UNIVERSITY OF CAPE COAST

LEVELS OF Hg AND Cd IN SELECTED BIOLOGICAL SPECIES FROM THE RIVER PRA - A STUDY OF POLLUTION OF THE RIVER PRA ESTUARY

BY

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Thesis submitted to the Department of Chemistry of the School of Physical Sciences, University of Cape Coast in Partial fulfillment of the requirements for the award of Master of Philosophy Degree in Chemistry

DECEMBER 2007

DECLARATION

Candidate's Declaration

I herby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

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Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast

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ABSTRACT

This thesis determined the level of mercury and cadmium in selected biologicalspecies namely tilapia (*Tilapia zillii*), mudfish (*Clarias submarginatus*), prawns (*Penaeus monodon*), crabs (*Carcinus maenas*) and Molluscs Periwinkle (*Gastropod*) from the River Pra Estuary using.

The samples were taken every fortnight from four sampling sites from Beposo to Shama Beach in the Western Region of Ghana for two months. That is form 10/05/06 to 29/06/06. During the period a total of eighty (80) samples were collected and analyzed and the levels of mercury determined in the muscles of the various biological species were between 0 and 0.00808 ± 0.00097µg/g while the levels of cadmium were between 0.00101 ± 0.00013 µg/g and 0.02024 ± 0.00263 µg/g. But all the values are well below European Union acceptable limits of 0.05µg/g.

ACKNOWLEDGEMENTS

I wish to acknowledge Rev. Prof. D. K. Dodoo of Chemistry Department (UCC), my principal supervisor, who took time off his numerous engagements in giving expert advice and valuable recommendations.

My sincere appreciation goes to Mr. D. K. Essumang who co-supervised this work to the end and offered valuable pieces of advice and encouragement.

My special thanks go to my wife Loretta for her moral support and prayers that made this dream came true.

I also thank the Director and staff of Ghana Atomic Energy Commission, especially the staff of GARR 1 for their tremendous support during the analysis of the samples.

Finally but not the least, I wish to acknowledge Mr. Anddy Wofesor, a lab technician in the Chemistry Department, who occasionally drove me to the sampling sites in his personal car when the faculty car was not available.

TABLE OF CONTENTS

	PAGES
ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
CHAPTER ONE INTRODUCTION	1
Estuaries	1
Types of Estuaries	2
The River Pra Estuary	3
Objectives of the Study	6
Statement of the Problem	6
Justification of the Study	7
CHAPTER TWO LITERATURE REVIEW	8
Environmental Issues	8
Definition of Pollution	9
Heavy Metals	12
Methods of analysis of Hg and Cd	12
Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)	13
Inductively Coupled Plasma Mass Spectrometer	13
Flame-Atomic Absorption Spectroscopy (F-AAS)	14
Cold Vapour Technique	15
Neutron Activation Analysis (NAA)	16
Occurrence of Cadmium	18

Uses of Cadmium 19

Disposal of Cadmium 20

Human Intake of Cadmium 22

Ingestion 22

Inhalation 24

Biochemical Effect of Cadmium 26

Documented Methods for Determination of Cadmium In Environmental Samples 27

Occurrence and Application of Mercury 30

Atmospheric Mercury 33

Aquatic Mercury 35

Biochemical Effect of Mercury 36

Toxic Effects of Mercury 38

Biological Methylation of Mercury: Amplification in Food Chain 41

Absorption of Mercury Species 43

Distribution of Mercury Species in the Human Body 44

Biotransformation of Mercury Species in the Human Body 45

Elimination of Mercury Species from the Human Body 46

Documented Methods for Determination of Mercury in Biological Materials 47

Documented Methods for Determination of Mercury in Environmental Samples 47

Feeding Habits of Selected Fish Samples 52

Tilapia 52

Mudfish 54

Crab 55

Prawns 58

Mollusks Periwinkle 59

Theory of Neutron Activation Analysis (NAA) 61

Neutron – Induced Reaction 62

Reaction Rate 63

Neutron Activation Analysis Calculations 65

Irradiation and Counting Procedures 69

Interferences in NAA 71

Accuracy and Precision 72

Sensitivity 75

CHAPTER THREE EXPERIMENTAL 77

Chemicals/Reagents 77

Equipments 78

Sample collection 78

Sample Treatment in the laboratory 80

Standard Reference Material 81

Sample Irradiation 81

Irradiation Source 82

Data Processing 82

Quality Control 83

CHAPTER FOUR RESULTS AND DISCUSSION 85

Table of Results 85

Discussion 101

Concentration levels in various parts of the biological species 102

Comparing samples from the River Pra Estuary and the River Ayensu 103

Control 104

Concentration levels As against Rainfall of Sampling Sites 107

Levels of mercury and cadmium in the muscles flesh as against lethal level 109

Statistical treatment of data 110

Pooled standard deviation for tilapia 110

Confidence limit of the work done 111

CHAPTER FIVE CONCLUSION AND RECOMMENDATION 113

Conclusion 113

Recommendation for Feature Research 114

REFERENCES 116

LIST OF TABLES

1. The Chemical-Physical State and Some Characteristics of Mercury 34
2. Chemical Species of Mercury 39
   Chemical Species of Mercury (continuation) 40
3. Validation of Results 83
4. Concentration of Mercury in various parts of selected Biological species from four sampling sites 1st sampling (10/05/06) 84
5. Concentration of Mercury in various parts of selected Biological species from four sampling sites 2nd sampling (24/05/06) 86
6. Concentration of Mercury in various parts of selected Biological species from four sampling sites 3rd sampling (8/06/06) 87
7. Concentration of Mercury in various parts of selected Biological species from four sampling sites 4th sampling (29/06/06) 89
8. Concentration of cadmium in various parts of selected Biological species from four sampling sites 1st sampling (10/05/06) 90
9. Concentration of cadmium in various parts of selected Biological species from four sampling sites 2nd sampling (24/05/06) 92
10. Concentration of cadmium in various parts of selected Biological species from four sampling sites 3rd sampling (8/06/06) 94
11. Concentration of cadmium in various parts of selected Biological species from four sampling sites 4th sampling (29/06/06)
12. Concentration of Mercury and Cadmium in various parts of selected Biological species collected River Kakum at Akotokyir (control sampling) 104
13. Concentration of Mercury in various parts of selected Biological species

collected from River Ayensu near Winneba (control sampling) 105

14. Concentration of Cadmim in various parts of selected Biological species collected from River Ayensu near Winneba (control sampling) 106

15. Rainfall – Mercury concentration correlation 108

16. Rainfall – Cadmium concentration correlation 108

17. Calculation of standard deviation 110

LIST OF FIGURES

1.	Map of Pra Estuary	5
2.	Metabolism of Cadmium	27
3.	Mercury Cycle in the environment	31
4.	Mechanism of Mercury Propagation	42
5.	*Tilapia zilli*	52
6.	Mudfish (*Clarias submarginatus*)	54
7.	Crab (*Carcinus maenas*)	55
8.	Prawns (*Penaeus monodon*)	58
9.	Molluscs Periwinkle (*Gastropod*)	60
10.	The researcher is removing life crab	79
11.	The researcher is removing life crab	79
12.	The researcher is picking life mollusc	80
13.	The researcher is observing life mollusc	80
14.	Variation of Hg concentration in selected Biological Species collected on (10/05/06)	86
15.	Variation of Hg concentration in selected Biological Species collected on(24/05/06)	88
16.	Variation of Hg concentration in selected Biological Species collected on (8/06/06)	90
17.	Variation of Hg concentration in selected Biological Species collected on (29/06/06)	92
18.	Variation of Cd concentration in selected Biological Species collected on (10/05/06)	94

19. Variation of Cd concentration in selected Biological Species collected on (24/05/06) 96

20. Variation of Cd concentration in selected Biological Species collected on (8/06/06) 98

21. Variation of Cd concentration in selected Biological Species collected on (29/06/06) 100

22. Variation of Cd concentration in selected Biological Samples collected from River Ayensu near Winneba 107

CHAPTER ONE

INTRODUCTION

Protein plays an important role in the growth and development of human body. Lack of it in children for example, leads the disease called "Kwashiorkor". Protein sources include animals and plants and most Ghanaians depend largely on animals for protein than for plants. Actually Ghanaians obtain about 60% of their dietary animal protein from fish (Environment Group, African Region).

However, our water bodies that serve as home and source of fish are under serious threat from chemical pollution. The Ghanaian newspapers, especially the Daily Graphic, carry frequent news items on water pollution episodes from the mining sector. Pollutants from the industries turn to destroy the aquatic environment where fish for protein comes from. These fishes in the polluted waters are exposed to chemical stressor some of which include Cd, Cu, Hg and Zn, (Dodoo *et al,* 1991).

Estuaries

An estuary is an inlet of the sea at the mouth of a river developed especially in areas, which have been submerged by the sea. Alyn (1991) also described an estuary as a portion of the ocean that is semi-isolated by land and is diluted by drainage. Both isolation and dilution are requirement for an estuary.

Therefore, all estuaries are coastal embayment. Differences in net circulation and current patterns are determined by tidal and river flows. Circulation and vertical distribution of salinity, or salt content, are used to categorize estuaries.

Types of Estuaries

Different types of estuary exist. These include:

(i) the Salt Wedge Estuaries, which occur within the mouth of a river flowing directly into salt water;

(ii) the Well-Mixed (or vertically homogenous) estuaries, which have a strong tidal mixing and low river flow;

(iii) the Partially-mixed estuaries which have a strong net seaward surface flow of fresh water and a strong inflow of seawater at depth and;

(iv) the Fjord-type estuaries which are the deep estuaries that have a moderately high river input but little tidal mixing (Alyn, 1991)

Ghana has many rivers, some of which enters the sea and thus form estuaries, among them is the Pra Estuary. High upstream of these rivers or along the profiles of these rivers exist many economic activities such as farming, mining and others, some of which tend to pollute these water resources.

Estuaries are uniquely sensitive to discharges from both the land and the sea. By acting as a trap, they filter contaminated sediment and act as a buffer between land and the coastal zone. They are of high fertility and act as a nursery grounds for many marine organisms (Biney, 1985). Estuary-dependent fish spend

the majority of their first year in habitats where the food supply is abundant, where they may as well be exposed to a variety of physical and chemical stressors. Among the chemical stressors are transition metals such as Cu, Hg and Zn. These metals can enter the estuary naturally through the weathering process or from the discharge of domestic industrial or agricultural effluents. (Dodoo et al, 1991).

The River Pra Estuary

The River Pra-estuary is of the Fjord– type and is located at 5° N, 1° 35" – 1°40", W. It takes its source from the Kwahu plateau in the Eastern Region of Ghana and flows down to join River Offin from Dunkwa, before it gradually empties itself into the Atlantic Ocean at Shama in the Western Region. Some major activities that take place along the banks of the river are:

(i) Intensive farming along the banks in the interlands. The main crops produced are plantain, banana, kolanut and cocoa.

(ii) Wood processing industries at Kede, Dunkwa and Daboase.

(iii) 'Galamsey' (illegal) mining around Dunkwa Offin

Also the river is used by the inhabitants who live along its banks as a means of transporting their goods from one place to another on market days, and for fishing. It is also a source of drinking water to some of the communities along its bank.

The River Pra has a catchments area of 29,000km^2 and is one of the largest rivers entering the Gulf of Guinea on the Ghanaian continental shelf. It is

also the second largest source of fresh water inflow into the coastal waters after the Volta River (Vanden-Bossche, and Bernnesek, 1990). It discharges $1.4x10^6$ t/yr. This was however before the river was dammed in 1967, (Donald, 1981).

The Hemang dam, on the Pra River has considerably reduced sediment input into the Gulf of Guinea. The reduction in sedimentation reaching the coastline has contributed to the rate of coastal erosion along the coast bordering the Gulf of Guinea. Hence there is the need for a regional appraisal of the sediment discharge into the Gulf. This will ensure effective management of the Pra and Offin rivers and subsequent coastal zone management. At present, the River Pra is one of the rivers in Ghana with the largest basin in the closed forest. It flows over pre-Cambrian, metamorphic, igneous and younger sedimentary rocks. These form the major source of sediment into the Gulf of Guinea shelf (Dickson, and Beneh, 1988)

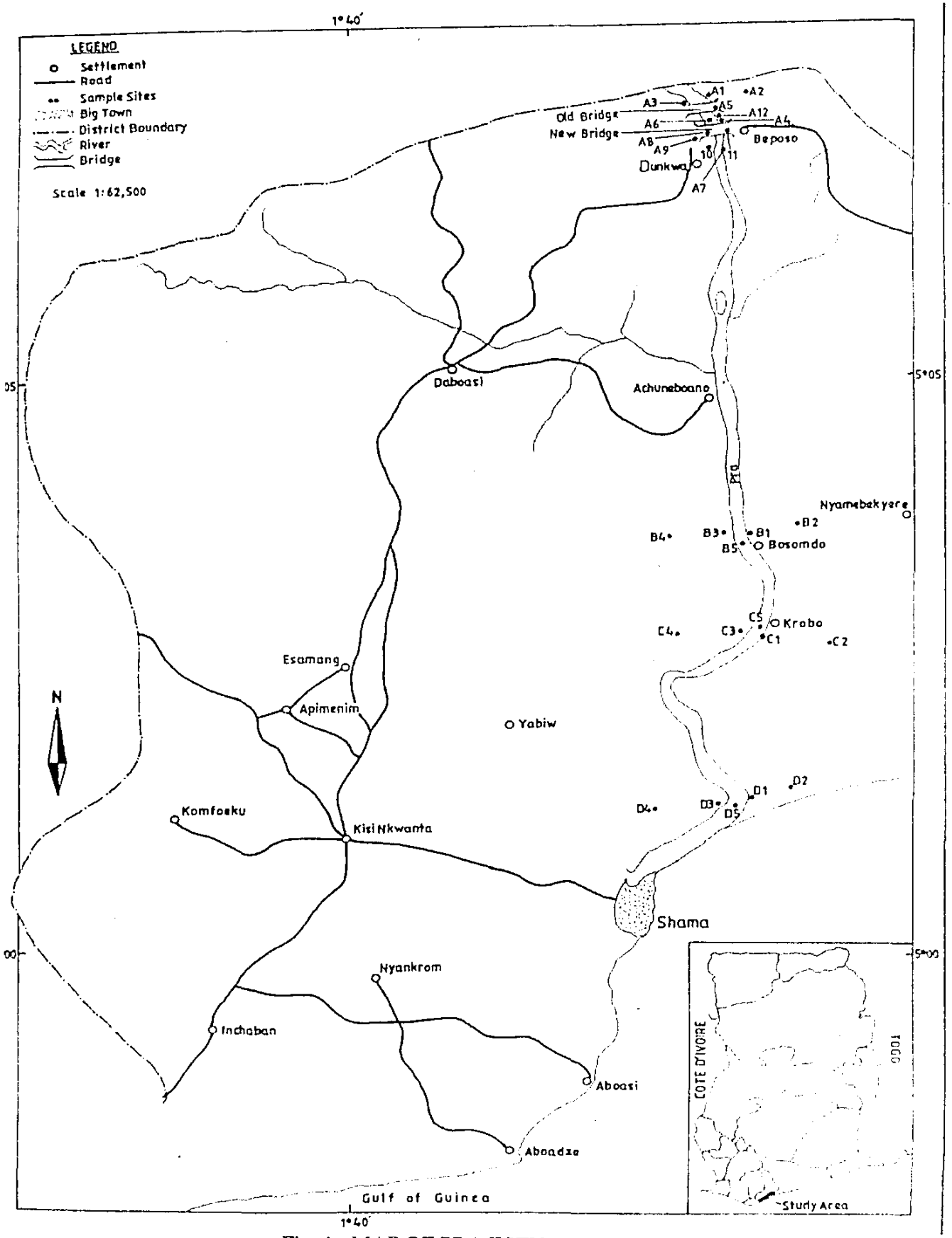


Fig. 1 MAP OF PRA ESTUARY

Objectives of the Study

The objectives are to:

1. determine mercury and cadmium levels in some selected biological species in the River Pra Estuary.
2. find out whether or not it is safe to consume fish and any other biota from the estuary.
3. give suggestions as to how the pollution, if any, could be manage.
4. find out whether the river is polluted or not.

Statement of the Problem

All over the country pollution is on the increase. Many of the rivers in the country are dying because toxic chemicals are constantly being dumped into them. Consuming fish from these water bodies could be a risk.

River Pra gets polluted up stream from the "galamsey" (illegal) mining, and agricultural activities and down stream from the vehicular traffic at the toll bridge. This could make fish and other biota from the River Pra Estuary unsafe for human consumption.

People consuming fish and other biota from the river may not be aware of the risks they are exposed to.

Justification of the Study

There are a lot of "galamsey" activities up stream, around Dunkwa Offin which tends to pollute the river with heavy metals. The bridge and the toll office at Beposo also encourage pollution from the vehicles that ply the road. These elements are toxic; with some of being carcinogenic and therefore may have an impact on human health for a fish-eating population, and hence must be a concern to all well meaning Ghanaians and sympathizers.

CHAPTER TWO

LITERATURE REVIEW

Environmental Issues

Many environmental problems are intimately linked to broader developmental concerns such as unplanned settlement, overpopulation, or resource use for economic activities. Poor domestic sanitation is one of the most widespread and pervasive of all the problems. It contributes to poor health, contaminated ecosystems, and unsightly conditions that hinder economic development opportunities such as tourism. In fact more than 1.5 million children, worldwide, die each year because they lack access to safe drinking water and proper sanitation (Daily Graphic, 2006).

The causes for poor sanitation are manifold: lack of local institutional capacity, low levels of education, unfettered urban growth and sprawl, and the lack of basic infrastructure. All these contribute to the deposition of excrement on beaches and untreated sewage in coastal lagoons or other areas. With few exceptions, all of these problems are strongly linked to general population and economic growth (Environment Group, African Region, 1996).

Pollution of the marine environment is the most serious of all environmental pollution and it poses a major threat to the health and well being of millions of people and the global ecosystem. Other major environmental problems

including global warming, climatic changes and loss of biodiversity through the extinction of many species are either wholly or partly caused by the presence of pollutants.

What is less generally appreciated, but is much more important in the long run, is that environmental pollution is insidious and its harmful effects only become apparent after long periods of exposure. Many people are exposed to the pollutants that may cause cancer ten or twenty years later without they realizing it. Gradual increase in atmospheric pollution could be causing chronic toxic effect in trees but the damage, which may be irreversible, may not appear for twenty or more years. Likewise, lakes can become much polluted, and species die out without any obvious signs at least in the early states. For these reasons environmental monitoring has become recognized as being vitally important in detecting where insidious pollution is occurring, the pollutants involved and their sources.

Environmental monitoring has benefited from the development of rapid and accurate methods of chemical analysis, such as gas chromatography, for organic pollutants, atomic absorption spectrometry for metals and neutron activation analysis for elements, including the metals.

Definition of Pollution

A widely used definition of pollution is "the introduction by man into the environment of a substance or energy liable to cause hazards to human health, harm to living resources and ecological system damage to structures or amenities,

or interference with legitimate uses of the environment. Some experts make a distinction between contamination and pollution. Contamination is used for situations where a substance is present in the environment but not causing any obvious harm, while pollution is reserved for cases where harmful effects are apparent.

However, the problem with this distinction is that with improved methods of analysis and diagnosis it may become apparent that harmful effects have been caused and so situations initially described, as contamination may really have been pollution.

Pollutants are basically of two types: primary pollutants, which exert harmful effects in the form in which they enter the environment, and secondary pollutants which are synthesized as a result of chemical processes, often from less harmful precursors, in the environment. Although highly toxic substances are responsible for many causes of environmental pollution, under some circumstances materials which are normally considered harmless may cause pollution if they are present in excessive quantities or in the wrong place at the wrong time.

For all cases of pollution, there is:

(i) a source of pollutant

(ii) the pollutants themselves

(iii) the transport medium (air, water or direct dumping onto land or river) and

(iv) the target (or receptor), which include ecosystems, individual organisms (e.g. humans) and structures.

Pollution can be classified in several ways according to

(i) the source (e.g. agriculture pollution)

(ii) the medium affected (e.g. air pollution or water pollution)

(iii) nature of the pollutant (e.g. heavy metal pollution)

Where the volume, the rate of emission and the toxicity of pollutants are relatively low, environmental processes can usually degrade or assimilate these excesses to a much greater extent than the more toxic air, water and land pollutants produced in large quantities by more technologically advanced cultures.

Given that environmental pollution poses one of the greatest threats to man's health and food security, the need for greater understanding of the problem becomes even more urgent. In recent times, there have been increasing demands for monitoring the environment for organochlorines, heavy metals and petroleum hydrocarbons (Daily Graphic, 2006). Monitoring programmes have been developed at both national and international levels, involving various types of sample matrixes from which scientists are expected to obtain accurate and reliable data.

The present work involves analysis of mercury and cadmium in selected biological species from the Pra estuary and determines the effect on human health of eating those selected biological species.

Heavy Metals

Every natural water body contains certain amounts of heavy metals. Their presence is usually dependent on the geochemistry of the soil, the topography, the climate and the land use of the river basin. Where land use involves urbanization, industrialization together with its concomitants pollution, the levels of metal ions in the water may considerably be increased and the possibility of introducing new foreign ones to that particular body of water is inevitable. Each heavy metal has a specific physiological function in a living organism. Consequently the level and types of heavy metals in a body of water will have some effect on the users of the water as well as the biota.

Methods of Analysis of Hg and Cd

The type of analytical method used to determine the levels of mercury and cadmium in biological and environmental samples is very important because it leads to a success or otherwise of a research.

Various methods are used to quantify mercury and cadmium in the environmental samples. These includes: inductively coupled plasma mass spectrometry (ICP-MS), graphite furnace atomic absorption spectroscopy (GF-AAS), flame-atomic absorption spectroscopy (F-AAS), cold vapour atomic fluorescence spectroscopy (CV-AFS), cold vapour atomic absorption spectroscopy (CV-AAS) and Neutron Activation Analysis (NAA), Berg et al, (2000).

Graphite Furnace Atomic Absorption Spectroscopy (GF- AAS)

Graphite furnace atomic absorption spectroscopy (GF-AAS) is a powerful technique suitable for trace analysis. The technique has high sensitivity (analyte amounts 10^{-8}-10^{-11} g absolute), the ability to handle micro samples (5-100 uL), and a low noise level from the furnace. Matrix effects from components in the sample other than the analyte are more severe in this technique compared to flame-AAS. The precision is typically (5-10) % using GF-AAS.

A graphite tube is located in the sample compartment of an AA spectrometer with the light from an external light source passing through it. A small volume of sample is placed inside the tube, which then is heated by applying a voltage across its ends. The analyte is dissociated from its chemical bonds and the fraction of analyte atoms in the ground state will absorb portions of light. The attenuation of the light beam is measured. As the analyte atoms are created and diffuse out of the tube, the absorption raises and falls in a peak-shaped signal. Beer-Lamberts law describes the relation between the measured attenuation and concentration of analyte. A detailed description of the GF-AAS technique can be found in various textbooks, Montaser, (1998).

Inductively Coupled Plasma Mass Spectrometry

ICP-MS is a multi-element technique that is suitable for trace analysis. The technique offers a long linear range and low background for most elements. The detection limits obtained are better or comparable to what is obtained by graphite furnace atomic absorption spectroscopy (GF-AAS). The technique is

prone to some interference that will be described below. Different sample introduction devices may be used in combination with ICP-MS to allow introduction of non-liquid samples such as solid samples, slurries and gaseous samples. In this chapter, only conventional solution introduction will be described.

ICP-MS is a technique where ions produced in inductively coupled plasma, are separated in a mass analyser and detected. The sample solution is fed into a nebulizer by a peristaltic pump. The nebulizer converts the liquid sample into a fine aerosol that is transported into the plasma by an Ar gas flow, most often called carrier gas or nebulizer gas. With an ordinary pneumatic nebulizer, only 1-2% of the sample reaches the plasma. In the plasma the sample is evaporated, dissociated, atomised and ionised to varying extent. The produced positive ions and molecular ions are extracted into the mass analyser. A simple quadrupole gives a resolution of 1 amu or more at a peak width of 10% of the peak height. The ions are separated by mass to charge ratio (m/z) and measured by a channel electron multiplier. Detailed description of the ICP-MS technique can be found in various textbooks (Jarvis *et al.* 1992; Montaser, 1998).

Flame-Atomic Absorption Spectroscopy (F-AAS)

F-AAS is a very specific technique prone to little interference. F-AAS is a single element technique with analyte determinations in the mg L^{-1} region as routine for most elements.

A liquid sample is neutralized to form a fine aerosol, which is mixed with fuel and oxidant gasses and carried into a flame. In the flame the sample is dissociated into free ground state atoms. A light beam from an external light source emitting specific wavelengths passes through the flame. The wavelength is chosen to correspond with the absorption energy of the ground state atoms of the desired element. The measured parameter in F-AAS is attenuation of light. Lambert-Beers law expresses the relationship between the attenuation of light and concentration of analyte.

Cold Vapour Technique

The cold vapour technique is an atomization method of determination of mercury because is the only metallic element that has an appreciable vapour pressure at ambient temperature. The determination of mercury in various types of samples is of vital importance currently because of the toxicity of various organic mercury compounds and their widespread distribution in the environment. The method of choice for this analysis is cold vapourisation followed by atomic absorption spectrometry or atomic fluorescence spectroscopy. In per forming analysis of this type, mercury is converted to Hg^{2+} by treatment of sample with an oxidizing mixture of the nitric and sulphuric acids followed by reduction of Hg^{2+} to the metal with $SnCl_2$. The elemental mercury is then swept into a long-pass absorption tube by bubbling a stream of inert gas through the mixture from which the element was formed. The analysis is completed by measuring the absorbance

at 253.7nm. Detecting limits in the parts-per billion ranges are realized (Skoog *et al* 2000)

Neutron Activation Analysis (NAA)

Neutron Activation Analysis (NAA) is a quantitative and qualitative method of high efficiency for the precise determination of a number of main-components and trace elements in different types of samples. NAA, based on the nuclear reaction between neutrons and target nuclei, is a useful method for the simultaneous determination of about 25-30 major, minor and trace elements of geological, environmental, and biological samples in ppb-ppm range without or with chemical separation. In NAA, samples are activated by neutrons. During irradiation the naturally occurring stable isotopes of most elements that constitute the rock or mineral samples, biological materials are transformed into radioactive isotopes by neutron capture. Then the activated nucleus decays according to a characteristic half-life; some nuclides emit particles only, but most nuclides emit gamma-quanta, too, with specific energies. The quantity of radioactive nuclides is determined by measuring the intensity of the characteristic gamma-ray lines in the spectra. For these measurements a gamma-ray detector and special electronic equipment are necessary. As the irradiated samples contain radionuclides of different half-lives different isotopes can be determined at various time intervals.

Although the development of analytical techniques has led to the expansion of new methods (ICP-AAS, ICP-MS, etc.), which can also be widely applied in analytical chemistry, NAA is still competitive in many areas. The

indisputable advantage of the method is its sensitivity and accuracy especially in respect of some trace elements. The method is of a multielement character, i.e. it enables the simultaneous determination of many elements without chemical separation. In the case of instrumental determination, the preparation of samples involves only the preparation of representative samples, i.e. pulverization or homogenization in most cases, and this reduces the danger of contamination to a minimum and accelerates the whole analytical process. If the determination of some special elements or groups of elements can be carried out only through chemical separation, it is possible to carry out after irradiation. Thus the pollution caused by the different chemicals will not get activated, the chemical yield can be measured by feeding inactive carriers and the chemical processes can be better controlled. During NAA the neutrons get into interaction with the nucleus, therefore, the chemical composition and crystal structure of the substance under analysis will have an effect on the result only in exceptional cases.

The development of the method has contributed to the elaboration of some very simple and accurate methods of standardization, which lead to a surpassingly accurate analysis.

The widespread application of NAA is hindered, however, by some conditions. Among the different fields of application, the Instrumental Neutron Activation Analysis (INAA) following a reactor irradiation is the most competitive. In view of the increasing protest against nuclear energy, a number of research reactors have been shut down; therefore, the possibilities of irradiation

are limited in many countries. The equipment needed for the analysis is rather expensive and requires special laboratories and a highly qualified staff.

NAA has the following advantages:

(i) Samples for NAA can be liquids, solids or power;

(ii) NAA is non-destructive and, since no pre-chemistry is required, reagent-introduced contaminants are completely avoided;

(iii) NAA is a multi-elemental analytical technique in that many elements can readily be determined simultaneously,

(iv) NAA is sensitive to trace elements.

(v) NAA is also totally unaffected by the presence of organic material in the sample. Organic material is significant matrix problem in many types of conventional chemical methods.

These factors have pushed the detection limits of many elements of interest to very low levels not readily achievable by other analytical techniques.

Occurrence of Cadmium

Cadmium is found in very low concentrations in most rocks, as well as coal and petroleum and always in combination with zinc minerals. Geologic deposits of cadmium can serve as source to groundwater and surface water contamination, especially when in contact with soft, acidic waters. Cadmium is used in electroplating, nickel-cadmium batteries, paint and pigments, and plastic stabilizers. It is introduced into the environment from mining and smelting operations and industrial operations, including electroplating, reprocessing

cadmium scrap, and incineration of cadmium containing plastics. Other cadmium emissions are from combustion of fossil fuel, fertilizer application, and sewage sludge disposal. Cadmium may enter drinking water as a result of corrosion of galvanized pipe. Landfill leachates are also an important source of cadmium in the environment, Shelton (2000); available at http://www.water.rutgers.edu/Fact_Sheets/e214.pdf. assessed 16/08/06).

Uses of Cadmium

Cadmium, its alloys, and its compounds are used in a variety of consumer and industrial materials. The use of cadmium compounds falls into five categories: active electrode materials in nickel-cadmium batteries (70% of total cadmium use); pigments used mainly in plastics, ceramics, and glasses (12%); stabilizers for polyvinyl chloride (PVC) against heat and light (17%); engineering coatings on steel and some nonferrous metals (8%); and components of various specialized alloys (2%) (Elinder, 1992; IARC, 1993; Thornton, 1992; USGS, 1997). Cadmium carbonate and cadmium chloride were used as fungicides for golf courses and lawns, but were banned by US EPA in the late 1980s (Farm Chemicals Handbook 1997). The significance of cadmium chloride as a commercial product is declining; however, it is used in the preparation of cadmium sulfide, in the manufacture of special mirrors, and in dyeing and calico printing (IARC, 1993). Cadmium-based colorants are used mainly in engineering plastics, ceramics, glasses and enamels. Cadmium sulfide and cadmium telluride are primarily used in solar cells and a variety of electronic devices which depend

on cadmium's semi-conducting properties (IARC, 1993; OECD, 1994). The photoconductive and electroluminescent properties of cadmium sulfide have been applied in manufacturing a variety of consumer goods (IARC, 1993). Though cadmium metal consumption for batteries has grown steadily since the 1980s other uses of cadmium began declining in the mid 1990s. Pigment, stabilizer, coating, and alloy markets have peaked in cadmium consumption (USGS, 1997). Excessive exports from Bulgaria and Russia in 1997 caused a drop in the average price of cadmium from $1.84 per pound in 1995 to $0.51 per pound in 1997. Also, Ni-Cd batteries have been replaced in some markets by lithium-ion and nickel metal hydride batteries (USGS, 1997). Regulations by local authorities have forced the recycling of cadmium in Ni-Cd batteries, further depressing the demand for primary cadmium metal (USGS, 1999).

Disposal of Cadmium

Incineration of municipal wastes, particularly from older, poorly controlled facilities, is a potential environmental source of cadmium. In modern incineration plants, about 99.9% of cadmium was captured in boilers and control equipment (OECD, 1994).

A range of physicochemical processes is available for treatment of cadmium in liquid waste process streams. These include ion exchange, electrolysis, cementation, and adsorption. Both ion exchange and sulfide precipitation are used as alternate processes aimed at achieving low cadmium residuals in liquid wastes (UN, 1985). Combining processes, for example,

conducting the primary precipitation of cadmium as hydroxide followed by secondary precipitation of residual cadmium as sulfide, has also been adopted. The more general application of the sulfide precipitation technique, however, is constrained due to a tendency for formation of colloidal precipitate, the toxicity and odor of hydrogen sulfide, and the necessity to oxidize residual sulfide occurring in emissions prior to discharge (UN, 1985).

The most widely used treatment process involves the alkaline precipitation of cadmium as hydroxide or basic salts (UN, 1985). Removal of specific metal species during hydroxide precipitation is pH-dependent, and some components of the waste stream can influence the solubility of cadmium hydroxide. After filtration, the sludge formed from the conversion of soluble cadmium compounds to insoluble compounds can be deposited in a suitable landfill (UN, 1985).

Various cadmium-bearing wastes are subject to aggressive leaching in refuse media, particularly under aerobic conditions (UN, 1985). While liquid wastes are banned from land disposal, the leaching tendency is accentuated in the presence of brine solutions. Also, the mobility of cadmium in landfill conditions could be enhanced in the presence of mineral acids, which tend to solubilize cadmium compounds, or amine containing materials, which tend to complex cadmium ions. Waste containing mineral acids, cyanides, organic solvents, and amine-type substances should not be land-filled near cadmium-bearing wastes (UN, 1985).

According to the data compiled in the TRI (TRI961998), in 1996, about 3,100 pounds of cadmium were sent to publicly owned treatment works

(POTWs). The data regarding manufacturing and processing facilities which reported releases to the environment indicate that 1,000 metric tons of cadmium-bearing wastes were transferred off-site, presumably for disposal or recovery (TRI96, 1998).

As an alternative to land disposal, scrap metals and batteries containing cadmium may be recycled (HSDB, 1994; UN, 1985). In the laboratory, a recommended method for recovering cadmium from small quantities of cadmium oxide wastes uses a minimum amount of concentrated nitric acid to form nitrates. The solution is evaporated in a hood to form a thin paste, then diluted with water and saturated with hydrogen sulfide. After the filtration, the precipitate is washed, dried, and returned to the supplier (UN, 1985). No information was located regarding the quantity of cadmium currently being recycled in the United States.

Human Intake of Cadmium

Ingestion

Much of the cadmium which enters the body by ingestion comes from terrestrial foods. This is to say, from plants grown in soil or meat from animals which have ingested plants grown in soil. Thus, directly or indirectly, it is the cadmium present in the soil and the transfer of this cadmium to food plants together with the cadmium deposited out of the atmosphere on edible plant parts which establish the vast majority of human cadmium intake. Some have estimated that 98% of the ingested cadmium comes from terrestrial foods, while only 1%

comes from aquatic foods such as fish and shellfish, and 1% arises from cadmium in drinking water (Van-Assche, 1998).

The cadmium content of terrestrial foods varies significantly as a function of the type of food crop grown, the agricultural practices pursued, and the atmospheric deposition of cadmium onto exposed plant parts. Cadmium levels in the soil principally derived from natural sources, phosphate fertilisers and sewage sludge will naturally impact upon this cadmium uptake. However, this effect is secondary to the type of crop grown and the agricultural practices followed with respect to tillage and crop rotation.

Many studies have attempted to establish the average daily cadmium intake resulting from foods. In general, these studies show that the average daily diet for non-smokers living in uncontaminated areas is at present at the low end of the range of 10 to 25 μg of cadmium per kg body weight (Elinder, 1985; OECD, 1994; ATSDR, 1997).

The World Health Organisation (WHO) has established a provisional tolerable weekly intake (PTWI) for cadmium at 7 μg/kg of body weight. This PTWI weekly value corresponds to a daily tolerable intake level of 70 μg of cadmium for the average 70-kg man and 60 μg of cadmium per day for the average 60-kg woman. Clearly, the daily cadmium intake for the general population from food, which is by far the dominant source of cadmium, is well below the guidelines established by the World Health Organisation. The average daily cadmium intake for the general population in the Western World has shown a distinct downward trend from 1970 through 1992 (Van-Assche and Ciarletta

1992), a reduction presumed to be due to the marked decreases in direct atmospheric deposition of cadmium onto crops and soils. Other studies have suggested that, over the timeframe of 1980 - 1985, levels of cadmium intake have been relatively constant (OECD, 1994). At an absorption rate of 5% from ingestion, the average person is believed to retain about 0.5 to 1.0 μg of cadmium per day from food.

There is considerable information in the literature regarding the cadmium contents of foods grown in contaminated areas (Elinder, 1985; WHO, 1992; OECD, 1994). Detailed studies have indicated that only a small percentage of these contaminated areas were actually utilised for growing foods which were subsequently consumed with the exception of rice fields in Japan where considerable cadmium did find its way into the average person's diet through rice grown on contaminated rice fields (Elinder, 1985). In specific cases, management measures to reduce the transfer of cadmium from historically contaminated soils into the local food chain have proven successful (Staessen *et al.*, 1991).

Inhalation

Cadmium inhalation is a far smaller contributor to total cadmium body burden except, as previously noted, in the cases of smokers or some highly exposed workers of the past. Today, the inhalation route is well controlled in the occupational setting, and is well-controlled from point sources such as those which directly pertain to the non-ferrous, cadmium or cadmium products industries. Ambient air emissions from fossil fuel power generation plants, the

iron and steel industry and other major industries where cadmium may be present as a low concentration impurity, on the other hand, may be substantial because the volumes of the waste gases generated are substantial.

Cadmium Intake From Cigarette Smoking - Smokers absorb amounts of cadmium comparable to those from food, about 1 to 3 µg of cadmium per day, from the smoking of cigarettes. It has been reported that one cigarette contains about 1 - 2 µg of cadmium and that about 10% of the cadmium content is inhaled when the cigarette is smoked (WHO, 1992). In cigarette construction, the use of filters and variations in the cadmium contents of tobaccos could decrease cadmium exposure by this route, but in general cigarette smoking is habits which can more than double the average person's daily cadmium intake. Cigarette smokers who are also occupationally exposed may increase their total cadmium intake even further.

Cadmium Intake From Occupational Exposure - Up to the 1960s, very elevated cadmium in air exposure levels were measured in some workplaces, sometimes as high as 1 mg/m^3. Since that time, workplace exposures and standards have decreased markedly so that most occupational exposure standards today are in the range from 2 to 50µg/m^3. The result has been that occupational exposures today are generally below 5 µg/m^3, and most cadmium workers are exposed at levels which are considered to be safe (ATSDR, 1997). Considering present levels of occupational exposure cadmium intake, general dietary intake, and cigarette smoking intake, it still would appear, however, that the average

daily cadmium intake is well below the values recommended by the World Health Organisation.

Biochemical Effects of Cadmium

Growing plants acquire Zn and they also take up and concentrated Cd with the same biochemical setup. The outbreak of Cd poisoning occurred in Japan in the form of itai or "Ouch ouch" disease. Many people suffered from this disease in which their bones become fragile. Acute and chronic exposure to cadmium in animals and humans causes kidney problems such as kidney dysfunction, hypertension, anemia, marrow disorders, and liver damage (De, 2000).

The major portion of Cd ingested into our body is trapped in the kidneys and eliminated. A small fraction is bound most effectively by the body proteins, *metallothionein*, present in the kidneys, while the excess stored in the body gradually accumulates with age. When excessive amount of Cd^{2+} are ingested, it replaces Zn^{2+} a key enzymatic sites, causing metabolic disorders. (De, 2000).

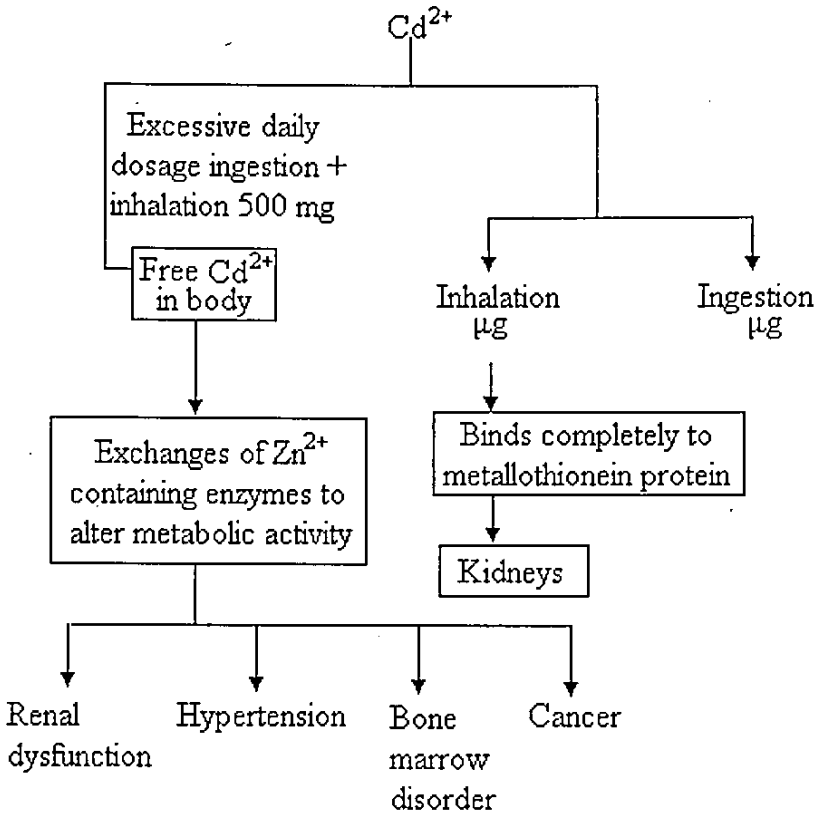


Fig. 2 Metabolism of cadmium

Documented Methods for Determination of Cadmium in Environmental Samples

Analysis for cadmium in environmental samples is usually accomplished by AAS or AES techniques, with samples prepared by digestion with nitric acid (APHA 1989; EPA 1982b, 1983a, 1983b, 1986b, 1986d, 1986e). Since cadmium in air is usually associated with particulate matter, standard methods involve collection of air samples on glass fiber or membrane filters, acid extraction of the filters, and analysis by AAS (APHA 1977; NIOSH 1984b). Adsorptive cathodic stripping voltametry (ACSV) (Nimmo and Fones 1994), differential pulse anodic stripping voltametry (DP-ASV) (Nam *et al.* 1994), and epithermal neutron

activation analysis (NAA) (Landsberger and Wu, 1993) have also been used for air analysis.

The accuracy of the analysis of cadmium in acid digested atmospheric samples, measured by ACSV, was evaluated and compared with graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma mass spectrometry (ICP-MS). The ASCV limit of detection for cadmium was 0.6 ng/mL, higher than that of GFAAS at 0.3 ng/mL but lower than that of ICP-MS for a l-minute collection period. ACSV has advantages for analysis of low concentrations of cadmium in aerosol acid digest samples (Nimmo and Fones 1994).

Three methods standardized by US EPA (1982b, 1983a, 1983b) are generally used for measuring concentrations of cadmium in water. The American Public Health Association (APHA) recommends similar methods for water: AAS/direct aspiration, AAS/graphite furnace technique, and inductively coupled plasma (ICP). In addition, the APHA describes a calorimetric method using dithizone (APHA, 1989). The graphite furnace AAS technique has greater sensitivity than the direct aspiration AAS and ICP techniques for cadmium. Techniques to compensate for chemical and matrix interferences in all three methods are described by APHA (1989) and EPA (1982b, 1983a, 1983b). Water analyzed by acid digestion and measured by the AAS/direct aspiration, AAS/furnace techniques or ICP/atomic emission method resulted in recoveries ranging from 90 to 110% (EPA 1982b, 1983a, 1983b). After soils and solid wastes are extracted or solubilised by acid digestion, they may be analyzed for

cadmium by the same AAS methods that are used for water (EPA 1986d, 1986e). Water can also be analyzed for cadmium by NAA methods (Saleh *et al.* 1993), potentiometric stripping analysis (PSA) methods (Ostapczuk, 1993) and anodic stripping voltametry (ASV) (Daih and Huang, 1992). Sediment and soil samples have been analyzed for cadmium using the methods of laser-excited atomic fluorescence spectroscopy in a graphite furnace (LEAFS) (Zhau *et al.* 1998), GFAAS (Klemm and Bombach, 1995), and ETAAS (Das and Chakraborty, 1997). Preparation of the samples is generally accomplished by treatment with HCl and HNO_3. The most common method for analysis of cadmium in foods is AAS (Bruhn and Franke, 1976; Dabeka, 1979; Muys, 1984), with GFAAS (ETAAS) being one of the most common AAS methods used (Cabrera *et al.*, 1995; Yang *et al.*, 1995; Zhang *et al.*, 1997). Electrothermal vaporization isotope dilution inductively coupled plasma mass spectrometry (ETV-ID-ICP-MS) has been utilized for the analysis of fish samples (Li and Jiang, 1998). Radiochemical neutron activation analysis (RNAA) (Greenberg *et al.*, 1979; Dermelj *et al.* 1996), differential pulse anodic stripping voltametry (ASV) (Satzger *et a*l., 1982, 1984), and the calorimetric dithizone method (AOAC, 1984) may also be employed. The AAS techniques appear to be

most sensitive, with recoveries ranging from 94 to 109% (Bruhn and Franke 1976; Muys, 1984). A method used to isolate cadmium by first extracting with bismuth diethyldithiocarbamate (Bi[DDC],) and then with zinc diethyldithiocarbamate ($Zn[DDC]_2$) in chloroform and then measuring by RNAA showed 94-106% recovery (Greenberg *et al.*, 1979).

Occurrence and Application of Mercury

As a naturally occurring, chemically active element, mercury is pervasive in both environmental media and biota. As it cycles between the atmosphere, land, and water (figure 2.2), mercury undergoes a series of complex chemical and physical transformations, many of which are not completely understood. However, scientists do agree that mercury's cycle in the environment is a result of both natural and human (anthropogenic) activities. In general, current scientific understanding of the relative contribution of mercury from anthropogenic sources is limited due to uncertainties regarding the level of natural emissions as well as the amount and original source of mercury that is re-emitted to the atmosphere from soils, watersheds, and ocean waters (US EPA 1997; Commonwealth of Massachusetts Department of Environmental Protection, 1996).

Elemental mercury is a silver-white, heavy, mobile, liquid metal at ambient temperatures. Virtually insoluble in water, elemental mercury is a volatile metal with fairly low viscosity and high surface tension. The vaporization rate of elemental mercury approximately doubles for every 10 degrees centigrade increase in temperature and its saturation level in air increases logarithmically with increasing temperature (Commonwealth of Massachusetts Department of Environmental Protection, 1996). Other forms of mercury such as mercuric acetate and mercuric chloride are white, heavy powders or crystal solids, which are more soluble in water and have higher vapour pressures depending on the compound (Commonwealth of Massachusetts Department of Environmental Protection, 1996; Perry and Chilton, 1973).

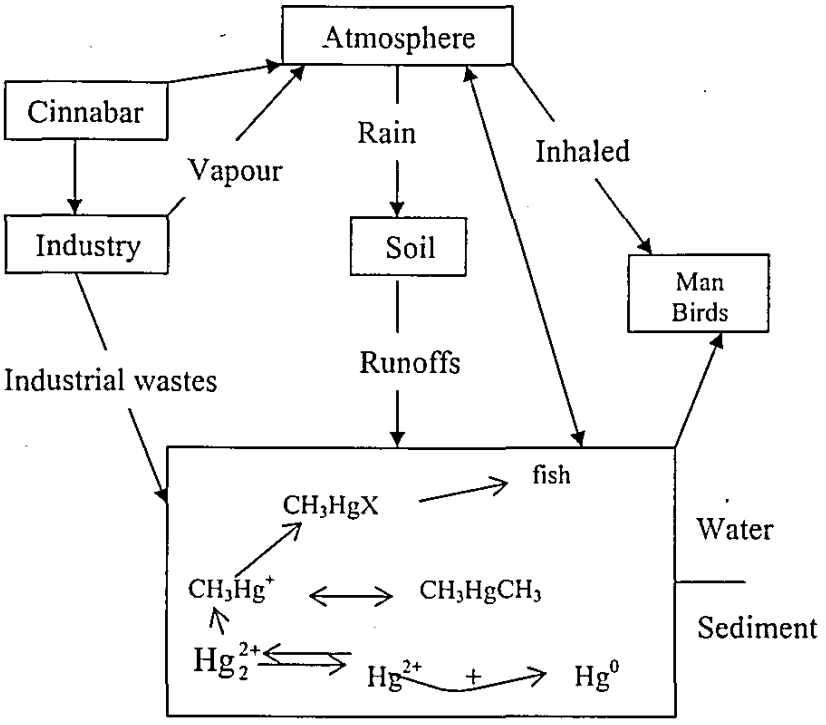


Fig. 3 Mercury cycle in the environment

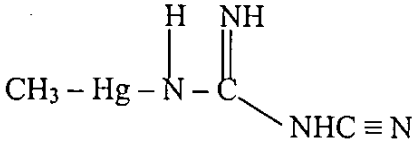
In nature, mercury occurs as a trace component of many minerals, continental rocks containing an average of about 80 parts per billion of mercury. The principal ore is Cinnabar, HgS. It is also found in soils, rivers, seas, lakes, oceans, and atmosphere and in tissues of living organisms. Fossil fuels, coal and lignite contain about 100 parts per billion of mercury. The natural abundance in soil is 0.1 parts per million. Sewage effluent sometimes contains up to 10 times the level of Hg in natural water (0.001-0.0001ppm), (De, 2000).

Mercury finds a wide variety of applications. The largest consumer is the chlor–alkali industry which manufactures Cl_2 and NaOH by an electrolytic process using Hg electrodes. The second largest consumption of mercury is in the

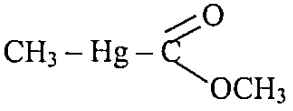
production of electrical apparatus, e.g. Hg vapours lamp, electrical switches, Hg batteries etc. The third largest consumer is the agricultural industry using large number of fungicides for seed dressings.

Some typical compounds of this category are:

$CH_3 - Hg - C \equiv N$ methylmercurynitrile



Methylmercurydicyandiamide



Methylmercuryacetate

$C_2H_5 - Hg - Cl$ Ethylmercurychloride

The impact of seed dressing is enormous since it is applied to a large volume of seed, which is subsequently sowed over millions of acres, thereby causing a widespread dispersal of mercury compounds. Furthermore, mercury undergoes translocation in plants and animals and then finds its way into the human food chain.

Once mercury is absorbed on sediments of water bodies and streams, it is slowly released into the water and constitutes a reservoir which is likely to cause chronic pollution long after the original source of mercury is removed. Natural

addition of mercury to the oceans is about 5000 tonnes per annum, and a further 5000 tonnes is added via human activities. (De, 2000)

Atmospheric Mercury

In the atmosphere mercury may occur as vapour or may be adsorbed on dust particles. Elemental mercury is the most common form of mercury found in the atmosphere whereas in all other environmental media, mercury is found in the form of inorganic mercuric salts and organomercury compounds (US EPA 1997; Commonwealth of Massachusetts Department of Environmental Protection, 1996). In soils and surface waters, predominately the mercuric and mercurous states of mercury exist as ions with varying solubility. Since mercurous mercury is rarely stable under ordinary environmental conditions, mercuric chloride, a simple salt, is the predominant form in many surface water bodies (US EPA 1997; Commonwealth of Massachusetts Department of Environmental Protection, 1996). This is due to the differences in physical and chemical properties between the various forms of mercury.

The Table of Chemical-Physical state of mercury and some characteristics which are important to atmospheric transportation is shown in table 1 below:

Table 1

The Chemical-Physical State and Some Characteristics of Mercury

Type of Mercury	Residence Time in Atmosphere	Transport Distance	Method for Deposit
Elemental mercury vapour	One year	Global	Wet precipitation
Mercury in the atmospheric particulates	Hours to years, depending on particle size	Local, regional and global	Dry and wet precipitation
Divalent mercury	Days	Regional	Dry and wet precipitation

The global atmospheric mercury from anthropogenic sources is estimated between 40 to 75% of the total atmospheric mercury content. This is a 2-4 times increase in atmospheric mercury. The long residence time in the atmosphere results in mercury vapour and some mercury particles being distributed globally. Divalent mercury and majority of particulate mercury precipitate out of the atmosphere and deposits close to the release source. Thus, mercury air emissions haves have both a local deposition effect and a global distribution dependent on the type of mercury being released (available at http://enhs.umn.edu/hazards/hazardssite/mercury/mercdose.html. assessed 12/11/2006).

Aquatic Mercury

Mercury in water exists in two basic forms; the inorganic salt and organic mercury compounds (methyl mercury or dimethyl mercury). Methyl mercury is somewhat soluble in water and is readily absorbed by biota and can be passed up the aquatic food chain, resulting in bio-concentration of mercury in the predator species. Dimethyl mercury, another organic mercury compound, is much less soluble (Commonwealth of Massachusetts Department of Environmental Protection, 1996; Perry and Chilton, 1973). Inorganic mercury can be methylated by microorganisms indigenous to soils, sediments, fresh water, and salt water, to form organic mercury. Invariably, microbial processes can also lead to demethylation, where methyl mercury is reduced back to elemental mercury. However, elemental mercury that has been reduced back from methyl mercury is believed to volatilize into the atmosphere (US EPA 1997; Commonwealth of Massachusetts Department of Environmental Protection, 1996).

Although most of the mercury in aquatic systems is in the inorganic form, greater than 95% of the mercury accumulated by fish is in the form of methyl mercury (US EPA 1997). The high affinity of methyl mercury for sulfhydryl groups of proteins causes rapid absorption in living organisms. The elimination of methyl mercury by fish is very slow relative to the rate of uptake, allowing it to accumulate (Laarman *et al.*, 1975; McKim *et al*, 1976). Biological retention of methyl mercury in pike has been shown to be in the order of several years (Olsson, 1976). Inorganic mercury, while absorbed almost as readily as methyl mercury, is depurated at a much faster rate (Hidebrand, *et al*, 1980). Therefore,

almost all of the mercury found in animal tissues is in the form of methyl mercury (US EPA 1997; Commonwealth of Massachusetts Department of Environmental Protection, 1996).

Biochemical Effects of Mercury

Minamata Incident – Mercury is a well – known toxic metal which came to limelight only after the incidence of Minamata disease in 1953 – 60 in Japan. At Minamata Bay in Japan more than 100 people lost their lives and many thousands were permanently paralyzed from eating mercury contaminated fish. In a particular village facing the bay (population 1100) 15% of the villagers were either killed or permanently crippled. Genetic defects are observed in some 50 babies whose mothers had consumed the contaminated fish from Bay. The sea fish in the Bay were found to contain 27 – 102 ppm of Hg in the form of methyl mercury. The source of Hg was the effluent discharged into the Bay from a vinyl chloride plant, Minamata Chemical Company. The Hg was deposited on the bottom of the Bay and remained there since 1950s.

In the long history of water pollution, the Minamata incident was unique. The mystery of the existence of methyl mercury in sea fish was baffling at first since the source was inorganic mercury compounds discharged into the Bay by the Minamata Chemical Plants. The missing link between inorganic mercury in Bay water and methyl mercury in sea fish was bridged only after extensive research since the 1950's. This is the first known case where the natural

bioaccumulation (in fish) of a toxic material (methyl mercury) killed hundred people and genetically damaged a large population (De, 2000).

The fate of Hg was traced by measuring some 300 Hg concentrations in the surface sediments in Yatsushiro Sea (outside the Bay) during 1970 – 85. Twenty four sampling stations were set up to collect samples at the same location every year. The dispersion of Hg from the Bay was documented: on the average, 3.7 tonnes of Hg was transported outside from the Bay every year. The total Hg concentration in the Bay water ranged from 125 ng L^{-1} (0.125 ppb) at the centre of the Bay to 22 ng L^{-1} (0.022 ppb) near the exit to Yatsushiro sea. The amount of organic Hg ranged from 1.8 to 5.5 ng L^{-1} in the Bay water while the total Hg in Yatsushiro sea water was 16 – 25 ng L^{-1} (25 – 40 kilometres from effluent point) in 1985. A decontamination project ($400 million project) in 1984 dredged about one million cubic metres of contaminated sediment having Hg greater than 25ppm and dramatically decreased the flow of Hg from the Bay to the sea and protected the environment of the Tasushior sea for years to come. The clean – up operation was evident from decrease in Hg concentration in Bay and sea water in 1985 which were considered reasonable for inland waters (De, 2000).

The Minamata incident was followed by a more tragic report of Hg poisoning from Iraq in 1972 where over 450 villagers died after eating wheat which had been dusted with mercury-containing pesticide. These two tragic events boosted the awareness of Hg as a pollutant so that it was studied more extensively than any other toxic element (De, 2000).

Toxic Effects of Mercury

Mercury has been responsible for a number of poisoning calamities in the world. The most notable examples of mercury poisoning from industrial sources occurred in Minamata Bay in Japan (1953-1950) and Niigata Prefect also in Japan. Mercury compounds in fish caused the death of 41 people near Minamata Bay in Japan. Similar poisoning of subpopulations has occurred in Canada, where subsistence Indian populations consumed fish from contaminated waters. In Iraq (1971-72), where mass poisonings occurred from the consumption of wheat treated with mercurial fungicide, over 6000 people were hospitalized and over 450 deaths occurred, largely due to central nervous system (CNS) failure. In all episodes, neurotoxicity was the most common adverse health effect noted Other cases of mercury poisoning were Pakistan, Guatamala, Sweden in 1960, America and Canada in 1970 (Klein and Goldberg, 1970; Nadakavukaren, 2000).

Mercury is known to be very toxic to fetuses of mothers exposed to methyl mercury in pregnancy. Archived umbilical cord blood from 151 placentas (saved by women due to Japanese tradition) were correlated with the methyl mercury exposures and showed a dose-response effect between the concentration of methyl mercury and the degree of neurological damage diagnosed in offspring (Nadakavukareen, 2000; Harada *et al.*, 1999). Both organic and iorganic mercury accumulates in the heart and has been associated with elevated blood pressure and abnormal heart rhythms such as, tachycardia and ventricular heart rhythms (NAS, 2000).

Mercury can exist in three oxidation states: metallic or elemental (Hg^0); mercurous (Hg_2^{2+}); and mercuric (Hg^{2+}). Mercury's properties and chemical behaviour strongly depend on its oxidation state. Mercury can form ionic bonds with inorganic compounds as well as many stable complexes with carbon-containing, or organic, compounds. Depending on the chemical form and the dose received, mercury can be toxic to both humans and wildlife. In people, toxic doses of mercury can cause developmental effects in the foetus, as well as effects on the kidney and the nervous system in children and adults (US EPA 1997; Code of Federal 1999).

The toxicity of Hg depends on its chemical species as shown in table 3

Table 3

Chemical Species of Mercury

Species	Chemical and biochemical properties
Hg	Elementary mercury: Relatively inert and non-toxic; vapour highly toxic when inhaled.
Hg_2^{2+}	Mercurous ion: Insoluble as chloride: low toxicity.
Hg^{2+}	Mercuric ion: Toxic but not easily transported across biological membranes
RHg^+	Organomercurials: Highly toxic, particularly CH_3Hg^+ (methyl mercury); causes irreversible nerve and brain damage; easily transported across biological membranes; stored in fat tissue.

R_2Hg	Diorganomercurials: Low toxicity but can be converted to RHg^+ in acidic medium.
HgS	Mercuric sulfide: Highly insoluble and non – toxic; trapped in soil in this form.

Elemental mercury is fairly inert and non–toxic. If swallowed, it is excreted without serious damage. It has a fairly high vapour pressure and so the vapour, if inhaled, is quite toxic. Hence Hg should be handled only in well-ventilated areas and spills should be cleaned up as a quickly as possible. Hg vapour, when inhaled, enters the brain through the blood stream, leading to sever damage of the central nervous system.

Hg_2^{2+} forms an insoluble chloride with chloride ions. As our stomach contains a fairly high concentration of chloride, Hg_2^{2+} is not toxic. Hg^{2+} (mercuric ion) however, is fairly toxic. Because of its high affinity for Sulphur atoms, it easily attaches itself to the sulphur-containing amino acids of proteins. It also forms bonds with haemoglobin and serum albumin, both of which contain sulphydryl groups. This ion, however, does not travel across biological membranes and hence does not get access into biological cells.

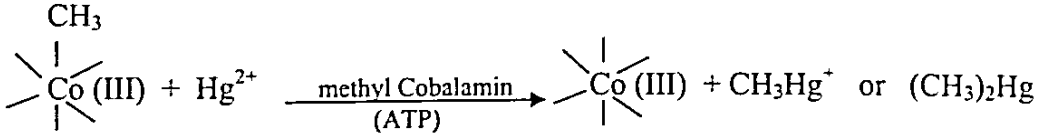
The most toxic species are the organomercurials, particularly CH_3Hg^+ (methyl mercury) which are soluble in fat, the lipid fraction on membranes and brain tissue. The covalent Hg–C bond is not easily disrupted and the alkyl mercury is retained in cells for prolonged periods of time. The most dangerous

aspect is the ability of RHg^+ (organomercurials) to move through the placental barrier and enter foetal tissues.

Attachment of Hg to cell membranes is likely to inhibit active transport of sugar across the membranes. In case of brain cells, this will result in energy deficiency in the cell and disorders in the transmission of nerve impulses. This will explain why babies born to mothers subjected to methyl mercury poisoning suffer from irreversible damage to the central nervous system, including cerebral palsy, mental retardation and convulsions. Methyl mercury poisoning also leads to segregation of chromosomes, symptoms of mercury poisoning set in at blood levels of 0.5 ppm of CH_3Hg^+ (De, 2000).

Biological Methylation: Amplification in Food Chain

The Minamata Chemical Company discharged elemental mercury (Hg) into Minamata Bay, but the fish in the Bay were found to contain CH_3Hg^+. This missing link was filled up by subsequent research. Hg or its slats can be converted to methyl mercury by anaerobic methane – synthesizing bacteria in water. This conversion is facilitated by Co(III) – coenzyme which transform methyl cobalamin to Hg^{2+}, yielding CH_3Hg^+ or $(CH_3)_2Hg$, enzymatically:



An acidic medium promotes the conversion of dimethyl mercury to methyl mercury which is soluble in water. It is methyl mercury which enters the food

chain through plankton, and is concentrated by fish by a factor 10^3 or more as it passes up the food chain, as shown in fig. 4

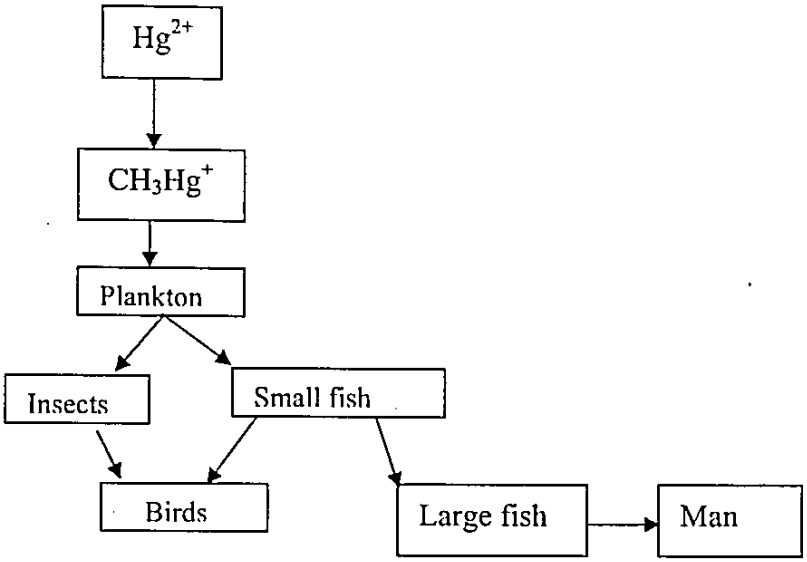


Fig. 4 Mechanism of mercury propagation

Bioavailability of methyl mercury is determined by a combination of physicochemical characteristics of the aquatic system, the amount and rate of contamination, and the biological structure and function within the system (Boudou *et al*, 1991). However, methyl mercury is one of the most highly toxic forms of mercury and is also the one most easily accumulated in the aquatic food chain. Therefore, humans and wildlife–such as bald eagles, kingfishers, otter and mink–that feed on fish are particularly at risk because of the potential for methyl mercury to bio accumulate in freshwater fish (US EPA 1997; Commonwealth of Massachusetts Department of Environmental Protection, 1996).

The mercury concentration builds up at each level of the food chain. This is valid even in uncontaminated waters. Mercury has always been part of our environment and mercury cycles existed long before any industry developed. Large fish of ancient ages, preserved in some museums, have been found to contain significant level of mercury. However, mercury pollution considerably enhances the mercury concentration in each level of the food chain.

Soon after the Minamata disaster, it was reported that fresh – water fish from Erie and St. Chair showed high levels of Hg (0.1 – 3.5ppm) in the form of methyl mercury in their living tissues. As a result, important commercial fisheries in those areas were closed down.

Absorption of Mercury Species

Studies have shown that 75-85% of an inhaled dose of the elemental mercury vapor is absorbed by the body (human studies) and 97% of absorption occurs through the lungs. Orally, less than 0.01% of an ingested dose is absorbed from the gastro intestinal (GI) tract (rat study). Dermal absorption rate is 0.024 ng Hg/cm^2 skin for every 1 mg/m^3 in the air (human study), while less than 3% of total amount of elemental Hg absorbed by the body is from dermal exposure. (Clarkson, Thomas W., 2002; US EPA, 1997; NAS, 2000)

Data on inhalation of ionic mercury are limited however absorption via inhalation is estimated at 40% (dog study). Absorption from the GI tract following oral dose is estimated at 7-15% (human study). A mouse study indicates that absorption is approximately 20%. In the case of dermal studies

approximately 2-3% of a dermally applied dose of mercuric chloride was absorbed during a 5-hour period (guinea pig study). (Clarkson, Thomas W. 2002; US EPA, 1997; NAS, 2000)

Methyl mercury vapors can be absorbed, but the amount is unknown. However in the case of oral dose an approximately 95% of methyl mercury in fish is absorbed from the GI tract (human studies). The exact site of absorption is unknown.

Distribution of Mercury Species in the Human Body

Absorption of elemental mercury in lungs results in rapid diffusion across the lungs and entrance into the bloodstream, where it is distributed throughout the body (because it is lipophilic), including the blood-brain barrier and the placenta.

For ionic mercury the ingested dose is rapidly distributed from the GI tract to the blood and organs. Mercuric mercury has a high affinity for sulfhydryl groups in the RBCs and plasma. The highest concentration is in the kidneys. Mercuric mercury induces metallothionein production in the kidneys, which may contribute to the kidney's accumulation of mercuric mercury. It does not readily cross the blood-brain barrier or the placenta because of its ionic charge.

The percentage of absorbed methyl mercury from the GI tract that is distributed to the blood ranges from 1% to 10%. About 5% is absorbed into the bloodstream and is distributed to all tissues within a few days. The concentration in RBCs is roughly 20 times the concentration in plasma. Maximum levels

(approximately 10%) occur in the brain, in 5-6 days. It is also readily transferred to the fetus and the fetal brain. The high mobility of methyl mercury in the body is not due to lipid solubility. It is present in the body as water-soluble complexes mainly attached to the sulfur atom of thiol ligands. Methyl mercury transport across the blood-brain barrier occurs via a MeHg-L-cysteine complex, which is transported by the L-system (leucine preferring) amino acid carrier.

Biotransformation of Mercury Species in the Human Body

Elemental mercury is oxidized in the red blood cells by catalyses and hydrogen peroxide to Hg^{2+}. Hg^{2+} is unstable in vivo and has been shown to convert to elemental mercury (rat study). It can also be methylated by intestinal flora, but cannot be methylated in body tissues.

Methyl mercury is stable in the body compared to other species. It is slowly demethylated to Hg^{2+} in tissue macrophages, intestinal flora, and the fetal liver. Although these sites of demethylation are known, the enzymes in mammalian tissues responsible for the biotransformation have not yet been identified. It is metabolized to ionic mercury at a rate of around 1% of the body burden per day. The mercuric mercury resides for long periods of time in the central nervous system (CNS), probably in an inert form.

Elimination of Mercury Species from the Human Body

Approximately 7-14% of inhaled mercury vapor is exhaled within a week after exposure. The rest of the elemental Hg is either excreted via sweat and saliva, or is excreted as mercuric mercury. It is about 80% of the elemental mercury that is excreted as mercuric mercury via faeces and urine. Half-life elimination is approximately 58 days. This is slightly more than 1% of the body burden per day.

For ionic mercury, approximately 85% of an oral dose is excreted via faeces within a of couple days. Most of the absorbed ionic mercury is excreted in urine. Smaller amounts are excreted in saliva, bile, sweat, exhalation, and breast milk. Half-life excretion ranges from 49-96 days.

The major routes of excretion of methyl mercury are bile and feces. It undergoes enterohepatic cycling where it is secreted into bile, and then partly reabsorbed and returned to the liver. Most methyl mercury is eliminated by demethylation and then excretion of the ionic form in the faeces (approximately 90% in faeces as mercuric mercury). This process does not occur in nursing infants due to incomplete development; their process of elimination is not understood. Breast milk is also a route of excretion.

The range of half-life elimination has been estimated at 45-90 days (although much faster for lactating females). Individuals who are exposed regularly to methyl mercury reach a steady-state body burden in about 5 half-lives (approximately 1year).

Documented Methods for Determination of Mercury in Environmental Samples

Mercury levels have been determined in numerous environmental matrices, including air, water (surface water, drinking water, groundwater, sea water, and industrial effluents), soils and sediments, fish and shellfish, foods, pharmaceuticals, and pesticides. The sample preparation varies with the complexity of the matrix, but most complex samples require decomposition of the matrix and reduction of the mercury to its elemental form. For biological samples, special sample preparation methods need to be employed if inorganic and organic mercury are to be determined separately, or if the individual species of the organic mercury fraction are to be determined.

Both CVAAS and CVAFS have been used to monitor air and suspended particulates in air for mercury (Baeyens and Leermakers, 1989; Bloom and Fitzgerald, 1988; Friese et al., 1990; NIOSH, 1994; Paudyn and Van, 1986; Sengar et al., 1990; Stockwell et al., 1991; Temmerman et al., 1990). Both methods are sensitive, accurate, and precise, although slightly greater sensitivity was reported with AFS (low ppt) than with AAS (mid ppt); AFS is becoming a more common method of analysis (Horvat, 1996). When AAS or AFS was combined with gas chromatography (GC), the different mercury species (inorganic mercury, dimethylmercury, diethylmercury, and methylmercury chloride) present in the air could be separated (Bloom and Fitzgerald, 1988; Paudyn and Van, 1986). A colorimetric method, based on the formation of a colored complex formed in the presence of mercury, has been used as a quick and

simple field test that can detect mercury present at the mid-ppb level (Cherian and Gupta, 1990).

Numerous methods, including CVAAS, ASV, inductively coupled plasma (ICP) MS, ICP atomic emission spectrometry (AES), microwave-induced plasma (MIP) AES, NAA, GC/AFS, high performance liquid chromatography (HPLC)/UV, HPLC/ECD, and spectrophotometry, have been used to determine mercury levels in aqueous media. Mercury has been measured in drinking water, surface water, groundwater, snow, waste water effluents, and sea water. Of the available methods, CVAAS is the method of choice (Baxter and Frech, 1989, 1990; Birnie, 1988; Eaton et al., 1995; Goto et al., 1988; Lee et al., 1989; Mateo et al., 1988; Munaf et al., 1991; Paudyn and Van, 1986; Ping and Dasgupta, 1989; Robinson and Schuman, 1989; Schintu et al., 1989; Shkinev et al., 1989) and the method recommended by EPA and AOAC (AOAC, 1984; Beckert et al., 1990; EPA, 1994f, 1994g). This method is very sensitive for mercury in water (sub- to low-ppt) and has been proven to be reliable. Water samples generally do not require digestion, but mercury in the samples is usually reduced to the elemental state and preconcentrated prior to analysis. When combined with GC, CVAAS has been used to separate and determine individual mercury species in aqueous samples (Paudyn and Van, 1986). Spectrophotometry has often been used to determine mercury in aqueous matrices (Abbas et al., 1989; Ajmal et al., 1989; Eaton et al., 1995; Raman and Shinde, 1990; Singh et al., 1989). Sample preparation methods vary and have included separation by thin-layer chromatography (TLC) (Ajmal et al., 1989) or column chromatography (Yan et

al., 1989), selective extraction (Abbas et al. 1989), and ligand formation (Raman and Shinde, 1990; Singh et al., 1989). While recoveries were good, spectrophotometry is not as sensitive a technique as CVAAS. Tests of additional methods, including ASV (Liu et al., 1990), ICP/MS (Haraldsson et al., 1989), NAA (Itawi et al., 1990), AES-based techniques (Kitagawa and Nishimoto, 1989; Mahanti, 1990; Nakahara et al., 1988), HPLC based techniques (Evans and McKee, 1988; Shofstahl and Hardy, 1990), and graphite-furnace (GF) AAS (LeBihan and Cabon, 1990) indicate that these methods may also be useful for determining mercury in water samples. One of the most promising methods is GC/AFS, which has the advantages of increased sensitivity and precision compared to CVAAS and can also be used to isolate individual mercury species (Bloom, 1989). A colorimetric assay has also been developed that is useful for rapid preliminary screening of field samples (Cherian and Gupta, 1990).

CVAAS is the most commonly used technique for determining the mercury concentration of sediments, soils, and sludge (Bandyopadhyay and Das, 1989; Beckert et al., 1990; EPA 1994g; Van Delft and Vos, 1988). As with other matrices, it is sensitive, reliable, and requires little sample preparation beyond digestion of the matrix and reduction of the mercury to its elemental form. It is the method recommended by EPA for solid matrices (Beckert et al., 1990; EPA 1994g). A method based on CVAFS that uses flow injection analysis with on-line microwave digestion for the determination of total mercury has been described recently (Morales-Rubio et al., 1995). Good sensitivity (90 ppt) and precision (4% RSD) was demonstrated. Gas chromatography in conjunction with atomic

emission detection (GC/AED) has been used to determine organomercury species in soils and sediments (Liu et al., 1994). Direct current ASV (DCASV) has been tested for use in determining mercury levels in river sediment (Lexa and Stulik, 1989). The accuracy and sensitivity of this method are good, but it is less precise than CVAAS. A field method using XRF has been developed to monitor soil contamination (Grupp et al., 1989). This method is rapid and portable, but its high detection limit (low-ppm) makes it useful only for on-site screening.

Methods have been developed for the determination of mercury in fish, shellfish, foods, food sources, and pharmaceuticals. AAS, usually with cold vapor generation (CVAAS), is one of the primary methods used to measure mercury in these complex matrices (Carrillo et al., 1986; Friese et al., 1990; Landi et al., 1990; Navarro et al., 1992; Odukoya, 1990; Vermeir et al., 1988, 1989), because of its sensitivity and reliability.

Although the sensitivity (sub- to low-ppb), accuracy, and precision are not as good as with less complex gaseous and aqueous media, it is still one of the best methods available for analysis of mercury in any matrix. Flameless AAS without cold vapor generation has also produced good results when used to determine ppb levels of mercury in wine (Cacho and Castells, 1989) and fish (Filippelli, 1987); it is also one of the methods recommended by AOAC for fish and food (AOAC, 1984). When combined with high resolution GC (HRGC), the individual organic mercury species in fish could be determined (Jiang *et al*., 1989). Sub-ppt levels of mercury in powdered milk and oyster tissue were reliably determined using AFS (Vermeir *et al*., 1991a, 1991b). NAA was used to measure mercury levels in

copepod homogenate and tomato leaves, but the sensitivity (mid- to low-ppb) and reliability were not as good as that of CVAAS or AFS (Taskaev *et al.*, 1988; Zhuang *et al.*, 1989). Several other methods, including IDSSMS (Moody and Paulsen, 1988), HPLC/ICP/MS (Bushee, 1988), square-wave voltametry (ASV) (Mannino *et al.*, 1990), ASV (Golimowski and Gustavsson, 1983), MIP/AES (Natajaran, 1988), GC/ECD (Ahmed *et al.*, 1988; AOAC, 1984), and spectrophotometry (Agrawal and Desai, 1985; Marquez *et al.*, 1988) have also been used to analyze fish, plant material, and pharmaceuticals for mercury. HPLC/ICP/MS has the additional advantage of permitting separation and quantitation of individual mercury species (Bushee, 1988). An AOAC-recommended colorimetric method is available for screening food samples (AOAC, 1984). Several other environmental matrices have been analyzed for mercury content. These include coal fly ash (Horvat and Lupsina 1991; Lexa and Stulik, 1989), coal dust (Wankhade and Garg, 1989), minerals (Bichler, 1991), pesticides (Sharma and Singh, 1989), gasoline (Costanzo and Barry, 1988), and oily waste (Campbell and Kanert, 1992). The methods used include CVAAS, DCASV, NAA, spectrophotometry and GC/alternating current plasma detection (ACPD). The data on each method for each matrix were insufficient for making comparisons.

Feeding Habits of Selected Fish Samples

Tilapia (*Tilapia zillii*)

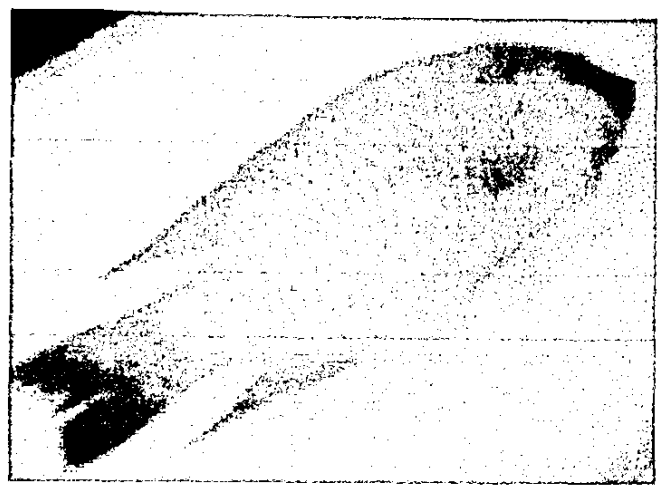


Fig 5 *Tilapia zillii*

Tilapias belong to the family *Cichlidae*, in the order *Perciformes* (Encarta, 2004). They constitute a large group of food fish widely distributed in mainly the tropical and subtropical areas of the world. They are native to tropical freshwaters of Africa, but some commercially important species have been introduced and cultivated in Israel and several Asian countries, including Indonesia, Malaysia, Thailand, and the Philippines (Encarta, 2004). They were introduced into the United States of America for biological vegetation control, in place of chemical and mechanical control methods. They are not carnivorous. (Dupree and Huner, 2005). Most tilapias (including *tilapia zillii*) are obligate herbivores and are anatomically equipped for feeding on attached or matted algae, which they can graze, and on free floating algae. They have minimal ability to consume submergent macrophytes because grazing is limited to very soft material (Noble,

1989). They are herbivores and/or detritivores. They feed on phytoplankton and zooplankton (Zohary et al., 1994; Cailteux, 1988). Whetstone (2002) reported that blue tilapia, stocked at 400 fish/acre and 200 fish/acre in experimental ponds, controlled filamentous algae in one-two months and three months, respectively. Blue tilapia also controlled watermeal *Wolffia spp.* after three months, but did not effectively control duckweed *Lemna valdiviana.* Pierce and Yawn (1965) and Childers and Bennet (1967) reported that the vegetative control of Mozambique tilapia *Tilapia mossambica* decreased substantially in the presence of largemouth bass.

Tilapias feed on a wide variety of food, including insect larvae, various shrimp-like creatures, fish fry, worms, plants, and detritus (Microsoft Encarta Encyclopaedia Standard, 2004). Mallin (1985) described that over 99 percent of the stomach contents in sixty blue tilapia (42-286 mm) were comprised of organic and inorganic detritus. Mallin (1985) also reported that as this species becomes larger, they begin to consume a significantly larger amount of zooplankton and phytoplankton. Due to the wide range of findings, Mallin (1985) probably described blue tilapia most accurately in his study on Lake Julian when he stated that they were found to be opportunistic omnivores. A study by Gu et al. (1997) on carbon and nitrogen isotopes agrees with Mallin (1985), in that blue tilapia consume a wide array of potential food sources due to the broad ranges of ^{13}C and ^{15}N that were found in gut contents. The diet of blue tilapia may also be dependent on the abundance and composition of foods available in different aquatic environments (Gu et al., 1997).

Tilapia zillii, the species used in this research, is the most attractive species. It has the olive-green body with the blue sheen, marked with six to seven dark vertical bars. The throat is red and during breeding this colour intensifies. The dorsal, anal and caudal fins are marked with yellow spots, and the edges of both dorsal and anal fins are usually outlined in orange.

This species feeds entirely on plants and is consequently found mainly in reedy areas. It reaches a maximum length of 270mm (Holden and Reed, 1991).

Mudfish (*Clarias submarginatus*)

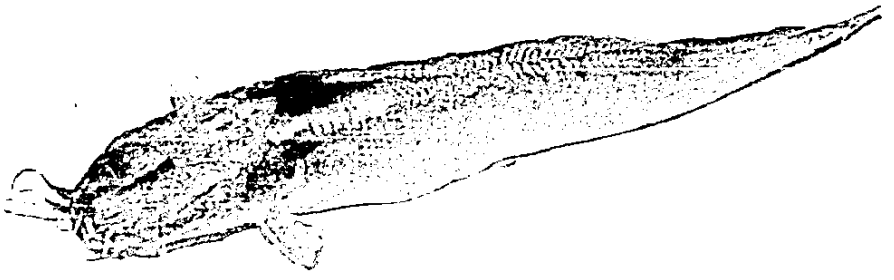


Fig. 6 *Clarias submarginatus*

Mudfish belong to the genus *Clarias* in the family *Clariidae*, our most numerous groups of freshwater fish. Like all *clariidae*, mudfish are elongate fish lacking scales, and have a thick leathery skin with a coating of mucus that protects the fish against infection, and also reduces desiccation when out of water. However, mudfish differ from other *clariidae* in a number of ways. They have

either very reduced or absent pelvic fins and are more eel-like in form. (Nicholas Ling, 2001). Mudfish prefers swamps, sloughs, pools, and backwaters of lowland streams. They are flat headed predators with a strong preference for life fish, and have a voracious appetite. The fish at all sizes are extremely cannibalistic (Dupree and Huner, 2005). About 80 percent of their diet consists of fish, with crayfish being the second most dominant food item. They stalk their prey using their senses of smell and sight (available at http://www.permissiontohunt.com/fishing_desc.asp?Fish_ID=17tm assessed 2/01/07). All species including the Canterbury mudfish are generalized opportunistic carnivores that will eat almost anything of appropriate size. The tiny newly hatched fry, only 5 to 7 mm long, are active by day, feeding in mid-water on any small aquatic zooplankton. After two months, when they have reached a length of about 35 mm, they become nocturnal like the adults and feed mostly on small crustaceans, earthworms, the larvae of aquatic insects, and forage for trapped insects at the water surface. (Ling 2001; also available at www.doc.got.nz/Publications/004~science-and-esearch/miscellaneous/PDF/mudfish.pdf (assessed 16/08/06)

Crab (*Carcinus maenas*)

Fig. 7 *Carcinus maenas*

The European green crab, *Carcinus maenas*, occasionally is not green. The dorsal (top) shell or carapace is mottled, dark brown to dark green in coloration, and has small, yellow patches. Its ventral surface (underside) colour may change from green to orange and then red during the moulting cycle. The most distinctive characteristic is the array of 5 spines on either side of the eyes on the front end of the carapace. The 3 rounded lobes (bumps) between its eyes may also be used to help identify the European green crab. An adult European green crab is typically about 63.5 mm long, but can range up to 100 mm. The last pair of hind walking legs is relatively flat.

The European green crab is a voracious predator that feeds on many types of organisms, particularly bivalve molluscs (e.g., clams, oysters, and mussels), polychaetes, and small crustaceans (available at http://wdfw.wa.gov/fish/ans/

greenerb.htm). The European green crab is capable of learning and can improve its prey-handling skills while foraging.

In its native range, the feeding activity of the European green crab greatly impacts populations of mussels (*Mytilus* spp.), dogwhelks (*Nucella lapillus*), and cockles (*Cerastoderma edule*). In Scotland, the crab acts as an intermediate host of the acanthocephalan worm, *Profilicollis botulus*, which causes heavy mortalities in common eiders (*Somateria mollissima*). Along the east coast of North America, the European green crab preys on quahogs (*Mercenaria mercenaria*), a hard shell clam, and has been implicated in the demise of the Atlantic soft-shell clam fisheries of the 1950s. In Bodega Bay, California, there has been a significant reduction in the populations of native clams (*Transennella* spp.) and a shore crab (*Hemigrapsus oregonensis*) since the arrival of the European green crab in 1993. Furthermore, laboratory studies show that European green crabs readily prey on Dungeness crabs (*Cancer magister*) of equal or smaller size. Dungeness crabs spend part of their juvenile life in the intertidal zone, and may therefore be at risk from European green crab predation.

The European green crab is capable of learning and can improve prey-handling skills while foraging. The crab is quicker, more dexterous, and can open shells in more ways than other species of crabs. Two colour varieties exist: red and green. Red-colour European green crabs prefer larger bivalves and usually dominate green-colour European green crabs in aggressive disputes over prey. The crusher claws (the larger of the two claws) of red European green crabs exert more force, on average, than those of green European green crabs. In Denmark,

the foraging activity of the European green crab is about 20 times higher in summer and fall than in winter and spring. In summer, large numbers of European green crabs move up and down the shore with the tides. European green crabs usually forage during high tide, whereas females are active primarily at night, independent of the tidal phase.

Studies conducted on the European green crab in Europe indicate that when preying on bivalves, feeding rates generally decrease with increasing bivalve size and with decreasing crab size. Oysters are typically "crab-proof" at around 60 mm shell length, whereas mussels are free of predation at around 45 mm shell length. European green crabs, with a 25-75 mm carapace width, are capable of eating three oysters up to 60 mm shell length daily; a relatively low number compared to the nearly three-dozen mussels (up to 45 mm shell length) it is able to eat in the same period. Mussel populations located high in the intertidal zone tend to survive predation better than those lower in the intertidal zone.

Prawns (*Penaeus monodon*)

Fig. 8 *Penaeus monodon*

Prawns belong to the phylum **Crustacea**, class **Malacostraca**, order **Decapoda**. Like all the animals in their phylum, they have a hard shell named exoskeleton covering and protecting the whole body. The shell (or exoskeleton) is replaced during growth, through moulting.

They mature and breed only in tropical marine habitats and spend their larval, juvenile, adolescent and sub-adult stages in coastal estuaries, lagoons or mangrove areas. In the wild, they show marked nocturnal activity, burrowing into bottom substratum during the day and emerging at night to search for food as benthic feeders. Under natural conditions, the giant tiger prawn is more of a predator than an omnivorous scavenger or detritus feeder than other penaeid shrimp. After moulting, the new shell is still soft which causes prawns to become vulnerable and they may subsequently be eaten by their predators or companions. Adults are often found over muddy sand or sandy bottoms at 20-50 m depth in offshore waters.

Prawns at first stage in larval development do not feed but live on their yolk reserve and passes rapidly through various larvae stages they change their habit to feed on benthic detritus, polychaete worms and small crustaceans (available at http://www.fao.org/figs/servlet/static?dom=culturespecies&xml=Penaeu and http://www.edge-of-reef.com/maccruri/macrurien.htm (assessed 2/01/07).

Molluscs Periwinkle (*Gastropod*)

Fig. 9 *Gastropod*

Periwinkles belong to the family *Littorinidae* of the order *Mesogastropoda*. The common periwinkle is classified as *Littorina littorea*, the rough periwinkle as *Littorina saxatilis*. Periwinkles (*gastropod*), also winkle, common name applied to gastropod molluscs are common on seashores around the world. Periwinkles crawl about underwater but usually remain passive when left uncovered by the tide. Many gastropods are herbivores; they feed on microscopic diatoms and other algae. With multi-toothed radulas, they scrap algae from various substrates (rocks). Among the carnivorous, species is the conch, which feeds on smaller molluscs, and the cone shells (*Conus*), which feed on fish and annelid worms that they first paralyze with poison contained in their hollow radula teeth. The poison is also toxic to humans, causing paralysis and sometimes death. Gastropods have a complex nervous system with ganglia.

The edible species produce eggs that develop outside the maternal body, but in the rough periwinkle, a species found nearer the high-water mark, the

young are hatched and have a hard shell before they leave the mother (Encarta, 2004; htt://columbia.thefreedictionary.com/periwinkle; http://en.wikipedi.org/wiki/Periwinkle_(mollusc)).

Theory of Neutron Activation Analysis (NAA)

NAA can be divided into non-destructive and destructive analysis, i.e. Instrumental (INAA) and with radiochemical separation, respectively. Although the detection limits in the latter are usually at least one order of a magnitude lower than in the former, instrumental NAA still plays an important role in the field because no time-consuming separation work is needed. In particular, NAA is ideally suited for the analysis of small sample sizes, such as air-borne particulate mater, which can commonly have weights less than a milligram.

Neutrons used in NAA can be produced in several ways: by a nuclear reactor or a particle accelerator or from artificial isotopes, such as plutonium-beryllium. The most common source is a fission reactor, owing to its high neutron flux.

Usually the reactor neutrons can be obtained by using a filter which is made of material with high neutron absorption for low energy neutrons, such as cadmium and boron, so that virtually all low energy neutrons, known as epithermal neutrons, activate the sample. Fast neutrons from particle accelerators can also induce reactions.

Neutron-Induced Reactions

When a neutron collides with a nucleus, the following reactions may occur: elastic scattering (n, n), inelastic scattering (n, n′), radiative capture (n, γ), charge particle reaction (n, α) or (n, 2n), and fission (n, f). The most useful reaction in NAA is radiative capture, which can be denoted as;

$$ {}^{1}_{0}n + {}^{A}Z \longrightarrow {}^{A+1}Z^{*} \longrightarrow {}^{A+1}Z + \gamma \qquad (1) $$

Where ${}^{A}Z$ is the target nucleus and ${}^{A+1}Z^{*}$ is called the compound nucleus and is usually in an excited state. It can de-excite with the emission of a gamma-ray called a prompt gamma. In normal NAA this gamma-ray is not used in the analysis; instead, delayed gamma-radiation from the product ${}^{A+1}Z$ is employed. This means that this product ${}^{A+1}Z$ is required to be radioactive, emitting at least one gamma-ray photon. It can be detected by a high resolution gamma-ray detector for both energy and intensity. If the product ${}^{A+1}Z$ is a stable isotope it can be detected. For example, although magnesium has in nature three stable isotopes, ${}^{24}Mg$, ${}^{25}Mg$, and ${}^{26}Mg$, only the last one, which has an abundance of only 11%, can be used in neutron activation analysis employing the ${}^{26}Mg$ (n, γ) ${}^{27}Mg$ reaction. Neutron-induced reactions of ${}^{24}Mg$ and ${}^{25}Mg$ give the stable isotopes ${}^{25}Mg$ and ${}^{26}Mg$, respectively.

Since stable isotopes of an element can undergo different reactions depending on the neutron energy, it is possible that the same product ${}^{A+1}Z$ can be produced via different nuclear reactions. For example, ${}^{28}Al$ is produced by the following three reactions:

$$^{27}Al + n \longrightarrow {}^{28}Al + \gamma \qquad (2)$$

$$^{28}Si + n \longrightarrow {}^{28}Al + p \qquad (3)$$

$$^{31}P + n \longrightarrow {}^{28}Al + \alpha \qquad (4)$$

The first reaction (2) is the expected reaction in thermal NAA; (3) and (4) are referred to as primary uncle interference reactions.

Reaction Rate

Neutron capture cross-section is defined as the probability of a radioactive capture reaction occurring in a neutron collision with a nucleus given in terms of an area, which depends on the incident neutron energy. The reaction rate R of a particular element in the sample can be evaluated from:

$$R = N \int_{\substack{\text{w hole} \\ \text{energy} \\ \text{range}}} \sigma(E)\Phi(E)dE \qquad (5)$$

where $\Phi(E)dE$ is the neutron flux of neutrons with kinetic energy between E and $E + dE$, in n $cm^{-2}s^{-1}$, $\sigma(E)$ is the neutron capture cross-section, in cm^2, and N is the number of atoms of the element in the sample.

Equation (5) can be separated into three terms: thermal, epithermal, and fast neutron ranges:

$$R = R_{th} + R_{epi} + R_{fast} \qquad (6)$$

For many nuclides, particularly those of low atomic number, the neutron capture cross-section decreases linearly with increasing velocity of the neutron (known as 1/υ absorbers) for low energies (<1 eV). Thermal neutrons have a

velocity of 2200 m/s corresponding to an energy of 0.025 eV. The thermal reaction rate is:

$$R_{th} = N \int_{thermal} \sigma(E)\Phi(E)dE$$

$$= N\sigma_{th}\Phi_{th} \qquad (7)$$

where Φ_{th} is the thermal neutron flux in n $cm^{-2}s^{-1}$. At medium energies (0.5eV<E<0.5 MeV), the neutron capture cross-section varies rapidly, with resonance peaks. It is assumed that the neutron flux follows a 1/E distribution in the epithermal range. In practice, the neutron radiative capture resonance integral (I_o) is used to refer to the neutron capture cross-section in the medium energy range. The epithermal reaction rate can evaluate by:

$$R_{epi} = N \int_{epithermal} \sigma(E)\Phi(E)dE$$

$$= N \int_{epithermal} \sigma(E)\Phi_{epi}dE/E$$

$$= N\Phi_{epi}\left[\int_{epithermal} \sigma(E)dE/E\right]$$

$$= N\Phi_{epi}\left[\int_{epithermal} \sigma(E)d(\ln E)\right]$$

$$= N\Phi_{epi}I_o \qquad (8)$$

where Φ_{epi} is the epithermal flux per unit ($\ln E$). At high energy, the neutron capture cross-section is usually very small, whereas the cross-sections for other reactions such as (n,p) are dominant and the fast radiative capture reaction rate R_{fast} is negligible. Values for the thermal neutron capture cross-section and the

resonance integral are given by Mughabghab *et al*, (1984) and IAEA (1987). The reaction rate can thus be expressed as:

$$R = N\sigma_{th}\Phi_{th} + NI_o\Phi_{epi} \quad (9)$$

In neutron irradiations, the reaction rate is governed by the two terms shown in equation (9). The first term is usually dominant in thermal irradiations, but in epithermal irradiations this term becomes less important. Some elements which have high ratios of I_o/σ_{th} include antimony, arsenic, barium, cadmium, indium, gallium, molybdenum, selenium, tungsten and uranium. The epithermal technique is often useful for samples containing sodium, since its activity is greatly reduced in epithermal NAA resulting in lower background and better sensitivities. A detailed evaluation of the benefits of epithermal NAA has better made by Rowe and Steinnes (1977) and several other authors have successfully used this method in a wide variety of investigations. Some of these authors are Landberger (1988), Parry (1982), Tobler *et al*, (1990); Dowalti and Jervis(1991); Lavi, *et al* (1989); Zaghloul *et al*, (1987).

Neutron Activation Analysis Calculations

Neutron activation analysis is based on the measurement of characteristics gamma-rays from a radionuclide formed by a specific neutron reaction. The corresponding radioactivity is governed by the usual radioactive decay law. If other factors are considered in the measurement, the final equation would be [7].

$$A = \sigma\Phi(m/M)N_A SDC\,\theta\,P_\gamma\,\varepsilon \quad (10)$$

where A is the measured activity (Bq) from the product of an expected reaction,

σ is the activation cross-section of the reaction (cm^2)

Φ is the activating flux (n $cm^{-2}s^{-1}$),

m is the amount of the element determined (g),

M is the atomic weight of the element to be determined (g/mol),

N_A is the Avogadro constant ($6.022x10^{23}$ molecules/mol),

$S = [1-\exp(-\lambda t_i)]$ is the saturation factor (λ is the decay constant of the radioactive product, t_i is the duration of irradiation),

$D = \exp(-\lambda t_d)$ is the decay factor (t_d is the duration of the decay),

$C = [1-\exp(-\lambda t_c)]$ is the correction factor for nuclide decay during the counting time (t_c is the duration of counting)

θ is the relative natural isotopic abundance of the activated isotope,

P_γ is the probability of emission of a photon with energy, E

ε is the detector efficiency for the measured radiation energy.

The above equation can be greatly simplified by using the comparator method. If equal weights of both sample and standard (with a known concentration of the element of interest) have the same irradiation, decay and counting times, then equation (10) becomes:

$$\dot{C}_{sam} = \dot{C}_{std}\,(A_{sam}/A_{std}) \qquad (11)$$

Where $\dot{C}_{sam}$ is the unknown concentration of the element in the sample, $\dot{C}_{std}$ is the known concentration of the element in the standard, A_{sam} is the activity of the sample, and A_{std} is the activity of the standard.

In this case, the unknown concentration of the element in the sample can be obtained by comparing the activities of the gamma-ray peaks. However,

usually the terms D and C in equation (10) have to be used as well, since they often vary. Normalization of the weight between standard and unknown must also often be done. The overall equation is then as follows:

$$\dot{C}_{sam} = \dot{C}_{std}\,(A_{sam}/A_{std})\,(D_{std}/D_{sam})\,(Cstd/Csam)(W_{std}/W_{sam}) \qquad (12)$$

where W_{sam} and W_{std} are the weights of the sample and the standard, respectively (Alfassi, 1994).

There are essentially two different ways to calibrate the NAA system for elemental analysis. The first involves the use of liquid standards which are prepared from either commercially available solutions or the basic chemicals. If the basic chemicals are chosen extreme care should be given to the stoichiometry of the chemical, including any hydrated forms. Moody et al (1988) have reviewed the inorganic chemicals recommended for use in calibration. All chemicals used should be over-dried in order to remove moisture. Both polyethylene and quartz vials can be utilized for the encapsulation of liquid samples (Alfassi, 1994). Liquid standards are pipetted into acid-washed vials, which are heat-sealed. If polyethylene vials are chosen, liquid samples should be doubly encapsulated for irradiation owing to inherent leakage. The heat-sealed vial needs to be placed in a larger vial which is then also heat-sealed. If larger vial cannot be used, the heat-sealed smaller vial can be wrapped and taped in polyethylene sheeting or heat-sealed in a small polyethylene bag. After irradiation, all liquid standards are transferred to labelled inert vials for counting. In some reactors there are no facilities available for liquid irradiations, or the neutron flux is so high as in turn

to cause evaporation and expensive leaking due to thermal heating (Alfassi, 1994).

In the comparator method, calibration can also be done by pipetting a known amount of one or more elements onto a filter paper. This methods is commonly used but several factors must be taken into consideration. The filter paper used should contain only minimal concentrations of contaminants when compared with the amount pipetted (hence the need for trace element characterization of the filter paper before use). When combining two or more standard solution of two elements, it has to be ascertained that the solution one element does not contain trace or minor quantities of the second element. For instance, it is quite conceivable that commercially bought or laboratory prepared compound may contain unspecified quantities of other elements. Filters need to be placed in acid-washed vials for irradiation, and then transferred to labelled inert vials prior to counting. However, some types of filter paper may not be transferable because of their condition following irradiation, particularly for high neuron flux conditions. Filter papers can also be prepared in the form of a pellet, but care must be taken to prevent contamination from the apparatus used for the procedure. The advantage of pelletization is that an exact geometry can be reproduced for irradiation and counting procedures. (Alfussi, 1994).

Another mean of calibration in the comparator method involves the use of references materials. In general, reference materials are not used for the calibration procedure but rather for testing the analytical accuracy of the methods and techniques. Many geological or biological reference materials have trace and

minor elements certified with errors to ±20% (and in some cases even more), or may not be certified with known elemental concentrations. This means that the final derived elemental concentration in the actual samples will have accuracies no better than the reported errors of the individual elemental concentrations. The existence of interferences may also limit the application of certified reference material made from geological material. For instance, the 136.0 keV and 264.7 keV gamma-rays coming from the ^{74}Se (n,γ) ^{75}Se activated product are susceptible to substantial interference from the same photo peaks arising from the ^{180}Hf (n,γ) ^{181}Hf and $^{181}Ta(n,\gamma)$ ^{182}Ta reactions, respectively. Other elements, such as nickel, exhibit poor sensitivities in thermal NAA and therefore should not be used in any calibration procedure, (Alfussi, 1994).

The second method calibration is the k_o method. Essentially, this is an absolute technique in which the amount of material is calculated from the measured counts by means of equation (10). The nuclear constants of each nuclide are combined together to give the k_o constant. As there are some uncertainties in the nuclear constants, the k_o values are determined experimentally (De-Cort . and Simonits, 1989).

Irradiation and Counting Procedures

Depending on the neutron flux density, the mass of the sample and the efficiency of the germanium detector, irradiation, decay and counting time may vary but generalized schemes can be adhered to. Typically two irradiations are performed: one to determine short-lived radionuclide (several minutes) and one

for medium/long live radionuclide (several hours to several weeks). If any epithermal NAA is required to augment the sensitivities of some radionuclide, then an additional irradiation is necessary. For activation analysis to produce short lived radionuclides, irradiation times varying from 5s to 10 min, depending on the type of sample, are employed. For many samples a decay period of at least five minutes is needed to let the usually high ^{28}Al activity decrease by two half-lives. High backgrounds due to the Compton Effect are caused by this high activity. Counting period of 5 -15 min are normally used to determine the rest of the element. To improve detection limits for barium, iodine, indium, potassium, longer decay (30-60 min) and counting (30-60 min) period are utilized. Detection limits for these elements and silicon are significantly improved by using epithermal neutrons.

For the determination of medium and long lived activities, irradiations of one to several hours are needed to activate the samples sufficiently to achieve good counting statistics. The other main limiting factor for good detection limits is the high background resulting from ^{24}Na and ^{82}Br photo peaks. Improved detection limits for arsenic, antimony, gallium, molybdenum and tungsten can be achieved by epithermal NAA, as the Compton background due to ^{24}Na is significantly decreased. This, however, is at the expense of not having enough activity for the long-lived isotopes (since the epithermal flux density can be one order of magnitude less than the thermal flux density). If the samples can be split, with one portion being used for the medium-lived nuclides and one for the long-lived activation products, then epithermal NAA may be advantageous.

Interferences in NAA

When properly performed, neutron activation analysis can be among the most precise and accurate of all analytical methods for the determination of trace elements. Several matrix interferences in NAA result in increased analytical errors. The primary nuclear interference reaction discussed in section 2.4.1 is potentially a major problem, particular if the target element has a high cross-section. Therefore, the method of choosing a suitable reaction, reducing the interference reaction, or making the proper correction must be seriously considered.

Another common nuclear interference reaction arises from uranium fission. This is particularly true for many geological sample containing tens of parts per million of uranium. In uranium fission, some of the fission products are exactly the same radionuclides as those produced by the desire (n,γ) reaction. This is particularly important for elements with atomic masses around 95 and 140. For example, analysis of Mo and La involves ^{99}Mo and ^{140}La, which are two common radionuclides produced from uranium fission. Thus, a correction is necessary if the concentration of uranium is high in the sample. An overview of such interferences and update of published data have been compiled by Landberger (1986, 1989).

A gamma-ray spectral interference occurs when two radionuclides emit gamma-rays of the same, or nearly the same, energy. For example, ^{56}Mn has an 846.8 keV gamma-ray and ^{27}Mg has an 843.8 keV gamma-ray. This problem can

be solved in several ways: using a high resolution germanium detector, choosing suitable decay and counting times, or finding another photopeak. In the case of ^{203}Hg, which emits a single gamma-ray at 279 keV, its separation from the 279keV gamma-ray belonging to ^{75}Se is impossible, so a correction based on other selenium photopeak must be made. Uranium and thorium interferences in the detection of samarium have also been experimentally examined by Landsberg and Simsons (1987)

It is often wrongly assumed that NAA methods are completely independent of matrix. The presence of high-*Z* elements, such as various combinations of zinc, iron, cobalt, lead, nickel, tin, copper, silver, antimony, etc. in ore concentrates, meteorites, and archaeological artifacts can severely limit the detection of lower energy gamma-rays owing to the self-absorption by the matrix. Bode, De Bruin and Korthoven (1981) and Zikovsky (1984) have described correction factors for these effects, and Jaegers and Landsberger (1990) have published a PC-based program for the determination of the self- absorption fractions of gamma-rays for three types of geometrical configuration. Other interferences, such as neutron self shielding owing to the high neutron capture cross-sections may occur if there are elevated concentrations of boron, cadmium, gold, silver or certain other elements in sample (Alfussi, 1994).

Accuracy and Precision

In NAA, counting statistics is the dominant factor for the precision of the analytical results. Therefore, to make an estimate of the analytical precision

several measurements of the sample must be made. Associated with the result of replicate determinations to estimate precision is the average standard deviation. Precision can thus be 1, 2 or 3 standard deviations, represented 68 % 95%and 99% confidence limits. As in the other chemical techniques there are numerous pitfalls which can hamper reliable measures. An estimate of the accuracy of the measurement is reliable measurement is achieved by analyzing certified reference materials. This indicates how good ones techniques and methods are when the result are compared with known concentrations of major, minor, and trace elements in similar or other matrices.

There are several factors which can severely limit the accuracy of NAA including:

1) Changes in neutron flux profile which are not carefully monitored.

2) Inaccurate placement of samples in front of detectors, including volume variations between standards and samples. Radiation from the sample to the detectors decreases a $1/r^2$. This is particularly significant when counting at short distance from the detector. Sample positioning is crucial, and very efforts should be made to retain a nearly identically geometry between the sample and standards. Vertical germanium detectors are usually more susceptible to efforts of small changes in the counting position than the horizontal ones. Automatic turning of the

sample in front of the detector can also be used to ensure better reproducibility of the counting. A quality control check can be performed by turning the sample 180 and seeing if the new calculated concentrations are significantly different. With the use of palletized samples unknown either vertical or horizontal oriented detectors can be used;

3) Unknown changes in relative natural abundances of certain elements such as boron, sulphur, and lead;
4) Inaccurate correction for dead time losses and pulse pile-up by either software or hardware methods;
5) Poor deconvolution of overlapping peaks by computer programs;
6) Unforeseen nuclear or spectral interferences;
7) Human factor errors, including incorrect readings of irradiation, decay or counting time and sample weights. Exact time for short irradiations are crucial;
8) Trace impurities in counting vials which have been activated along with the sample (this is particularly crucial for biological and some environmental sample);
9) Volatilization of certain elements such as mercury during irradiation;
10) Incorrect drying procedures for bioenvironmental sample which may have high moisture content (Alfussi, 1994).

Sensitivity

There have been numerous definitions for sensitivity and detection limits. Generally, as the sensitivity increases, the detection limit decreases and conversely. Ultimately it is the background radiation in a gamma-ray spectrum that determines how little material is needed to be adequately detected. In many NAA applications, detection of trace constituents can be limited by the complexity of the gamma-ray spectrum or by the masking effect of bremsstrahlung radiation from beta emitting radionuclide present at high levels (e.g. phosphorus in biological samples) or Compton scattering. Lower energy can often exhibit a poor signal-to-noise ratio, making the analyses of these peaks difficult. In particular, this is result of Compton scattering due to the presence of elements at the percent level (e.g. aluminium, chlorine, iron and sodium) as well as at minor levels (e.g. bromine, manganese, scandium, zinc and cobalt). Most methods to enhance elemental sensitivities are straightforward and usually revolve around optimizing irradiation, decay and counting time (including cyclic NAA), employing loss-free counters, larger detectors or X-ray detectors, using alternative photo peaks, or ultimately using radiochemical means to isolate the radionuclide of interest. Increases in analytical sensitivity for certain elements can also be augmented by using epithermal NAA, employing cadmium or boron or a combination of the two, as filters (Alfussi, 1994).

Ideally one would like to diminish the effect of Compton scattering so that photo peaks which cannot be seen, or have poor statistics, can be analyzed with

relatively good precision. Two non-destructive techniques which are able to accomplish this are Compton suppression (also referred to as anti-Compton) and gamma-gamma coincidence. (Suzuki and Hirai, 1990; Cumming *et al*, 1988; Millard, 1988). While reducing the Compton Effect, Compton suppression also provides detectors needed. This technique has been successfully used to determine very low levels (1-2 ng/g) of arsenic and cadmium in biological reference materials (Petra *et al*, 1990; Landsberger, 1992) and cadmium in environmental tobacco smoke (Landsberger, Larson and Wu, 1992).

Gamma-gamma coincidence methods have also been utilized in numerous nuclear physics experiments, but only in a very few cases for delayed NAA. Previous work has included the detection of selenium in biological sample (Wangen, Gladney and Hensly, 1980), copper in ecological samples (Michelsen and Steinnes, 1968) and various trace elements in rice flour (Suzuki and Hirai, 1990). There has also been some interest in gamma-gamma directional correlation measurement to obtain information on the chemical environment of trace elements in solutions (de Bruin and Bode, 1983).

CHAPTER THREE

EXPERIMENTAL

Chemicals, Reagents and Equipment Used

Chemicals/Reagents

- Acetone - AnalaR Reagent grade. Refractive index $n_D^{20°}$ - 1.3580 to 1.3600; weight per ml at 20°C 0.789 – 0.791g; BDH – England
- Absorbent cotton wool by Cotton Dressing Industry Ltd, Accra
- Standard Reference Material 3108 Cadmium (Cd) standard solution (contain HNO_3). Lot No. 890312, US Dept of Commerce, National Institute of standards & Technology Gaithersburg, MD 20899.
- Standard Reference Material 3133 Mercury (Hg) standard solution (contain HNO_3). Lot No. 991304, US Dept of Commerce, National Institute of standards & Technology Gaithersburg, MD 20899.
- Sucrose, SigmaUltra, > 99.5% GC; Batch no. 014 Koo 10; SIGMA-ALDRICH, Inc., USA

Equipment

- Fishing net
- Fish board; BDH – England
- Ersa TE 40, 24V, 60W/350°C soldering rod; BDH – England
- Stainless steel forceps; BDH – England
- Plastic descaller – Interplast Ghana
- Eppendof Research pipette max. 20ml; BDH – England
- Rabbit plastic capsules (containers); BDH– England
- Sample polythene bag with seal
- Plastic sample containers with lids – Interplast Ghana
- GARR-1 Nuclear reactor at Ghana Atomic Energy Commission, Kwabenya- Accra

Sample Collection

Five different biological species namely: tilapia (*Tilapia zillii*), mudfish (*Clarias submarginatus*), crabs (*Carcinus maenas*), prawns (*Penaeus monodon)* and molluscs (*Gastropod*) were collected from the river Pra Estuary. Four collection points were marked. These were Beposo, Bosomdo, Krobo and Shama Beach, all being fishing communities along the Pra Estuary, with Beposo being the point where the toll bridge on Cape Coast - Takoradi high way is located. The river enters the sea at Shama Beach (see page 5 for study the area map). Samples were taken every fortnight for two and half months. The first and the last samples were taken on 26th April 2006 nd 29th June 2006 respectively.

The fish samples were caught by net which was set the evening before the sampling day. Each sampling centre has its own net and the fish were caught alive with the help of experienced fishermen. The molluscs were hand picked at the shore or the river bank and were collected at the time the fishes were removed from the net.

All the fish samples were put into polythene bags with seal. Each fish sample was put into a different polythene bag with a seal and appropriately labelled and packed into ice-chest and ice blocks packed onto them before shipping to the laboratory.

Analytical control samples were also taken from the River Kakum estuary at Akotokyir on University of Cape Coast campus. At Akotokyir and up stream of the River Kakum vehicular activities are minimal. Other samples were also taken from the River Ayensu Estuary near Winneba (Winneba is about 152 Km from Beposo). There is no mining activity upstream of the River Ayensu. This makes this estuary a good sampling site for the purpose of comparism.

Fig. 10 The researcher is removing life crab

Fig. 11 The researcher is removing life crab

In figs 10 and 11 the researcher is removing life crab from a net with the help of an experienced fisherman.

Fig 12 The researcher is picking life mollusc

Fig 13 The researcher is observing life mollusc

Sample Treatment in the Laboratory

Samples were stored in small plastic sample containers with lids and later in a freezer. In the laboratory, samples were removed from the freezer, allowed to defreeze and the water lingering on each sample was removed with tissue paper before they were wet-weighed and their lengths taken. The samples within a specific range of mass and length were selected and processed as described below.

The tilapia samples of length between 116mm – 139mm and mass from 96.70g to 105.20g were selected and the scales removed separately using a plastic descaller. Samples were dissected with a sharp stainless steel knife and the liver removed. The gills and the muscles were also put separately into small plastic containers with lid and appropriately labelled and kept in the freezer. The mudfish and the prawns where also treated similarly, except that for the prawns all the internal organs were used instead of the liver since the liver could not be identified separately and also most people consumed the whole fish. In the case of the crab and the mollusc, their shells and muscles were used.

The mudfish samples selected were of length ranging between 291mm and 367mm and of mass between 321.60g and 368.20g. The prawns selected were of length 115mm – 121mm and mass 25.40g – 28.44g whiles the crabs have their lengths ranging between 81mm and 105mm, and mass from 54.30g to 66.40g. Also, the molluscs used were of length 41mm to 52mm and mass 5.70g to 7.50g.

About 200mg of the various parts of the fish samples were wet weighed. Each weighed sample was parcelled in a polythene material, labelled appropriately and heat sealed using soldering iron for irradiation. Between eight and ten parcels were packed in rabbit plastic containers for the irradiation.

Standard Reference Material

103.7 g of mercury standard reference material (3133 mercury) was weighed into a small rabbit plastic container and about 200mg of sucrose added. Enough cotton wool was then added and the rabbit container heat sealed using the soldering rod for irradiation. In a similar way, 103.2 mg of cadmium standard reference material (3108 cadmium) was weighed and packaged for irradiation. The standard reference materials were used for validation of the results.

Sample Irradiation

Each of the rabbit capsules was sent by the pneumatic transfer system into the Am-Be source for irradiation. This is because the system allows only one capsules sample irradiation at a time. The irradiation scheme was chosen so as to take into account the half-lives of the radionuclides. In this regard, the irradiation

was done one (1) hour. At the end of each irradiation the sample was returned for counting, with a delay (cooling) time of twenty-four (24) hours. The activity of each irradiated samples was counting for 600 seconds.

Irradiation Source

The irradiation source is a 20 Curie Am-Be radioactive neutron source. It is cylindrically shaped and is fixed in a holder at the centre of a fiber-glass tank, filled with de-ionized water. The de-ionized water serves a dual purpose of moderator and also an absorber of neutrons. Extra shielding is provided by concrete blocks arranged round the tank. Transfer of sample to and from the neutron source is by means of a flexo-rabbit pneumatic transfer system operating under a pressure of 15 psi, giving a sample transfer time of 1.3 seconds (Tetteh, 1989). The thermal neutron flux at the irradiation site was 5×10^{11} ns^{-1} cm^{-2}

Data Processing

The detector type used for the counting of signals was an ENERTEC High Purity Germanium (HPGe) detector of 3000 (+ve) bias and a resolution of 2.55 KeV for 1332 photo peak of Co-60. The associated electronics are: high voltage supply (Canberra model 3105), spectroscopy amplifier (Canberra model 2010) and Canberra Multi-Channel Analyzer (MCA) Series 35-plus.

The signals from the detector were passed through the spectroscopy amplifier, and then accumulated by the MCA for a time. The spectra from the MCA were transferred to a DEC 350 microcomputer for analysis, using a Gamma

Spectrum Analysis software PRO/CEBAS supplied by the IAEA. This software identifies the various photopeaks and works out the area under them.

The concentration of mercury and cadmium in each sample were calculated. The validation of the analytical procedure was undertaken by irradiating a standard reference material (SARM) of mercury and cadmium, and counting under identical experimental conditions.

Quality Control

The following quality control methods were adopted throughout the analysis in order to get good and reliable results.

- Validation of the analytical procedure was undertaken by irradiating an IAEA standard reference material: Hg- 9.94µg/g and Cd- 9.5µg/g, with 5% uncertainty and counting under identical experimental conditions.

Table 3

Validation of Results

Standard Material	Concentration of Mercury and Cadmium in µg/g						
	Std	1	2	3	4	Mean	% Recovery
Hg	9.94	9.81	9.82	9.84	9.85	9.83	98.89
Cd	9.5	9.4702	9.4779	9.4700	9.4710	9.4727	99.70

- Control samples were taken from a river presumably least affected by mining activities, vehicular emissions and/or other forms of pollution.

These samples were treated, irradiated and counted under identical experimental condition as the real samples.

- Occasionally, at least two gamma-rays from the same nuclide were carefully analysed to determine if any spectrum or nuclear interferences exists.
- A careful evaluation of each individual spectrum using a Gamma Spectrum Analysis software PRO/CEBAS supplied by the IAEA to see if any potential interference exists.

CHAPTER FOUR

RESULTS AND DISCUSSION

Table of Results

Table 4

Concentration of Mercury in Various Parts Of Selected Biological Species from Four Sampling Sites - 1st Sampling (10/05/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
Tilapia	Gills ng/g	0.00096 ± 0.00013	0.00084 ± 0.00010	0.00079 ± 0.00012	0.00072 ± 0.00008
	Liver ng/g	0.00214 ± 0.00028	0.00208 ± 0.00025	0.00200 ± 0.00026	0.00198 ± 0.00024
	Muscles ng/g	0.00121 ± 0.00016	0.00111 ± 0.00013	0.00101 ± 0.00013	0.00102 ± 0.00012
	Scales ng/g	0.00068 ± 0.00009	0.00059 ± 0.00007	0.00042 ± 0.00005	0.00031 ± 0.00004
	Mean ng/g	**0.00125 ± 0.00016**	**0.00116 ± 0.00014**	**0.00106 ± 0.00014**	**0.00101 ± 0.00012**
Mudfish	Gills ng/g	0.00328 ± 0.00043	0.00291 ± 0.00035	0.00290 ± 0.00038	0.00258 ± 0.00031
	Liver ng/g	0.00527 ± 0.00069	0.00423 ± 0.00051	0.00398 ± 0.00051	0.00396 ± 0.00048
	Muscles ng/g	0.00436 ± 0.00057	0.00389 ± 0.00044	0.00354 ± 0.00046	0.00317 ± 0.00038
	Mean ng/g	**0.00430 ± 0.00056**	**0.00368 ± 0.00041**	**0.00347 ± 0.00045**	**0.00324 ± 0.00039**
Prawn	Internal organs ng/g	0.00377 ± 0.00049	0.00344 ± 0.00035	0.00308 ± 0.00041	0.00291 ± 0.00034
	Muscles ng/g	0.00301 ± 0.00039	0.00291 ± 0.00030	0.00242 ± 0.00031	0.00215 ± 0.00026
	Scales ng/g	0.00181 ± 0.00022	0.00169 ± 0.00020	0.00197 ± 0.00026	0.00153 ± 0.00018
	Mean ng/g	**0.00286 ± 0.00037**	**0.00268 ± 0.00032**	**0.00249 ± 0.00032**	**0.00220 ± 0.00026**
Crab	Muscles ng/g		0.00808 ± 0.00097	0.00759 ± 0.00099	0.00532 ± 0.00064
	Scales ng/g		0.00552 ± 0.00066	0.00483 ± 0.00063	0.00294 ± 0.00035
	Mean ng/g		**0.00680 ± 0.00082**	**0.00621 ± 0.00081**	**0.00413 ± 0.00050**
Mollusc	Muscles ng/g	0.00416 ± 0.00054	0.00393 ± 0.00037	0.00334 ± 0.00043	0.00309 ± 0.00037
	Scales ng/g	0.00340 ± 0.00044	0.00303 ± 0.00036	0.00259 ± 0.00034	0.00245 ± 0.00029
	Mean ng/g	**0.00378 ± 0.00048**	**0.00348 ± 0.00042**	**0.00297 ± 0.00039**	**0.00277 ± 0.00033**

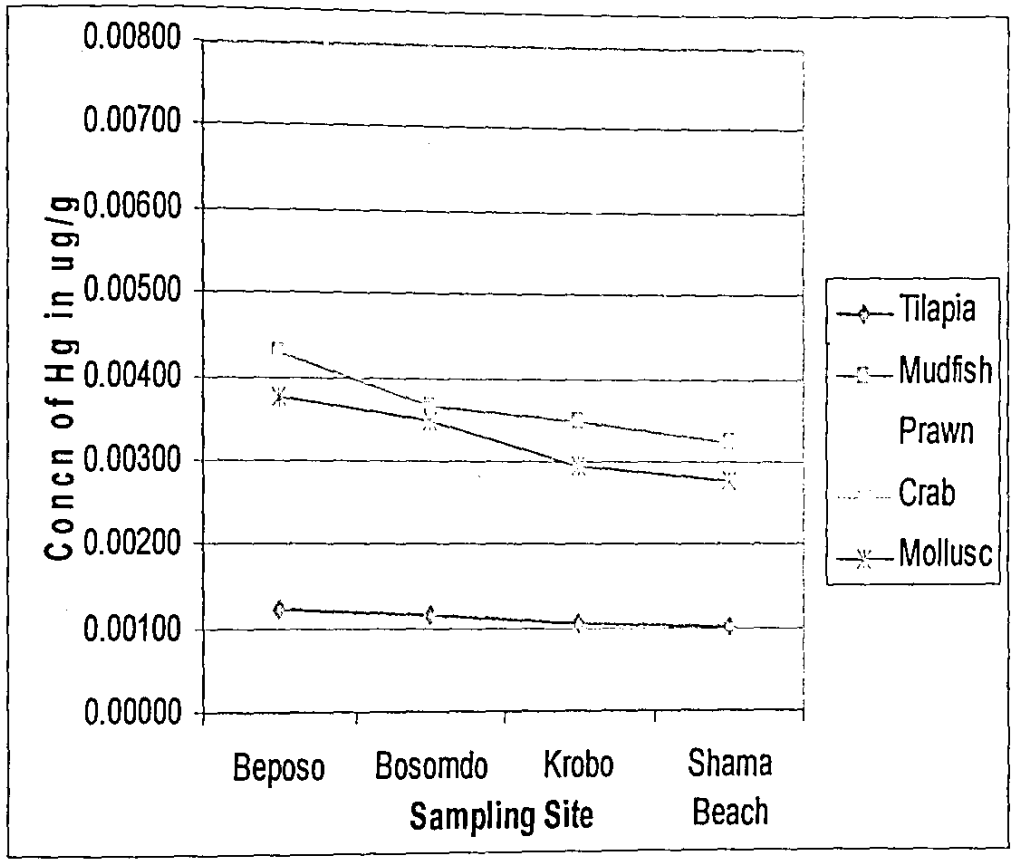


Fig 14. Variation of Hg Concentration in Selected Biological Species Collected on 10/05/06

Table 5

Concentration of Mercury In Various Parts of Selected Biological Species from Four Sampling Sites - 2nd Sampling (24/05/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
Tilapia	Gills µg/g	0.00055 ± 0.00007	0.00051 ± 0.00061	0.00041 ± 0.00005	0.00038 ± 0.00005
	Liver µg/g	0.00135 ± 0.00018	0.00132 ± 0.00016	0.00131 ± 0.00017	0.00131 ± 0.00016
	Muscles µg/g	0.00098 ± 0.00013	0.00098 ± 0.00012	0.00094 ± 0.00012	0.00090 ± 0.00011
	Scales µg/g	0.00037 ± 0.00005	0.00032 ± 0.00004	0.00029 ± 0.00004	0.00022 ± 0.00026
	Mean µg/g	**0.00081 ± 0.00011**	**0.00078 ± 0.00009**	**0.00074 ± 0.00009**	**0.00070 ± 0.00084**
Mudfish	Gills µg/g	0.00296 ± 0.00039	0.00210 ± 0.00025	0.00207 ± 0.00027	0.00201 ± 0.00024
	Liver µg/g	0.00468 ± 0.00061	0.00394 ± 0.00047	0.00390 ± 0.00051	0.00389 ± 0.00047
	Muscles µg/g	0.00397 ± 0.00052	0.00362 ± 0.00043	0.00312 ± 0.00041	0.00299 ± 0.00036
	Mean µg/g	**0.00387 ± 0.00051**	**0.00322 ± 0.00039**	**0.00303 ± 0.00039**	**0.00296 ± 0.00036**
Prawn	Internal organs µg/g	0.00295 ± 0.00038	0.00291 ± 0.00035	0.00286 ± 0.00037	0.00254 ± 0.00030
	Muscles µg/g	0.00208 ± 0.00027	0.00201 ± 0.00024	0.00198 ± 0.00026	0.00173 ± 0.00021
	Scales µg/g	0.00168 ± 0.00022	0.00153 ± 0.00018	0.00119 ± 0.00016	0.00103 ± 0.00012
	Mean µg/g	**0.00224 ± 0.00029**	**0.00215 ± 0.00027**	**0.00201 ± 0.00026**	**0.00177 ± 0.00021**
Crab	Muscles µg/g		0.00769 ± 0.00092	0.00637 ± 0.00083	0.00501 ± 0.00060
	Scales µg/g		0.00447 ± 0.00054	0.00405 ± 0.00053	0.00293 ± 0.00035
	Mean µg/g		**0.00608 ± 0.00073**	**0.00521 ± 0.00068**	**0.00397 ± 0.00048**
Mollusc	Muscles µg/g	0.00379 ± 0.00049	0.00287 ± 0.00034	0.00312 ± 0.00041	0.00254 ± 0.00030
	Scales µg/g	0.00314 ± 0.00041	0.00301 ± 0.00036	0.00264 ± 0.00034	0.00181 ± 0.00022
	Mean µg/g	**0.00347 ± 0.00045**	**0.00294 ± 0.00035**	**0.00288 ± 0.00037**	**0.00218 ± 0.00026**

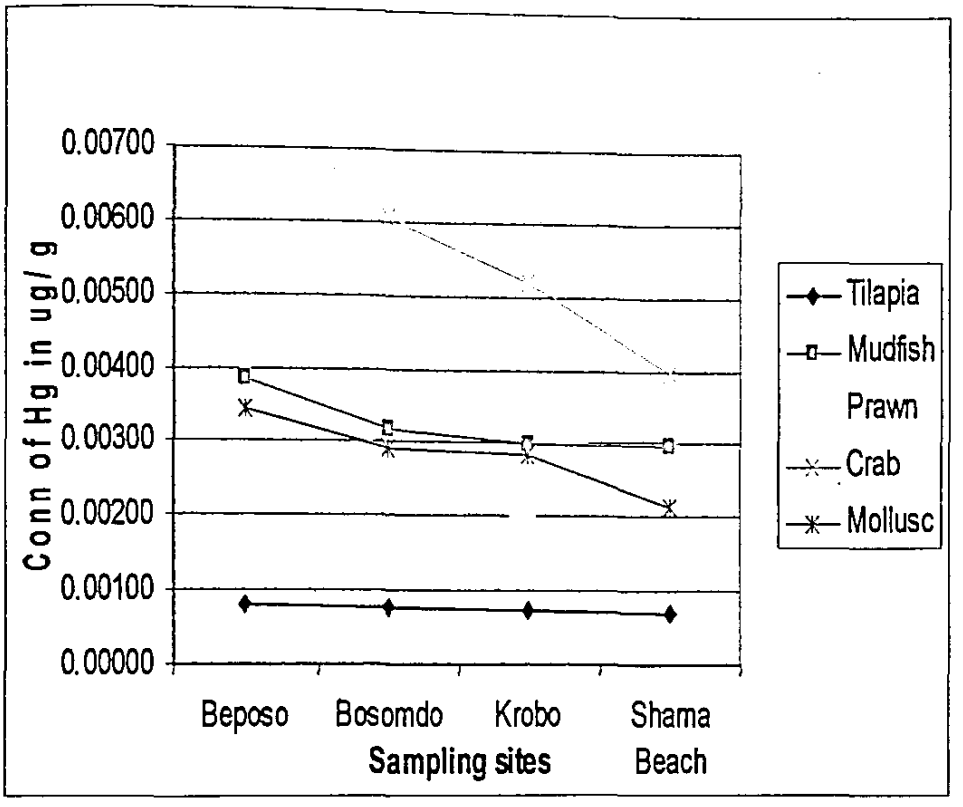


Fig 15. Variation of Hg Concentration in Selected Biological Species Collected on 24/05/06

Table 6

Concentration of Mercury In Various Parts of Selected Biological Species from Four Sampling Sites - 3rd Sampling (8/06/06)

BIOLOGICAL SPECIES		Bepomso	Bosomdo	Krobo	Shama Beach
Tilapia	Gills µg/g	0.00002 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.0000
	Liver µg/g	0.00009 ± 0.00001	0.00003 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000
	Muscles µg/g	0.00004 ± 0.00000	0.00001 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000
	Scales µg/g	0.00000 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000
	Mean µg/g	**0.00004 ± 0.00000**	**0.00001 ± 0.00000**	**0.00000 ± 0.00000**	**0.00000 ± 0.00000**
Mudfish	Gills µg/g	0.00160 ± 0.00019	0.00153 ± 0.00017	0.00125 ± 0.00029	0.00103 ± 0.00013
	Liver µg/g	0.00289 ± 0.00035	0.00272 ± 0.00030	0.00238 ± 0.00023	0.00206 ± 0.00027
	Muscles µg/g	0.00207 ± 0.00025	0.00199 ± 0.00022	0.00191 ± 0.00022	0.00162 ± 0.00021
	Mean µg/g	**0.00219 ± 0.00026**	**0.00208 ± 0.00023**	**0.00185 ± 0.00021**	**0.00157 ± 0.00020**
Prawn	Internal organs µg/g	0.00281 ± 0.00034	0.00264 ± 0.00029	0.00244 ± 0.00019	0.00173 ± 0.00023
	Muscles µg/g	0.00200 ± 0.00024	0.00186 ± 0.00021	0.00158 ± 0.00018	0.00099 ± 0.00013
	Scales µg/g	0.00149 ± 0.00018	0.00161 ± 0.00018	0.00115 ± 0.00016	0.00081 ± 0.00011
	Mean µg/g	**0.00210 ± 0.00025**	**0.00204 ± 0.00022**	**0.00172 ± 0.00020**	**0.00118 ± 0.00015**
Crab	Muscles µg/g		0.00379 ± 0.00042	0.00332 ± 0.00035	0.00237 ± 0.00031
	Scales µg/g		0.00164 ± 0.00018	0.00128 ± 0.00018	0.00098 ± 0.00013
	Mean µg/g		**0.00272 ± 0.00030**	**0.00230 ± 0.00028**	**0.00168 ± 0.00022**
Mollusc	Muscles µg/g	0.00264 ± 0.00032	0.00236 ± 0.00026	0.00211 ± 0.00023	0.00103 ± 0.00013
	Scales µg/g	0.00121 ± 0.00015	0.00108 ± 0.00012	0.00085 ± 0.00009	0.00054 ± 0.00001
	Mean µg/g	**0.00193 ± 0.00023**	**0.00172 ± 0.00019**	**0.00148 ± 0.00017**	**0.00079 ± 0.00010**

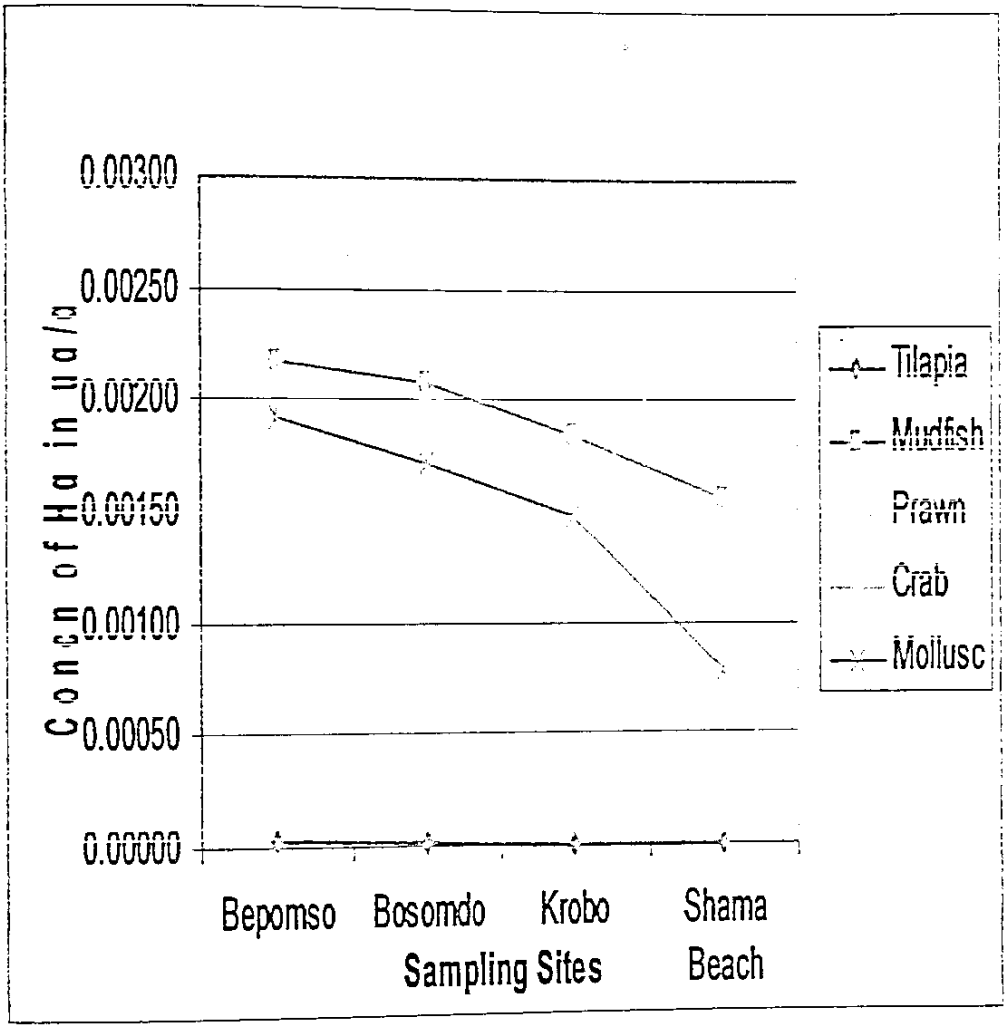


Fig 16. Variation of Hg Concentration in Selected Biological Species Collected on 8/06/06

Table 7

Concentration of Mercury in Various Parts of Selected Biological Species from Four Sampling Sites - 4th Sampling (29/06/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
	Gills µg/g	0.00006 ± 0.00000	0.00001 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000
	Liver µg/g	0.00031 ± 0.00003	0.00020 ± 0.00002	0.00014 ± 0.00001	0.00011 ± 0.00001
	Muscles µg/g	0.00018 ± 0.00002	0.00012 ± 0.00001	0.00010 ± 0.00001	0.00007 ± 0.00001
	Scales µg/g	0.00004 ± 0.00000	0.00001 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000
Tilapia	Mean µg/g	**0.00015 ± 0.00002**	**0.00009 ± 0.00001**	**0.00006 ± 0.00000**	**0.00005 ± 0.00000**
	Gills µg/g	0.00203 ± 0.00024	0.00211 ± 0.00023	0.00197 ± 0.00017	0.00198 ± 0.00017
	Liver µg/g	0.00366 ± 0.00044	0.00359 ± 0.00040	0.00319 ± 0.00027	0.00311 ± 0.00026
	Muscles µg/g	0.00294 ± 0.00035	0.00282 ± 0.00031	0.00279 ± 0.00035	0.00273 ± 0.00030
Mudfish	Mean µg/g	**0.00288 ± 0.00034**	**0.00284 ± 0.00031**	**0.00265 ± 0.00032**	**0.00261 ± 0.00032**
	Internal organs µg/g	0.00293 ± 0.00035	0.00275 ± 0.00030	0.00261 ± 0.00029	0.00212 ± 0.00025
	Muscles µg/g	0.00202 ± 0.00024	0.00190 ± 0.00021	0.00177 ± 0.00019	0.00169 ± 0.00019
	Scales µg/g	0.00152 ± 0.00018	0.00169 ± 0.00019	0.00136 ± 0.00016	0.00124 ± 0.00015
Prawn	Mean µg/g	**0.00216 ± 0.00030**	**0.00211 ± 0.00023**	**0.00191 ± 0.00020**	**0.00168 ± 0.00019**
	Muscles µg/g		0.00621 ± 0.00068	0.00507 ± 0.00052	0.00481 ± 0.00051
	Scales µg/g		0.00162 ± 0.00018	0.00159 ± 0.00017	0.00041 ± 0.00004
Crab	Mean µg/g		**0.00392 ± 0.00043**	**0.00333 ± 0.00039**	**0.00311 ± 0.00038**
	Muscles µg/g	0.00291 ± 0.00035	0.00278 ± 0.00031	0.00247 ± 0.00030	0.00227 ± 0.00033
	Scales µg/g	0.00180 ± 0.00022	0.00163 ± 0.00018	0.00156 ± 0.00017	0.00124 ± 0.00019
Mollusc	Mean µg/g	**0.00236 ± 0.00028**	**0.00221 ± 0.00024**	**0.00202 ± 0.00015**	**0.00176 ± 0.00021**

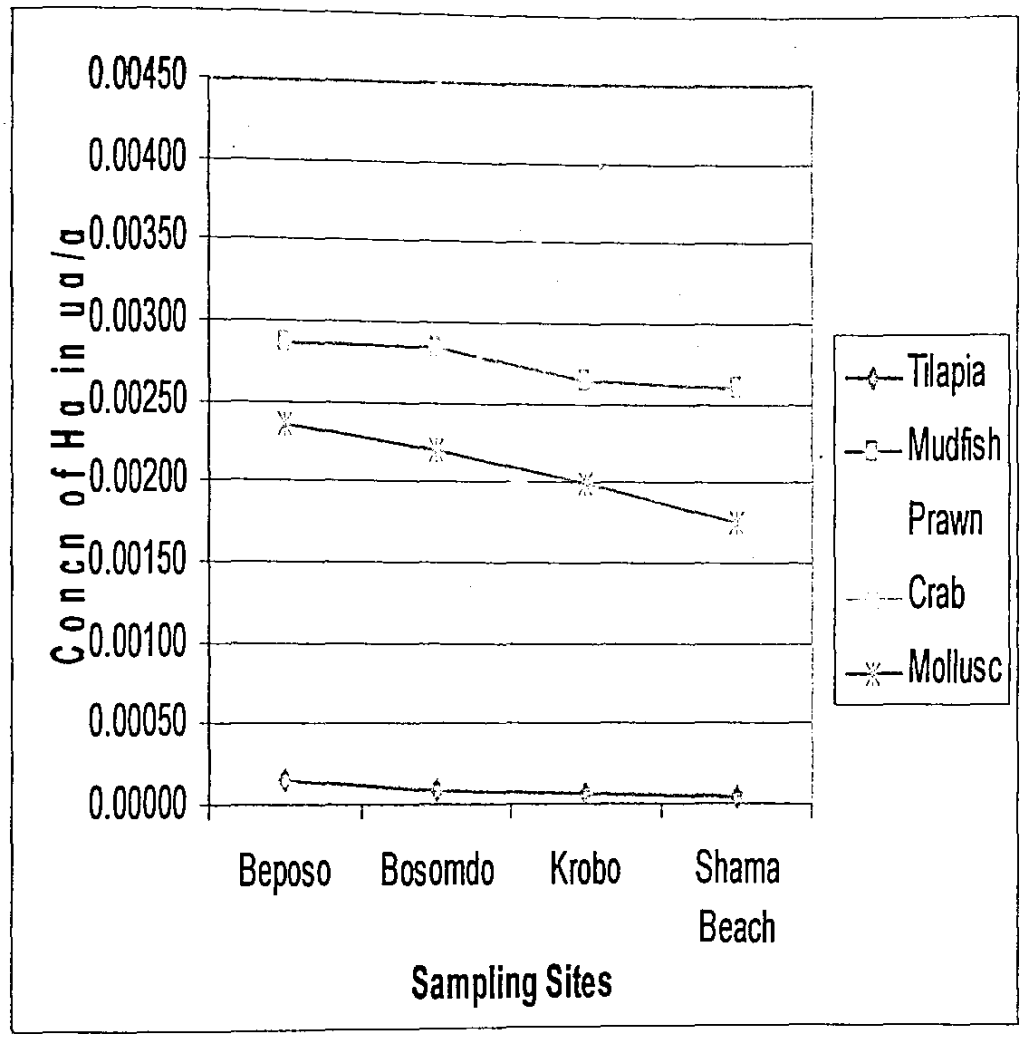


Fig 17. Variation of Hg Concentration in Selected Biological Species Collected on 29/06/06

Table 8

Concentration of Cadmium in Various Parts Of Selected Biological Species from Four Sampling Sites - 1st Sampling (10/05/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
Tilapia	Gills µg/g	1.95 ± 0.25	0.00182 ± 0.00024	0.00166 ± 0.00022	0.00104 ± 0.00014
	Liver µg/g	6.52 ± 0.85	0.00540 ± 0.00070	0.00491 ± 0.00064	0.00479 ± 0.00062
	Muscles µg/g	0.00449 ± 0.00058	0.00400 ± 0.00052	0.00348 ± 0.00045	0.00390 ± 0.00051
	Scales µg/g	0.00187 ± 0.00024	0.00171 ± 0.00022	0.00144 ± 0.00019	0.00098 ± 0.00013
	Mean µg/g	**0.00371 ± 0.00048**	**0.00323 ± 0.00042**	**0.00287 ± 0.00037**	**0.00268 ± 0.00035**
Mudfish	Gills µg/g	0.00947 ± 0.00123	0.00940 ± 0.00122	0.00922 ± 0.00120	0.00905 ± 0.00118
	Liver µg/g	0.02997 ± 0.00390	0.02097 ± 0.00273	0.01891 ± 0.00246	0.01172 ± 0.00152
	Muscles µg/g	0.01706 ± 0.00222	0.01243 ± 0.00162	0.01014 ± 0.00132	0.00991 ± 0.00129
	Mean µg/g	**0.01883 ± 0.00245**	**0.01427 ± 0.00186**	**0.01276 ± 0.00166**	**0.01023 ± 0.00133**
Prawn	Internal organs µg/g	0.01778 ± 0.00231	0.01283 ± 0.00167	0.00861 ± 0.00112	0.00809 ± 0.00105
	Muscles µg/g	0.01197 ± 0.00156	0.00755 ± 0.00098	0.00662 ± 0.00086	0.00625 ± 0.00081
	Scales µg/g	0.00291 ± 0.00038	0.00279 ± 0.00363	0.00261 ± 0.00034	0.00253 ± 0.00033
	Mean µg/g	**0.01089 ± 0.00142**	**0.00772 ± 0.00100**	**0.00595 ± 0.00077**	**0.00562 ± 0.00073**
Crab	Muscles µg/g		0.02024 ± 0.00263	0.01995 ± 0.00259	0.01495 ± 0.00019
	Scales µg/g		0.00968 ± 0.00126	0.00771 ± 0.00100	0.00706 ± 0.00092
	Mean µg/g		**0.01496 ± 0.00195**	**0.01383 ± 0.00180**	**0.01101 ± 0.00143**
Mollusc	Muscles µg/g	0.02031 ± 0.00264	0.01774 ± 0.00231	0.01098 ± 0.00143	0.00874 ± 0.00114
	Scales µg/g	0.01293 ± 0.00168	0. 00916 ± 0.00119	0.00592 ± 0.00077	0.00501 ± 0.00065
	Mean µg/g	**0.01662 ± 0.00216**	**0.01345 ± 0.00175**	**0.00845 ± 0.00110**	**0.00688 ± 0.00089**

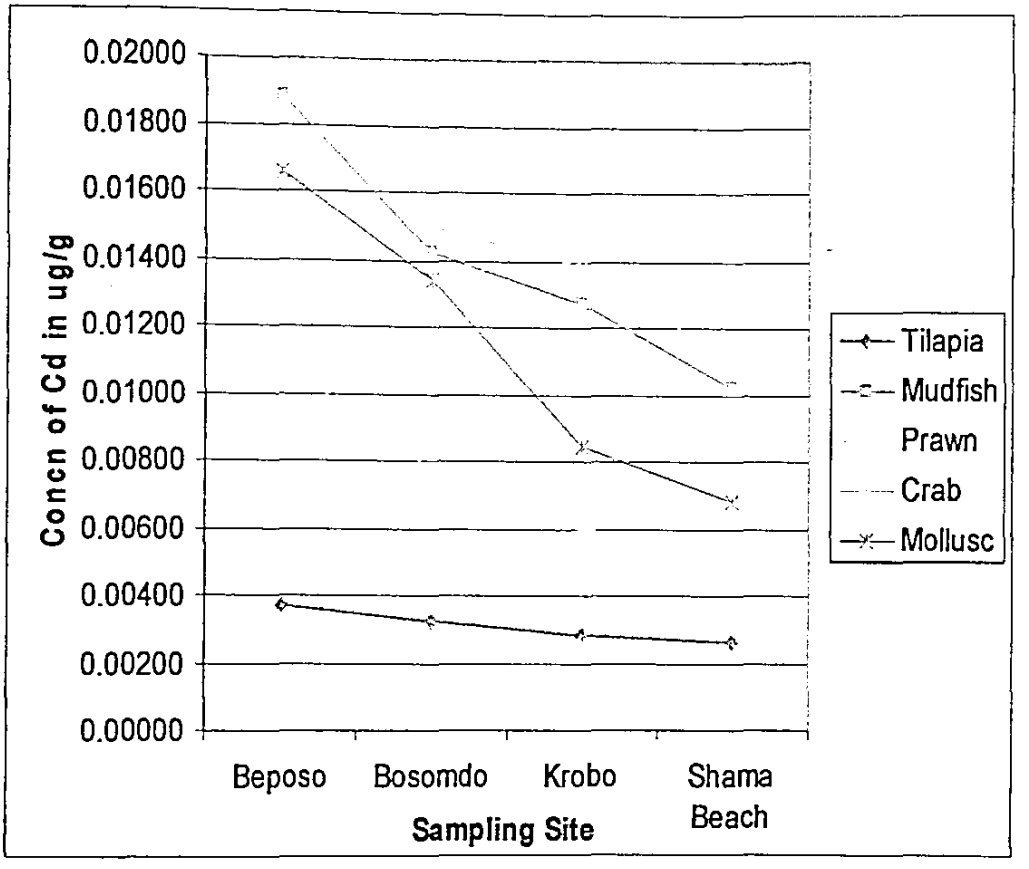


Fig 18. Variation of Cd Concentration in Selected Biological Species Collected on 10/05/06

Table 9

Concentration of Cadmium in Various Parts of Selected Biological Species from Four Sampling Sites - 2nd Sampling (24/05/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
Tilapia	Gills µg/g	0.00171 ± 0.00022	0.00165 ± 0.00022	0.00140 ± 0.00018	0.00129 ± 0.00017
	Liver µg/g	0.00449 ± 0.00058	0.00432 ± 0.00056	0.00419 ± 0.00055	0.00394 ± 0.00051
	Muscles µg/g	0.00308 ± 0.00040	0.00299 ± 0.00039	0.00290 ± 0.00038	0.00287 ± 0.00037
	Scales µg/g	0.00117 ± 0.00015	0.00111 ± 0.00014	0.00109 ± 0.00014	0.00101 ± 0.00013
	Mean µg/g	**0.00261 ± 0.00034**	**0.00252 ± 0.00033**	**0.00240 ± 0.00031**	**0.00228 ± 0.00030**
Mudfish	Gills µg/g	0.00918 ± 0.00119	0.00900 ± 0.00117	0.00891 ± 0.00116	0.00879 ± 0.00114
	Liver µg/g	0.01397 ± 0.00182	0.01246 ± 0.00162	0.01160 ± 0.00151	0.00934 ± 0.00121
	Muscles µg/g	0.01211 ± 0.00157	0.01004 ± 0.00131	0.01001 ± 0.00130	0.00993 ± 0.00129
	Mean µg/g	**0.01175 ± 0.00153**	**0.01050 ± 0.00137**	**0.01017 ± 0.001323**	**0.00935 ± 0.00122**
Prawn	Internal organs µg/g	0.01295 ± 0.00168	0.00981 ± 0.00128	0.00779 ± 0.00101	0.00762 ± 0.00099
	Muscles µg/g	0.00903 ± 0.00117	0.00589 ± 0.00077	0.00580 ± 0.00075	0.00567 ± 0.00074
	Scales µg/g	0.00276 ± 0.00036	0.00263 ± 0.00034	0.00244 ± 0.00032	0.00231 ± 0.00030
	Mean µg/g	**0.00825 ± 0.00107**	**0.00611 ± 0.00079**	**0.00534 ± 0.00070**	**0.00520 ± 0.00068**
Crab	Muscles µg/g		0.01850 ± 0.00241	0.01492 ± 0.00194	0.01379 ± 0.00179
	Scales µg/g		0.00501 ± 0.00065	0.00479 ± 0.00062	0.00403 ± 0.00052
	Mean µg/g		**0.01176 ± 0.00153**	**0.00986 ± 0.00128**	**0.00891 ± 0.00116**
Mollusc	Muscles µg/g	0.01324 ± 0.00172	0.01086 ± 0.00141	0.00879 ± 0.00114	0.00779 ± 0.00101
	Scales µg/g	0.00726 ± 0.00094	0.00613 ± 0.00080	0.00552 ± 0.00072	0.00408 ± 0.00053
	Mean µg/g	**0.01025 ± 0.00133**	**0.00914 ± 0.00119**	**0.00809 ± 0.00105**	**0.00734 ± 0.00095**

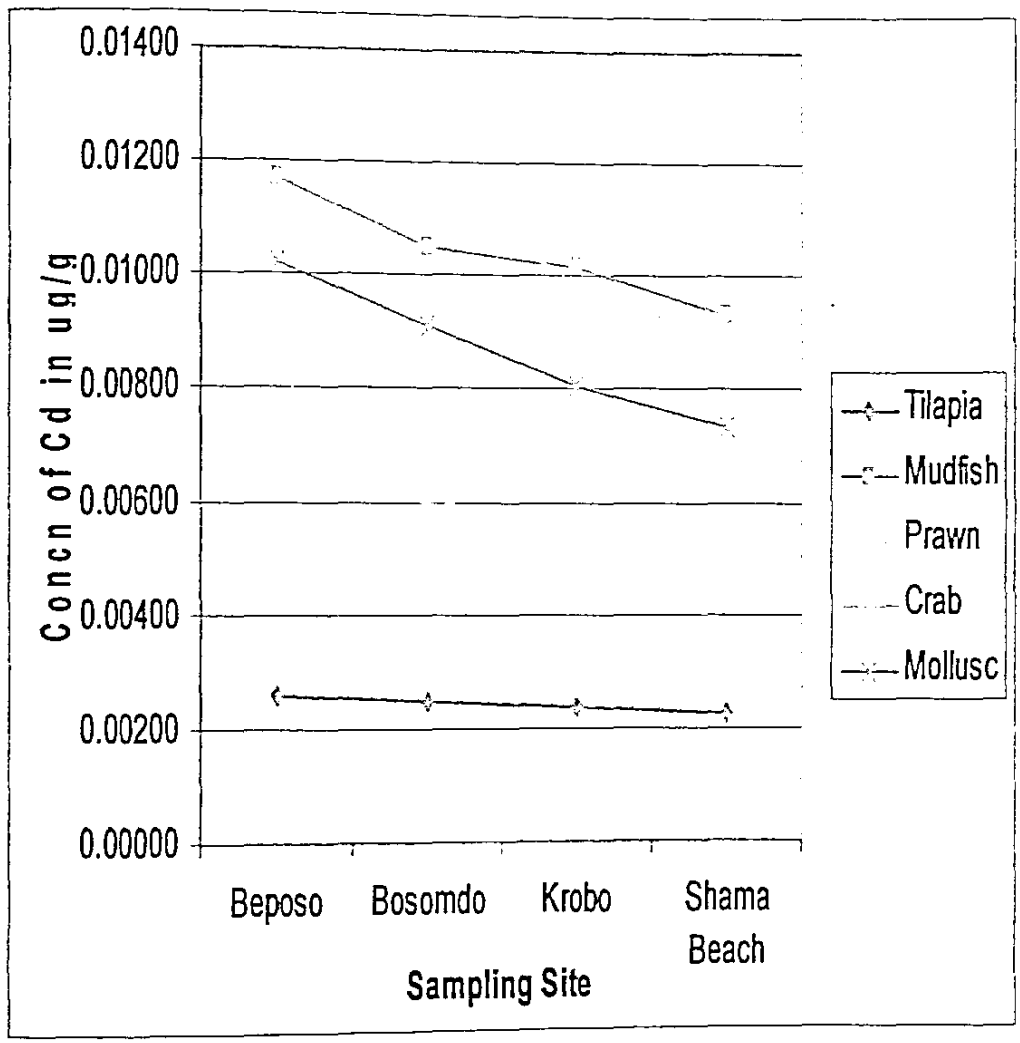


Fig 19 Variation of Cd Concentration in Selected Biological Species Collected on 24/05/06

Table 10

Concentration of Cadmium in Various Parts of Selected Biological Species from Four Sampling Sites - 3rd Sampling (8/06/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
Tilapia	Gills µg/g	0.00183 ± 0.00024	0.00169 ± 0.00022	0.00138 ± 0.00018	0.00128 ± 0.00017
	Liver µg/g	0.00400 ± 0.00052	0.00391 ± 0.00051	0.00363 ± 0.00047	0.00341 ± 0.00044
	Muscles µg/g	0.00337 ± 0.00044	0.00326 ± 0.00042	0.00312 ± 0.00041	0.00300 ± 0.00039
	Scales µg/g	0.00142 ± 0.00019	0.00138 ± 0.00018	0.00130 ± 0.00017	0.00125 ± 0.00016
	Mean µg/g	**0.00266 ± 0.00035**	**0.00256 ± 0.00033**	**0.00236 ± 0.00031**	**0.00224 ± 0.00029**
Mudfish	Gills µg/g	0.00501 ± 0.00 065	0.00487 ± 0.00063	0.00361 ± 0.00047	0.00346 ± 0.00045
	Liver µg/g	0.00968 ± 0.00126	0.00901 ± 0.00117	0.00890 ± 0.00116	0.00884 ± 0.00115
	Muscles µg/g	0.00816 ± 0.00106	0.00807 ± 0.00105	0.00768 ± 0.00100	0.00721 ± 0.00094
	Mean µg/g	**0.00762 ± 0.00099**	**0.00732 ± 0.00095**	**0.00673 ± 0.00088**	**0.00650 ± 0.00085**
Prawn	Internal organs µg/g	0.00712 ± 0.00092	0.00694 ± 0.00090	0.00687 ± 0.00089	0.00615 ± 0.00080
	Muscles µg/g	0.00578 ± 0.00075	0.00569 ± 0.00074	0.00562 ± 0.00073	0.00555 ± 0.000722
	Scales µg/g	0.00247 ± 0.00032	0.00232 ± 0.00030	0.00230 ± 0.00030	0.00221 ± 0.00029
	Mean µg/g	**0.00512 ± 0.00067**	**0.00498 ± 0.00065**	**0.00493 ± 0.00064**	**0.00464 ± 0.00060**
Crab	Muscles µg/g		0.01164 ± 0.00151	0.01009 ± 0.00131	0.00892 ± 0.00116
	Scales µg/g		0.00487 ± 0.00063	0.00384 ± 0.00050	0.00368 ± 0.00048
	Mean µg/g		**0.00826 ± 0.00107**	**0.00697 ± 0.00091**	**0.00630 ± 0.00082**
Mollusc	Muscles µg/g	0.00885 ± 0.00115	0.00772 ± 0.00100	0.00726 ± 0.00094	0.00597 ± 0.0078
	Scales µg/g	0.00416 ± 0.00054	0.00393 ± 0.00051	0.00364 ± 0.00047	0.00201 ± 0.00026
	Mean µg/g	**0.00651 ± 0.00085**	**0.00583 ± 0.00076**	**0.00545 ± 0.00071**	**0.00399 ± 0.00052**

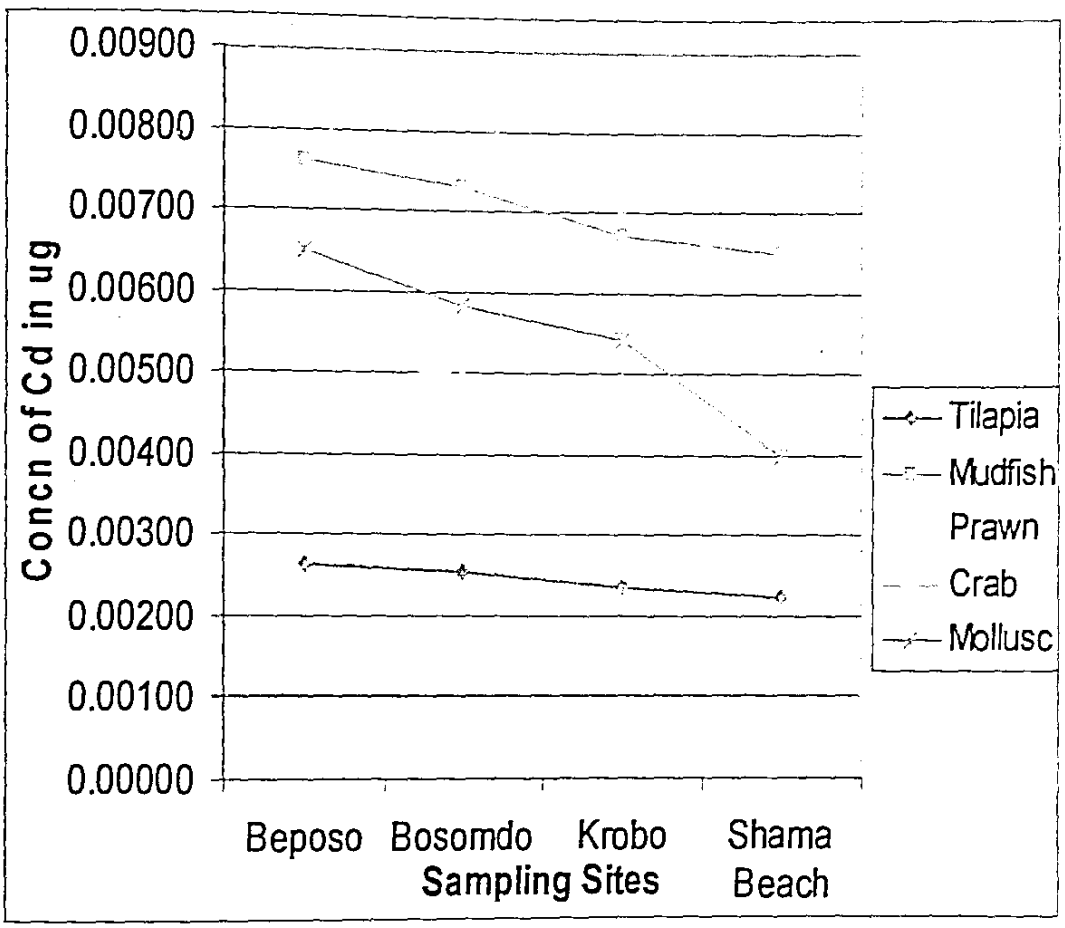


Fig 20. Variation of Cd Concentration in Selected Biological Species Collected on 8/06/06

Table 11

Concentration of Cadmium in Various Parts of Selected Biological Species from Four Sampling Sites - 4th Sampling (29/06/06)

BIOLOGICAL SPECIES		Beposo	Bosomdo	Krobo	Shama Beach
Tilapia	Gills µg/g	0.00190 ± 0.00028	0.00178 ± 0.00023	0.00160 ± 0.00021	0.00151 ± 0.00020
	Liver µg/g	0.00411 ± 0.00053	0.00400 ± 0.00052	0.00391 ± 0.00051	0.00387 ± 0.00050
	Muscles µg/g	0.00343 ± 0.00047	0.00339 ± 0.00044	0.00329 ± 0.00043	0.00321 ± 0.00042
	Scales µg/g	0.00148 ± 0.00019	0.00140 ± 0.00018	0.00137 ± 0.00018	0.00131 ± 0.00017
	Mean µg/g	**0.00273 ± 0.00036**	**0.00264 ± 0.00034**	**0.00254 ± 0.00033**	**0.00248 ± 0.00032**
Mudfish	Gills µg/g	0.00679 ± 0.00088	0.00603 ± 0.00078	0.00591 ± 0.00077	0.00564 ± 0.00073
	Liver µg/g	0.01099 ± 0.00143	0.01001 ± 0.00130	0.00989 ± 0.00129	0.00907 ± 0.00118
	Muscles µg/g	0.00891 ± 0.00116	0.00808 ± 0.00105	0.00778 ± 0.00101	0.00748 ± 0.00097
	Mean µg/g	**0.00890 ± 0.00116**	**0.00804 ± 0.00105**	**0.00786 ± 0.00102**	**0.00740 ± 0.00096**
Prawn	Internal organs µg/g	0.00879 ± 0.00114	0.00777 ± 0.00101	0.00732 ± 0.00095	0.00709 ± 0.00092
	Muscles µg/g	0.00631 ± 0.00082	0.00586 ± 0.00076	0.00577 ± 0.00075	0.00561 ± 0.00073
	Scales µg/g	0.00268 ± 0.00035	0.00252 ± 0.00033	0.00239 ± 0.00031	0.00213 ± 0.00028
	Mean µg/g	**0.00593 ± 0.00077**	**0.00538 ± 0.00070**	**0.00516 ± 0.00067**	**0.00494 ± 0.00064**
Crab	Muscles µg/g		0.01371 ± 0.00178	0.01291 ± 0.00168	0.00947 ± 0.00123
	Scales µg/g		0.00493 ± 0.0064	0.00384 ± 0.00050	0.00368 ± 0.00048
	Mean µg/g		**0.00932 ± 0.00121**	**0.00838 ± 0.00109**	**0.00658 ± 0.00086**
Mollusc	Muscles µg/g	0.00988 ± 0.00128	0.00879 ± 0.00114	0.00771 ± 0.00100	0.00640 ± 0.00083
	Scales µg/g	0.00487 ± 0.00063	0.00401 ± 0.00052	0.00399 ± 0.00052	0.00289 ± 0.00038
	Mean µg/g	**0.00738 ± 0.00096**	**0.00640 ± 0.00083**	**0.00585 ± 0.00076**	**0.00465 ± 0.00060**

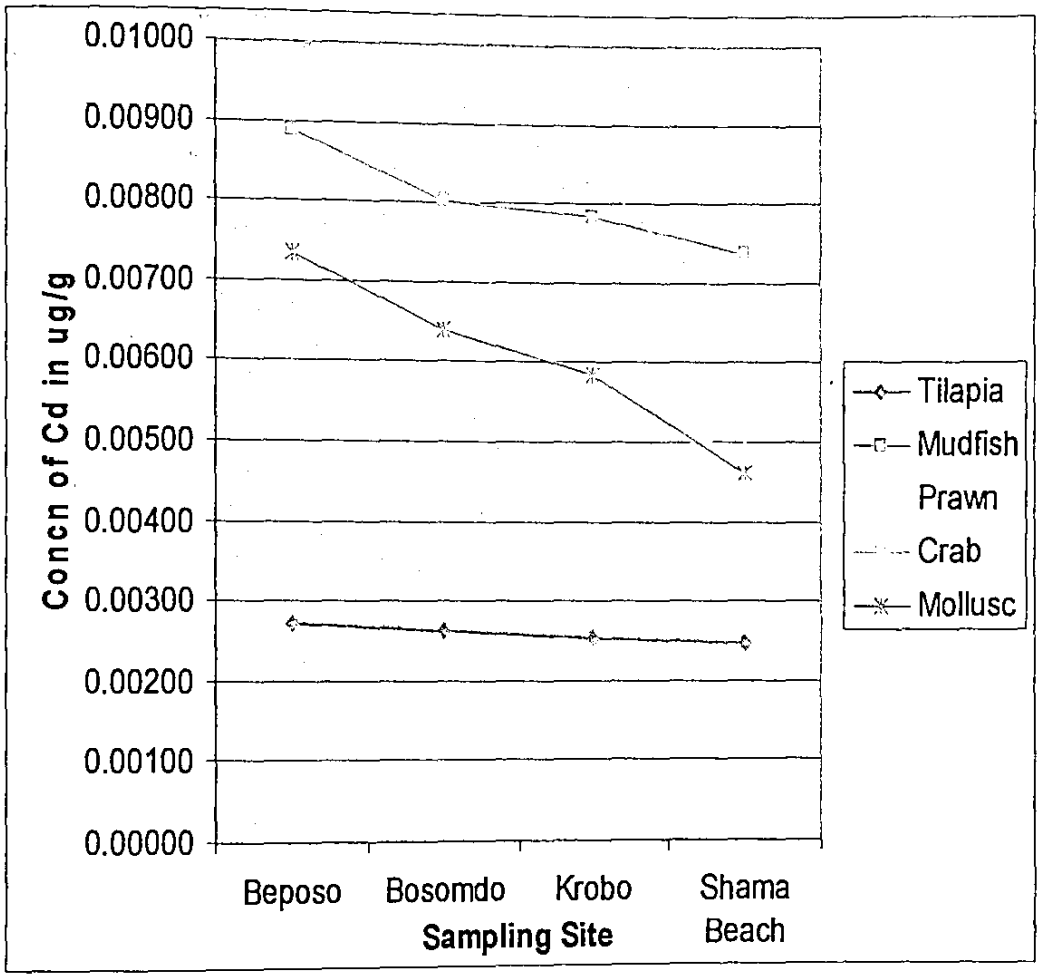


Fig 21. Variation of Cd Concentration in Selected Biological Species Collected on 29/06/06

Discussion

Generally the concentration of mercury and cadmium for each sampling day decreases from Beposo to Shama Beach (figs. 14, 15, 16, 17, 18, 19, 20 and 21). This general decrease in concentration of mercury and cadmium could be attributed to the fact that as the river flows from Beposo to Shama Beach some of the mercury and the cadmium are transformed into vapour (De, 2000) while portions are also taken up by the various biological species in the river.

Also some of these chemicals could also sink into the river bed. That is, by self cleansing, the heavy metals concentrations in the water column decrease as that of the sediment increases, Essumang *et al* (2007). It was also observed that at each sampling site, cadmium levels are higher than mercury levels. This is due to the fact that additional cadmium is being deposited from vehicles that ply the Cape Coast – Takoradi road and make momentary stop at Beposo to pay toll. In addition, mercury is very volatile, so it is possible that the lower concentration of mercury is due to the fact that portions of the mercury might have been evaporated.

From the research, as shown in figs 14, 15, 16, 17, 18, 19, 20 and 21 the concentration of mercury and cadmium in tilapia is the lowest with the crab having the highest levels of the metals. This is because crabs are carnivorous and bottom feeding animals (available at http://wdfw.wa.gov/fish/ans/greencrb.htm). That is there is a biomagnifications of the metals in the crabs as they (crabs) eat other biological species.

On the other hand, tilapia, which feeds mostly on plant and algae do not accumulate much of the metals (Zohary et al., 1994; Cailteux, 1988; Noble, 1989). They are also fast moving fish and therefore do not stay at one area so much, hence the least accumulation of the metals. Molluscs are bottom feeding species and always crawling on the river beds where the water is shallow. They largely feed on decaying organic matter (Encarta, 2004; http://en.wikipedi.org/wiki/Periwinkle_(mollusc)) and therefore also accumulate these metals to a higher level of concentration, although they do not biomagnify the metals as crabs do.

Concentration Levels in Various Parts of the Biological Species

The parts of the species considered were the gills, the liver, the scale (the shell in the case of the crabs and the mollusc) and the muscles. Among these parts, it is the muscles that are usually consumed. The gills of fishes are occasionally consumed. Scales are not consumed at all. However, shells of crabs and prawns are occasionally consumed, due to their high calcium content and delicacy.

For all the parts, the liver accumulated more of the metals than any other part. This is in accordance with the vital functions it plays by detoxifying the body of any toxic materials that enter the body (Abbey *et al* 2001).

The scales accumulated the least amount of the metals. This is due to the fact that the scales are hard refractory materials that resist penetration by metal ions. The levels of concentrations of the metals in the gills follow that of the scale. These

scale. These levels in the gills may be due to the fact that the gills help the fishes to get oxygen by allowing water which contain dissolved oxygen to pass over it so that it (the gills) can trap the oxygen for respiration. In this process some of these metals are also trapped by the gills. The accumulation in the gills is therefore from the process of trapping oxygen from the contaminated river and not through bioaccumulation.

The accumulation in the muscles is from bioaccumulation, and generally it is observed that the concentration levels in muscles follow that of the liver.

Comparing Samples from the River Pra Estuary and the River Ayensu

The results from the samples taken from the River Ayensu near Winneba showed non detectable (nd) concentration of mercury (table 13). However, appreciable levels of cadmium were detected in all the biological samples collected from this river (table 14). From the result (fig 22), Adawukwa, where the River Ayensu is bridged on Accra – Cape Coast road, had the highest concentration of cadmium while Sankro, closed to the sea had the minimum concentration of cadmium in all the biological samples collected. The results of Cd concentration in the biological samples collected from River Ayensu are of about the same value as those collected from the Pra Estuary and this may be due to the Police check point just after the bridge from Accra to Cape Coast.

Control

The control samples taken from the Kakum River at Akotokyir on the University of Cape Coast campus did not show any detectable levels of both mercury and cadmium (table 12).

Table 12

Concentration of Mercury and Cadmium in Various Parts of Selected Biological Species Collected from River Kakum at Akotokyir (Control Sampling)

Biological Species		Mercury	Cadmium
Tilapia	Gills ug/g	nd	nd
	Liver ug/g	nd	nd
	Muscle ug/g	nd	nd
	Scale ug/g	nd	nd
	Mean ug/g	nd	nd
Mudfish	Gills ug/g	nd	nd
	Liver ug/g	nd	nd
	Muscle ug/g	nd	nd
	Mean ug/g	nd	nd

nd = non detectable

Since the Accra – Cape Coast road is as busy as Cape Coast – Takoradi road, the control result, table 12 and the results from the River Ayensu, tables 13 and 14 showed that the source of cadmium in the River Pra might be due to vehicular emissions while mercury from the mining activities upstream.

Table 13

Concentration Mercury in Various Parts of Selected Biological Species Collected from River Ayensu at Winneba (Control Sampling)

BIOLOGICAL SPECIES		Adawukwa	Okyereko	Sankro
Tilapia	Gills ug/g	nd	nd	nd
	Liver ug/g	nd	nd	nd
	Muscle ug/g	nd	nd	nd
	Scale ug/g	nd	nd	nd
	Mean ug/g	nd	nd	nd
Mudfish	Gills ug/g	nd	nd	nd
	Liver ug/g	nd	nd	nd
	Muscle ug/g	nd	nd	nd
	Mean ug/g	nd	nd	nd
Mollusc	Muscle ug/g	nd	nd	nd
	Shell ug/g	nd	nd	nd
	Mean ug/g	nd	nd	nd

nd = non detectable

Table 14

Concentration of Cadmium in Various Parts of Selected Biological Species Collected from River Ayensu at Winneba (Control Sampling)

BIOLOGICAL SPECIES		Adawukwa	Okyereko	Sankro
Tilapia	Gills ug/g	0.00112 ± 0.000134	0.00101 ± 0.000121	0.00098 ± 0.000118
	Liver ug/g	0.00501 ± 0.000601	0.00489 ± 0.000587	0.00294 ± 0.000353
	Muscle ug/g	0.00389 ± 0.000467	0.00347 ± 0.000416	0.00189 ± 0.000227
	Scale ug/g	0.00098 ± .000118	0.00072 ± 0.000086	0.00069 ± 0.000083
	Mean ug/g	**0.00275 ± 0.000330**	**0.00252 ± 0.000303**	**0.00163 ± 0.000195**
Mudfish	Gills ug/g	0.00887 ± 0.001064	0.00741 ± 0.000889	0.00562 ± 0.000674
	Liver ug/g	0.01125 ± 0.001350	0.01099 ± 0.001319	0.00978 ± 0.001174
	Muscle ug/g	0.01089 ± 0.001307	0.00999 ± 0.001199	0.00819 ± 0.000983
	Mean ug/g	**0.01034 ± 0.001240**	**0.00946 ± 0.001136**	**0.00786 ± 0.000944**
Mollusc	Muscle ug/g	0.01007 ± 0.001208	0.01002 ± 0.001202	0.00821 ± 0.000985
	Shell ug/g	0.00725 ± 0.000870	0.00680 ± 0.000816	0.00534 ± 0.000641
	Mean ug/g	**0.00866 ± 0.001039**	**0.00841 ± 0.001009**	**0.00678 ± 0.000813**

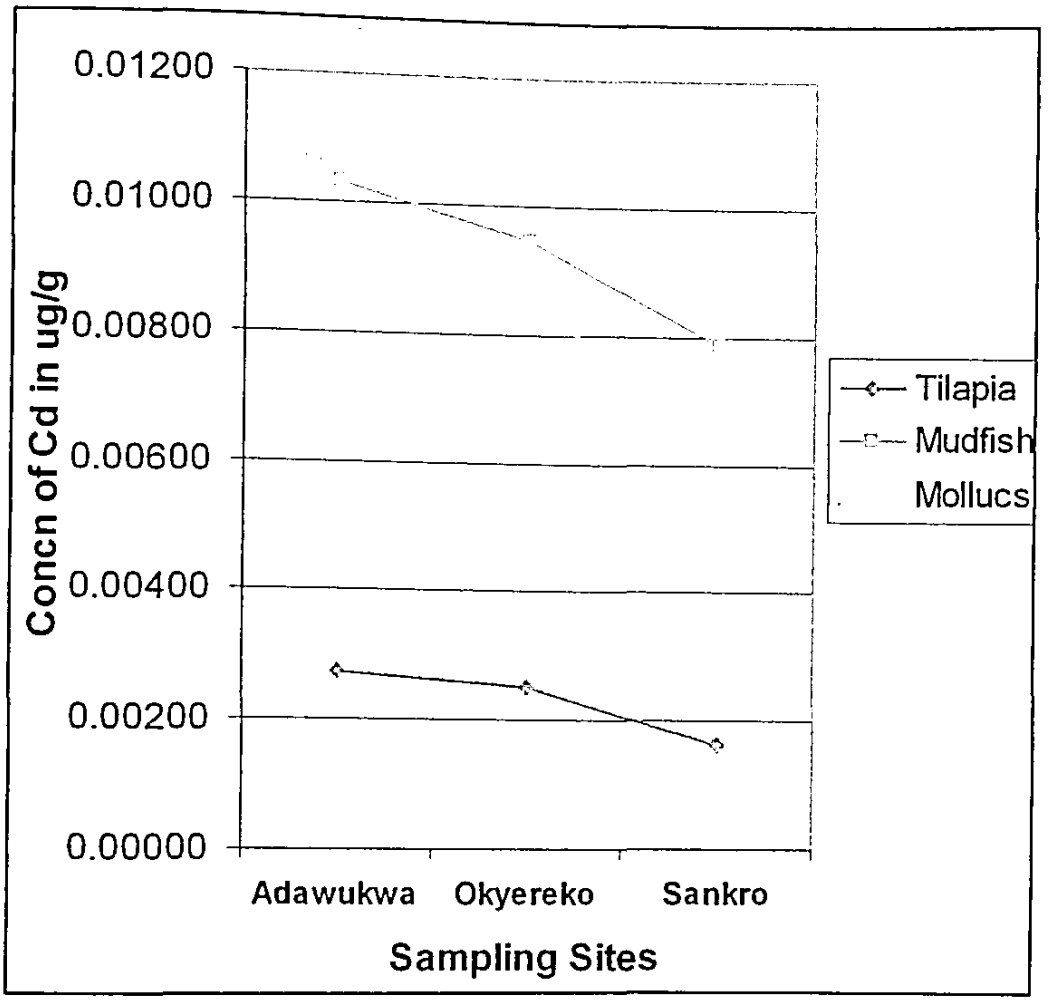


Fig 22 Variation of Concentration of Cd in Selected Biological Samples Collected from River Ayensu Near Winneba

Concentration Levels as Against Rainfall of the Sampling Sites

From the correlation tables, (tables 14 and 15) it was observed that the correlation between the rainfall figures a month before sampling dates and the concentrations of mercury and cadmium is weak and negative. The negative correlation means that as the amount of rainfall increases, the concentration of

shows that the concentration of the metals in various biological samples do not depend on the amount of rainfall. That is the amount of rainfall does not necessary contribute to the accumulation of the metals in the various biological samples. However it could be inferred from tables 11 and 12 that, generally as the rainfall figures increased the concentration of the mercury and the cadmium in the biological samples decreased. In fact, rainfall figures about a month before sampling were used to determine the correlation between the rainfall and the metals in the water column.

Table 15

Rainfall - Mercury Concentration Correlation Table

	Rainfall/mm	Tilapia	Mudfish	Prawn	Crab	Mollusc
1st week in April	263.7	0.00112	0.00355	0.00256	0.00571	0.00325
3rd week in April	206.2	0.00076	0.00314	0.00204	0.00509	0.00287
1st week in May	454.0	0.00001	0.00195	0.00176	0.00223	0.00149
4th week in May	1276.7	0.00008	0.00274	0.00197	0.00345	0.00208
Correlation coefficient		**-0.65616**	**-0.2894**	**-0.34203**	**-0.46555**	**-0.46823**

Table 16

Rainfall – Cadmium Concentration Correlation Table

	Rainfall/mm	Tilapia	Mudfish	Prawn	Crab	Mollusc
1st week in April	263.7	0.00312	0.01402	0.00755	0.01327	0.01135
3rd week in April	206.2	0.00245	0.00994	0.00623	0.01017	0.00796
1st week in May	454.0	0.00245	0.00689	0.00489	0.00717	0.00557
4th week in May	1276.7	0.0026	0.00787	0.00535	0.00843	0.00607
Correlation coefficient		**-0.17537**	**-0.50618**	**-0.51043**	**-0.47398**	**-0.54277**

Levels of Mercury and Cadmium in the Muscles (Flesh) as Against Lethal Level

The European Commission regulation 1881/2006 gave the maximum acceptable level of mercury in the muscles of fish products and muscle meat of fishes, crustaceans, including the brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans to be 0.50mg/kg (0.50μg/g) wet weight. Fortunately, the levels of mercury determined in the muscles of the various biological species were between 0 and 0.00808 ± 0.00097 μg/g. These levels are far below the maximum acceptable level. This means that eating the muscles of tilapia, mudfish, prawn, crab and mollusc, may not pose any serious health problem.

In fact, the concentrations in the muscles of the biological samples are in the decreasing order of crab >mollusk > mudfish > prawn > tilapia. This means that it is safer to consume tilapia than other fishes from the river Pra estuary. Also, consuming crab from the river Pra estuary may be relatively dangerous.

The maximum acceptable level of cadmium in muscles meat of the fishes considered above is 0.050 mg/kg (that is 0.050 μg/g) wet weight. But the levels determined in this work lie between 0.00101 ± 0.00013 μg/g and 0.02024 ± 0.00263 μg/g. Even though these levels also may not pose any immediate major health risk to the human beings who may eat any biological species from the river since it is below the European Commission regulation acceptable level, one must be conscious bioaccumulation.

Statistical Treatment of Data

Pooled Standard Deviation for Tilapia

The values in columns 4 and 5 for 1st sampling (table 3) were computed as follows:

Xi	I(Xi –)I	(Xi –)^2
0.00125	1.3×10^{-4}	1.69×10^{-8}
0.00116	4.0×10^{-5}	1.6×10^{-9}
0.00106	6.0×10^{-5}	3.6×10^{-9}
0.00112	1.1×10^{-4}	1.21×10^{-8}
0.00459	Sum of squares = 3.42×10^{-8}	

= = 0.00112

Table 17

Calculation of Standard Deviation

Sampling number	No. of samples measured	Hg Concentrations in ug/g	Mean in ug/g	Sum of squares of deviations from means
1st	4	0.00125, 0.00116, 0.00106, 0.00112	0.00112	3.42×10^{-8}
2nd	4	0.00081, 0.00078 0.00074, 0.00076	0.00076	6.875×10^{-9}
3rd	4	0.00004, 0.00000 0.00000, 0.00001	0.00001	1.075×10^{-9}
4th	4	0.00015, 0.00009 0.00006, 0.00009	0.00009	6.075×10^{-9}
Total number of samples measured (N) =16			Sum of sum of Squares = 4.82×10^{-8}	

The other data in columns 4 and 5 were obtained similarly.

Pooling data for Hg concentration in all tilapia samples analyzed

$$S_{pooled} = \sqrt{\frac{\sum_{i=1}^{N_1}(x_i - \bar{x})^2 + \sum_{j=i}^{N_2}(x_j - \bar{x})^2 + \sum_{k=1}^{N_3}(x_k - \bar{x})^2 + \ldots + \sum_{p=1}^{N_{n_i}}(x_p - \bar{x}_{n_i})^2}{N_1 + N_2 + N_3 + \ldots - n_i}}$$

$$S_{pooled} = \sqrt{\frac{3.42\times10^{-8} + 6.875\times10^{-9} + 1.075\times10^{-9} + 6.075\times10^{-9}}{16 - 4}}$$

$= 6.34 \times 10^{-5}$ or 0.0000634

Confidence Limit of the Work Done

Confidence limit (CL) for tilapia (1st sampling) is given by

$$95\%\ \text{CL} = \bar{x} \pm \frac{zs}{\sqrt{N}}$$

Where z is the value for 95% confident level, s is the pooled standard deviation and N is the number of samples measured.

$$\therefore\ 95\%\ \text{CL} = 0.00112 \pm \frac{1.96 \times 6.34 \times 10^{-5}}{\sqrt{4}}$$

$$= 0.00112 \pm 0.000062$$

Therefore the 95% confidence limits in ug/g for the concentration of Hg in tilapia for 2nd, 3rd and 4th sampling are

2nd sampling	0.00076 ± 0.000062
3rd sampling	0.00001 ± 0.000062
4th sampling	0.00009 ± 0.000062.

Similarly, pooled standard deviation for concentration of Cd in all the tilapia analyzed is 0.00031397 (3.1397×10^{-4}) and the 95% confidence limit in ug/g for Cd concentration in the tilapia are

1st sampling	0.0031225	0.00030769
2nd sampling	0.0024525	0.00030769
3rd sampling	0.002455	0.00030769
4th sampling	0.0022975	0.00030769.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Conclusion

From the work done, some amount of mercury and cadmium were found in the biological species collected from the River Pra Estuary, meaning that these metals find their way into the estuary. That is these biological species, which are delicacies among Ghanaians, tend to take up these pollutants (metals) from the river. However, the levels in the biological samples are below the maximum acceptable levels by European Commission standard.

It was observed in this research that the amount of rainfall at a particular time does not contribute to the levels of the metals the biological species could accumulate. Also, since the levels of cadmium and mercury were within the acceptable limit, these biological samples may not pose much health hazards to human being who eat them.

Since cadmium levels at Beposo and Adawukwaa, where bridges are located are high, the cadmium source into the river could be attributed the vehicles applying the road rather than any other sources.

It is clear from the research that the liver of the biological species accumulate more toxic materials than any other part of the body, while the scales and the shells accumulate the least

Among the biological species considered in this research, the bio-concentration in tilapia is the least while the crab is the highest. Therefore it is advisable to eat more tilapia than crab from the river Pra Estuary.

Recommendation for Feature Research

It is recommended that similar research should be carried out on other estuaries in the country in other to ascertain the safety or otherwise of eating fish from those estuaries. The research should include the seasonal variation and the scope of the pollutants to be covered in the research should be widen to cover other pollutants such as lead, copper, arsenic, cyanide, polyaromatic hydrocarbons (PAHs), persistent organic pollutants (POP), etc. Also, since the results from the river Ayensu (near Winneba) showed an appreciable levels of cadmium, it is important to analyze waters from all the major bridges on the trunk roads in the country to ascertain the contribution of the vehicular fall outs in polluting the water bodies in the country.

Intensive medical research should be carried out around polluted rivers/water bodies to find out if the pollution in the rivers has any serious health effect on the people living around those water bodies, especially the fishermen and the fish-mongers and their families.

Public education on preserving the environments especially those of water bodies should be intensified so as to control pollution of the water bodies so that eating fish and other biological species from the water bodies may be safe. Also, large scale fish farming should be promoted to make fish consumption safer in the country.

The mercury pollution is also more likely to be originating from the "galamsey" (illegal) mining at the up stream of the river.

The Ministries of Environment, Land and Mineral Resources, the Mineral Commission, the Ghana Chamber of Mines and the Ministry of Education, Youth and Sports should team up to educate the galamsey miners in and around the area about the effect of the pollution and how to minimize it. They should also monitor their activities in order to prevent further contamination of the estuary with these metals.

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