	0.552	0.302	0.089	670.59	48.1
0.31	0.125	0.163	0.178	0.154	0.083	317.32	36.4
0.18	0.086	0.105	0.123	0.104	0.187	288.27	35.8
0.292	0.119	0.156	0.171	0.134	0.443	312	39.7

Mercury Concentration in mudfish from Yapei (RAW DATA)

Muscle µg/g	Dorsal fin µg/g	Caudal fin µg/g	Pectoral fin µg/g	Pelvic fin µg/g	Anal fin µg/g	Weighg g	Length cm
0.292	0.383	0.094	0.204	0.232	0.517	830.1	47.4
0.180	0.171	0.284	0.178	0.243	0.123	201.38	34
0.310	0.086	0.117	0.084	0.089	0.101	301.93	36.5
1.033	0.417	0.320	0.314	0.182	0.114	962.5	55.2
0.172	0.097	0.156	0.100	0.560	0.135	355.7	39
0.140	0.086	0.262	0.552	0.119	0.305	183.77	31.7
0.626	0.145	0.134	0.195	0.134	0.355	358.01	39.4
0.737	0.390	0.472	0.171	0.111	0.117	628.04	47.5
0.228	0.093	0.091	0.123	0.101	0.386	304.2	36.6
0.432	0.194	0.222	0.584	0.229	0.343	403.65	42.4
0.343	0.125	0.190	0.163	0.104	0.823	357.76	39.6
0.808	0.077	0.163	0.213	0.669	0.089	670.59	48.1
0.433	0.162	0.439	0.153	0.302	0.083	317.32	36.4
0.282	0.359	0.286	0.210	0.154	0.187	288.27	35.8
0.458	0.119	0.105	0.472	0.212	0.443	312	39.7