AN ASSESSMENT OF HEAVY METAL CONTAMINATION OF SEDIMENTS AND TISSUES OF THE CLAM GALATEA PARADOXA (BORN 1778) IN THE VOLTA ESTUARY, GHANA

A THESIS SUBMITTED TO THE DEPARTMENT OF FISHERIES AND WATERSHED MANAGEMENT IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF PHILOSOPHY (MPHIL) IN FISHERIES SCIENCE AND WATERSHED MANAGEMENT

BY

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KUMASI

MAY, 2010

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STATEMENT OF ORIGINALITY

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Dear Prof. Dr. | Head of Department of Fisheries and Watershed Management, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

I am delighted to confirm that I have seen and read the thesis entitled " AN ASSESSMENT OF HEAVY METAL CONTAMINATION OF SEDIMENTS AND TISSUES OF THE CLAM *GALATEA PARADOXA* (BORN, 1778) IN THE VOLTA ESTUARY, GHANA" produced by Mr.\ **OBIRIKORANG KWASI ADU** from Department of Fisheries and Watershed Management, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology**,** Kumasi, Ghana**.**

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Best regards

Signature

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LIST OF ABBREVIATIONS AND UNITS

µg/g dw: Microgram per gram dry weight
µg/g: Microgram per gram
µg/ml: Microgram per millilitre
AAS: Atomic Absorption Spectrophotometer
AF: Accumulation Factors
Al: Aluminium
ANOVA: Analysis of variance
ANZECC: Australian and New Zealand Environment and Conservation Council As: Arsenic
AVFs: Acid-Volatile sulfides
BCF: Biological Concentration Factors
BSAF: Biosediment Accumulation Factors
Cd: Cadmium
CIFA: Committee for Inland Fisheries of Africa
Co: Cobalt
Cr: Chromium
Cu: Copper
DO: Dissolved Oxygen
DWAF: Department of Water Affairs and Forestry
EAF: East Africa Marine Pollution and Research Praogramme
FAO: Food and Agriculture Organisation
Fe: Iron g: gram
g/cm³: gram per centimetre cubed
GESAMP**:** The Group of Experts on Scientific Aspects of Marine Environmental Protection
H_2SO_4 : Sulphuric Acid
HCl: Hydrochloric Acid
$HClO_4$: Perchloric Acid
Hg: Mercury
HNO_3: Nitric Acid
IAEA: International Atomic Energy Agency
IOC: Intergovernmental Oceanic Commission
IQ: Intelligence Quotient
$KMnO_4$: Potassium Permanganate
LDPE: Low-density polyethylene m^2 /day: Meter squared per day
MEDPOL: Mediterranean Pollution Monitoring and Research Programme ml: Millilitre
Mg/kg: Milligram per kilogram mg/km: Milligram per kilometer mg/kg: Milligram per kilogram
Mg: Magnesium
Mn: Manganese

Mo: Molybdenum
NAS/NRC: National Academy of Science of the National Research Council
Nb: Niobium
ND: None Detected ng/g:
Nanogram per gram
Ni: Nickel
ºC: Degrees Celsius Pb: Lead
ppm: Part per million PTEs:
Potential Toxic Elements
Rb: Rubidium
RNA: Ribonucleic acid
Sb: Antimony
Se: Selenium
$SnCl_2.2H_2O$: Stannous Chloride
SPM: Suspended Particulate Matter
Sr: Strontium
TDS: Total Dissolved Solid
UNECE: United Nations Economic Commission for Europe
UNEP: United Nations Environment Programme
UNESCO
USEPA: United States Environmental Protection Agency
V: Vanadium
VRA: Volta River Authority
WACAF: West and Central Africa Marine Pollution and Research Programme
WHO: World Health Organisation
Y: Yttrium
Zn: Zinc
Zr: Zirconium

ABSTRACT

The concentrations of four heavy metals, Mn, Zn, Fe (essential heavy metals) and Hg (non-essential heavy metal) were determined in sediments and in whole soft tissue of the clam *Galatea paradoxa* (Born 1778) from two clam fishing locations, Ada and Aveglo at the Volta Estuary in Ghana over an 18-month period. Thirty (30) clams were obtained from each sampling location monthly and grouped into three size classes of 10 individuals each based on shell lengths as follows: small (25 40mm), medium (41-55mm), and large (above 55mm). The groupings were chosen based on the three dominant size groups in the natural population to give a broad and representative range of metal concentrations in the clams. All the results were expressed as total concentrations (μg/g dry weight (dw). Mean concentrations of analyzed metals in the tissue of the clams from the Ada sampling station were: Mn: 152.9 μg/g; Fe: 174.9 μg/g, Zn: 34.6 μg/g and Hg: 0.043 μg/g. The mean metal concentrations in the Aveglo clams were: Mn: 130.0 μg/g, Fe: 187.0 μg/g, Zn: 37.1 μg/g and Hg: 0.046 μg/g. Mean metal concentrations in the sediments were Mn: 186.0 μg/g, Fe: 1770.4 μg/g, Zn: 3.2 μg/g and Hg: 0.0086 μg/g for the Ada sampling station. The Aveglo sediments had mean metal concentrations as follows: Mn: 171.9 μg/g, Fe: 1758.5 μg/g, Zn: 3.7 μg/g and Hg: 0.0115 μg/g.

Metal concentrations in the tissues of the different clam size-classes (small vs. small, medium vs. medium and large vs. large) from the two sampling stations were almost identical and did not vary significantly ($p>0.05$). A comparative evaluation of the metal concentrations in the clams and sediments from the two stations, however revealed significant variations in concentrations for Zn Fe and, Hg. Concentration of Fe in the Ada sediment samples for June was as much as 18 times higher than the concentration in the clams and Hg concentrations were approximately five (5) times higher in the clam tissues than in the sediments during the study period. On the basis of calculated BSAFs the metal enrichment in the tissues of the clams rank in the following order Zn>Hg>Mn>Fe. The BSAFs indicated a significant accumulation of Zn and Hg in the clam tissues relative to the concentrations of these metals in the sediments although no clear relationships were established between the concentrations of the studied heavy metals in the clam tissues and sediments.

There were no significant differences ($p >0.05$) in Mn, Fe and Zn concentrations among the different size classes except for Hg concentration in clams from Ada, indicating a similar bioavailability of Mn, Fe, Zn at both locations and, possibly, an efficient metabolism to keep the concentrations of Mn, Fe and Zn relatively similar in the tissues of the different clam sizes. Spatial variations in metal concentrations in the clams (i.e., Ada small vs. Aveglo small, Ada medium vs. Aveglo medium, and Ada large vs. Aveglo large) were not significant for all four studied metals in the compared size classes. Results of the statistical test for spatial variations in the sediment samples from the two stations also revealed no significant differences ($p>0.05$) in the concentrations of Mn, Zn, Fe and THg during the study period.

To understand the relationships between metal concentration in the sediments and accumulation in the tissues of the three clam size classes as far as Mn, Zn, Fe and Hg were concerned, the monthly concentrations of the studied metals were graphed to observe distinct metal accumulation patterns

The graphs revealed no simple linear relationships between the concentrations of heavy metals in the clam tissues and the sediments at the two sampling stations although some distinct accumulation trends were observed as far as Mn was concerned.

Analysis of risks levels associated with the consumption of clams by humans revealed that the concentration of the Mn, Zn, Fe and Hg found in the clam tissues were within permissible limits using reference guides such as the WHO Safety Reference Standards for Bivalves and various indicators as the Tolerable daily Intake (TDI), rate of shellfish consumption (RSC), Risk Quotients (RQs) and levels of concerns (LOCs)

Based on geoaccumulation calculations, the sediments from the two sampling stations are unpolluted as far as the heavy metals, Manganese, Zinc, Iron and Mercury are concerned and the samples are similar to those observed in areas under low pollution impact.

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CHAPTER 1

INTRODUCTION

1.1 Background

Heavy metals may occur in aquatic environments from natural processes and from discharges or leachates from several anthropogenic activities (Connell *et al.*, 1999; Franca *et al.,* 2005). The contamination of natural waters by heavy metals negatively affects aquatic biota and poses considerable environmental risks and concerns (Cajaraville *et al.*, 2000; Ravera, 2001). Monitoring programmes and research on heavy metals in aquatic environments samples have become widely important due to concerns over accumulation and toxic effects in aquatic organisms and to humans through the food chain (Otchere, 2003). Contaminants can persist for many years in sediments in both freshwater and marine systems where they hold the potential to affect human health and the environment (Mackevičiene *et al.*, 2002).

Sediments are an important sink of a variety of pollutants, particularly heavy metals and may serve as an enriched source for benthic organisms (Wang *et al.*, 2002) especially in estuarine ecosystems. Metals may be present in the estuarine system as dissolved species, as free ions or forming organic complexes with humic and fulvic acids. Additionally, many metals e.g. Pb associate readily with particulates and become adsorbed or co-precipitated with carbonates, oxyhydroxides, sulphides and clay minerals. Consequently, sediments accumulate contaminants and may act as long-term stores for metals in the environment (Spencer and MacLeod, 2002). Exposure of sediment-dwelling organisms to metals may then occur via uptake of interstitial waters, ingestion of sediment particles and via the food chain (Luoma, 1989). The occurrence of elevated concentrations of trace metals in sediments found at the bottom of the water column can be a good indicator of man-induced pollution rather than natural enrichment of the sediment by geological weathering (Davies *et al.* 1991, Chang *et al.* 1998).

The analyses of water or sediment samples, however, are subject to a variety of shortcomings, in that the methods do not allow for the estimation of the quantity of the metal which is biologically available (Etim *et al.*, 1991). It is against this background that bio-indicators are preferred in environmental monitoring. Bivalves are effective biomonitors and have been widely used for heavy metal monitoring

purposes worldwide (Phillips and Yim, 1981, Etim *et al.*, 1991, Ferreira *et al.*, 2004, Otchere, 2003, Tay *et. al.*, 2004).

Of all the possible biomonitors available for monitoring aquatic environments, bivalves fulfil most of the above-mentioned characteristics. This is because they are widely distributed globally, easy to handle and sessile. They are also filter feeders that have the ability to accumulate high metal concentrations without metabolising the metals appreciably (Gunther *et. al.*, 1999; Nasci *et al.*, 1999; Olivier *et al.*, 2002), provide a time-integrated indication of environmental contamination (Regoli, 1998). Their ability to concentrate pollutants in their tissues at concentrations greater than the ambient water (El-Shenawy, 2002) and provides a solution to the problem of not being able to estimate the biologically available quantity of heavy metals using water or sediment samples (Butler *et al.*, 1971). Heavy metal accumulation in bivalves is however influenced by several abiotic and biotic factors (Phillips and Rainbow, 1994). Some of these include seasonality (Regoli, 1998), location (Blackmore and Wang, 2003) salinity (Chong and Wang, 2001), organic matter (Pan and Wang, 2004), sex (Sokolowski *et al.*, 2003), food acquisition capability (Saavedra *et al.*, 2004), stage of gonadal development (Bryan *et al.*, 1980) and size-weight relationships (Phillips, 1976, Riget *et al.*, 1996).

The clam, *Galatea paradoxa* (Born 1778) was chosen for this research because it satisfies most of the above-mentioned characteristics of bivalves as a possible biomonitor, and is a commercially-important bivalve species exploited mainly for its flesh. It is consumed either as boiled or fried. It is a filter-feeding organism with a wide distribution extending from the Gulf of Guinea to the Congo (Moses, 1990). Limited information about the prevalence and commercial exploitation of this clam is available from only a few countries, including Ghana, Nigeria and Cameroon, despite its extensive distribution in the wider north-west African region.

In Ghana, Ada and Aveglo represent the main fishing grounds for *Galatea paradoxa* in the Volta estuary. The clam fishing industry represents a viable source of income and livelihood for the local people. The clams serve as a protein source and are consumed by people in and around the estuary and even beyond. Calculated on a dry matter basis, the average protein content of the smoked clam is 46.5% (Kwei, 1965). It constitutes an important and affordable protein source to the riparian communities around the Volta (Amador, 1997).

1.2 Justification

Heavy metals are non-biodegradable and undergo a global eco-biological cycle in which natural waters are the main pathways (CIFA, 1994; Ukpebor *et al.*, 2005) and *G. paradoxa*, like all bivalves can accumulate these heavy metals in their tissues at concentrations greater than the ambient water and pose a health threat to humans who consume them.

In the human body, the metallic toxicants attack the proteins notably the enzymes (Ademoroti, (1996) and their toxic effects are cumulative and cause slow poisoning of the system over a period of time (Nriagu, 1988; Ukpebor *et al.,* 2005). Heavy metals have been implicated in the upsurge of liver and kidney diseases, and is believed to be responsible for a high proportion of mortality caused by kidney and liver morbidity (Friberg, *et al.*, 1986; Herber *et al.*, 1988; Ndiokwere, 2004), pains in bones (Tsuchiya, 1978), mutagenic, carcinogenic and teratogenic effects (Fischer, 1987; Friberg *et al.*, 1986, Kazantzis, 1987, Heinrich, 1988), neurological disorders, especially in the foetus and in children which can lead to behavioral changes and impaired performance in IQ tests (Lansdown, 1986; Needleman, 1987).

In recent years, there has been a proliferation of rural metal fabrication and agricultural industries along the Volta basin. Locations in the estuary including Ada and Aveglo have been implicated as areas impacted by these industries. Recent agricultural developments below the Akosombo Dam include irrigated rice, sugar, and vegetable cultivation in the areas immediately adjoining the Volta River and the estuary. The applications of sewage sludge, sewage water, pesticides and fertilisers to these agricultural lands contribute to the accumulation of the heavy metals in top soil layers and their subsequent spreading to large areas of the estuary through surface run-off. The smelting activities of the small scale metal fabricating industries activities not only release the target metals but also metals which are associated in the ores.

Anecdotal information suggests that the basin might be receiving a considerable range of polluting effluents, particularly heavy metals from these sources. The health risks associated with heavy metal poisoning in man and the environment are of concern to environmentalists and government agencies locally and globally and underscores the need for continuous research with a view to ameliorating the problems of environmental pollution by heavy metals. It is, therefore, very important for studies to be conducted on the levels of these heavy metals in the tissues of *G. paradoxa*, and in sediments of the Volta Estuary, and to ascertain whether or not the concentrations in the clams are within the

permissible limits for human consumption in comparison to Safety Reference Standards for the Consumption of Bivalves and Molluscs.

1.3 Objectives

This study was, therefore, be conducted to assess the concentrations of some heavy metals in the soft tissues of the clams and sediments of the Volta Estuary. Against this background, the specific objectives of this study were to:

1. Examine the level of some heavy metals in whole soft tissue of G*alatea paradoxa* and sediments of the Volta Estuary at Ada and Aveglo

2. To examine the variations in heavy metal concentrations in the tissue of *G. paradoxa* in relation to body size.

3. To examine the spatial and temporal trends of heavy metal levels in the tissues *of G. paradoxa* and sediment samples of the Volta Estuary.

1.4 Hypotheses

The following hypotheses were tested:

i) There are significant differences in metal concentrations in the tissues of *G. paradoxa* in relation to size

ii) Heavy metal concentrations are significantly different between the two sampled areas in accordance with local characteristics (substrate types, surface runoff and anthropogenic activities) iii) There is a seasonal variation in heavy metal concentrations during the year.

The studies on heavy metals at the Volta estuary were limited to Manganese (Mn), Zinc (Zn), Iron (Fe) and Mercury (Hg) because a preliminary study on seven (7) selected metals, including the above-listed ones and Copper, Lead and Cadmium indicated that the concentrations of the excluded metals (Copper, Lead and Cadmium) were in trace amounts below the detection limits of the Atomic Absorption Spectrophotometer (AAS) used for the analysis and registered as None Detected (ND).

CHAPTER 2

LITERATURE REVIEW

2.1 Environmental Pollution

Growing social concern about environmental quality can be observed in recent years, both on a global and local scale. Emission of harmful substances has negative effects on the natural environment, human health and agricultural production efficiency (Gadzała-Kopciuch, 2004). When the consequences of environmental pollution become visible, it is often too late to prevent them and chronic toxic effects, impossible to notice at the initial stage of the process, may manifest after many years (Alloway and Ayres, 1998). Toxic chemical substances introduced into the environment may be transported by the air, water and living organisms and may become a part of the natural biogeochemical cycle and accumulate in the food chain (Gadzała-Kopciuch, 2004). Water constitutes the "trouble spot" of all ecosystems, as many pollutants are waterborne and also plays an important role as a solvent of various substances, and as a medium in the cycle: air-soil-plantsanimals (Nałęcz-Jawecki and Sawicki, 1998).

Due to constant technological progress the natural environment undergoes numerous changes, deteriorating its quality, which often results in negative interactions between particular ecosystem components. Many of the heavy metals are toxic to organisms at low concentrations. However, some heavy metals, such as copper and zinc are also essential elements. Concentrations of essential elements in organisms are normally homeostatically-controlled, with uptake from the environment regulated according to nutritional demand. Effects on the organisms are manifest when this regulation mechanism breaks down as a result of either insufficient (deficiency) or excess (toxicity) metal (Duffus, 2002).

2.2 Heavy Metals

Heavy metal is a general collective term which applies to the group of metals and metalloids with an atomic density greater than 4g/cm^3 (Duffus, 2002). They are defined by the United Nations Economic Commission for Europe (UNECE) as "those metals or, in some cases, metalloids which are stable and have a density greater than 4.5 g/cm^3 and their compounds" (UNECE 1998). Alloway (1995a) defines heavy metals as "elements which have an atomic density greater than 6 g/cm^3." Another term,

Potential Toxic Elements (PTEs), has been used for this group of metals to avoid inconsistencies (Alloway 1995). Yet again many prefer the term „trace metals" when referring to metals of low natural concentrations. However the term „heavy metals" is still the most used and recognised term, and is therefore used throughout the thesis.

Although it is a loosely defined term, heavy metals are widely recognised and usually applies to the widespread contaminants of terrestrial and freshwater ecosystems. Examples of heavy metals are cadmium, chromium, copper, mercury, lead, zinc, arsenic, boron and the platinum group metals, which comprises Platinum, Palladium, Rhodium, Ruthenium, Osmium, and Iridium. Unlike almost all organic pollutants, such as organochlorines, heavy metals are elements which occur naturally in the Earth" s crust. They are therefore found naturally in soils and rocks with a subsequent range of natural background concentrations in soils, sediments, waters and organisms. Anthropogenic releases can give rise to higher concentrations of the metals relative to the normal background values. The most important anthropogenic releases of heavy metals to the environment come from metalliferous mining and smelting, agricultural materials (pesticides and fertilisers), irrigation and application of sewage water and sludge, fossil fuel combustion and metallurgical industries (Alloway 1995b)

As they are elements, they cannot be broken down; therefore heavy metals will persist in the environment. Unlike many organic pollutants, which eventually degrade to carbon dioxide and water, heavy metals will tend to accumulate in the environment, especially in lake, estuarine or marine sediments and can be transported from one environment compartment to another (Duffus, 2002).

Whether the source of heavy metals is natural or anthropogenic, the concentrations in terrestrial and aquatic organisms are determined by the size of the source and adsorption and/or precipitation in soils and sediments. The extent of adsorption depends on the metal, the absorbent, the physicochemical characteristics of the environment (e.g. pH, water hardness and redox potential) and the concentrations of other metals and complex chemicals present in the soil water, river or lake. Heavy metals also accumulate in organisms as a result of direct uptake from the surroundings across the body wall, from respiration and from food. Uptake *via* food is most important in terrestrial organisms and it may also be important in the aquatic environment. Dietary uptake can include heavy metals adsorbed on particulates present on the surface of leaves, which have not been absorbed by the plant (Duffus, 2002).

The free ion is generally the most bioavailable form of a metal, and the free ion concentration if often the best indicator of toxicity. However, there are exceptions, such as the well known case of mercury, where the organic form, (methylmercury) is more toxic than the inorganic ion. Metals exert toxic effects if they enter into biochemical reactions in the organism and typical responses are inhibition of growth, suppression of oxygen consumption and impairment of reproduction and tissue repair (Duffus, 2002).

2.3 The Toxic Effects of Heavy Metals to the Human Body

The toxicity of a metal is usually defined in terms of the concentration required to cause an acute response (usually death) or a sub-lethal response (Smith, 1986). Predicting the consequences of metal exposure on living organisms is complicated because metals may be essential or non-essential. Very low concentrations of essential metals can be as harmful as high concentrations (Figure 1, upper panel). Non-essential metals display more conventional toxicity curves, showing a sigmoidal increase in proportion of exposed individuals dying with an increase in metal concentration (Figure 1, lower panel) (Newman and Clements, 2008).

Understanding this dichotomy of essential and non-essential metal concentration–effect curves can still be insufficient for sound prediction of metal effects. For example, chronic exposure to the nonessential element cadmium can cause symptoms of zinc deficiency because cadmium displaces zinc in metalloenzymes. Excessive amounts of non-essential tungsten can cause an apparent deficiency of molybdenum, an essential and chemically similar element (Mertz 1981). Such an effect would appear as a shift to the left for the curve shown in the upper panel of Figure 1 (*x*-axis being the essential metal concentration). The bioactivity of some non-essential elements can also be affected by another element. For example, mercury toxicity is lowered if sufficient concentrations of selenium are also present. This would cause the curve in the lower panel of Figure 1 to shift to the right (Newman and Clements, 2008).

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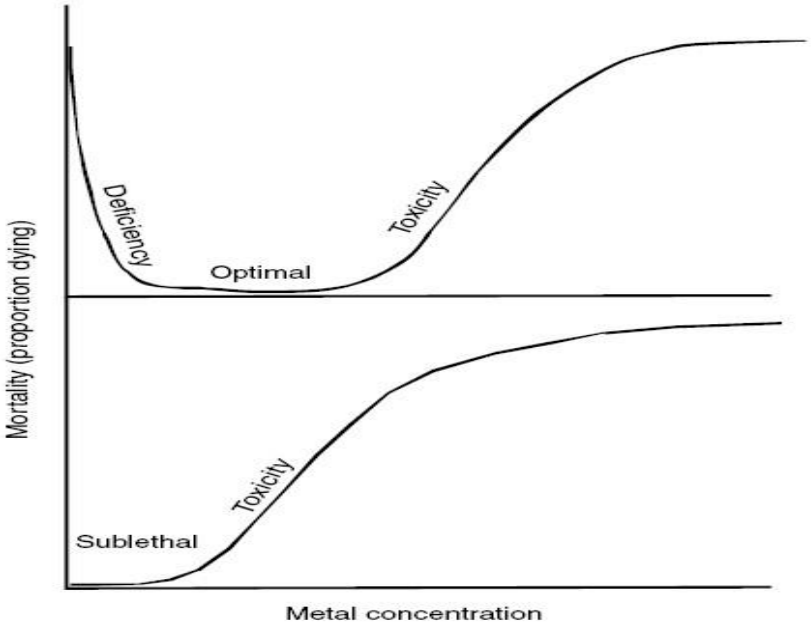

Figure 2.1 Mortality versus concentration for essential (upper panel) and non-essential (lower panel) metals (Newman and Clements, 2008)

The following subchapters review the toxic effects of heavy metals, especially the four studied metals on man and the environment.

Many of the heavy metals are toxic to organisms at low concentrations. However, some heavy metals, such as copper and zinc are also essential elements. Concentrations of essential elements in organisms are normally homeostatically-controlled, with uptake from the environment regulated according to nutritional demand. Effects on the organisms are manifest when this regulation mechanism breaks down as a result of either insufficient (deficiency) or excess (toxicity) metal (Duffus, 2002).

Copper is one of several heavy metals that are essential to life despite being as inherently toxic as non-essential heavy metals exemplified by Lead (Pb) and Mercury (Hg) (Scheinberg, 1991). Plants and animals rapidly accumulate it. It is toxic at very low concentration in water and is known to cause brain damage in mammals (DWAF, 1996). Interest in these essential metals which are required for metabolic activity in organisms lies in the narrow "window" between their essentiality and toxicity (Skidmore, 1964; Spear, 1981). Non-essential metals like Aluminium (Al), Cadmium (Cd) and Lead (Pb) exhibit extreme toxicity even at trace levels (Merian, 1991).

Cadmium (Cd) has been found to be toxic to fish and other aquatic organisms (Rao and Saxena, 1981; Woodworth and Pascoe, 1982). The effect of Cd toxicity in man includes kidney damage (Friberg, *et al.*, 1986; Herber *et al.*, 1988) and pains in bones (Tsuchiya, 1978). Cd also has mutagenic, carcinogenic and teratogenic effects (Fischer, 1987; Friberg *et al.*, 1986, Kazantzis, 1987, Heinrich, 1988).

Lead is defined by the United States Environmental Protection Agency (USEPA) as potentially hazardous to most forms of life, and is considered toxic and relatively accessible to aquatic organisms (USEPA, 1986). Lead is bioaccumulated by benthic bacteria, freshwater plants, invertebrates and fish (DWAF, 1996). The chronic effect of lead on man includes neurological disorders, especially in the foetus and in children. This can lead to behavioral changes and impaired performance in IQ tests (Lansdown, 1986; Needleman, 1987).

2.3.1 Mercury

There are three forms of mercury and among these the most toxic one is the organic form, methyl mercury. Methyl mercury is microbiologically transformed from inorganic mercury when it reaches aquatic environments, in water bodies or in soils (Zahir *et al.*, 2005). Inorganic- and organic mercury is toxic to the human body in different ways, effecting different organs in different ways. Inorganic mercury can cause neurological and psychological symptoms, such as tremor, changes in personality, restlessness, anxiety, sleep disturbance and depression. These symptoms are however reversible after ending of exposure to inorganic mercury. Inorganic mercury is also an allergen, which may cause contact eczema. The kidneys are the organs that accumulate the highest levels of mercury compared to brain and liver. This can cause kidney damage which is reversible after the exposure has stopped (Zahir *et al.*, 2005).

Methyl mercury, toxicity is not reversible as it is with inorganic mercury. Organic mercury affects the nervous system and the main symptoms of methyl mercury poisoning relate to damage of the nervous system. The earliest symptoms of poisoning are parestesias and numbness in the hands and feet. Later symptoms are coordination difficulties and concentric constriction of the visual field (Järup, 2003). Other symptoms are memory loss, shortfall in attention and Alzheimer" s disease like dementia (Zahir *et al.*, 2005). Hock *et al.* (1998) conducted a study on whether environmental factors may influence the risk of getting Alzheimer" s disease and found that Alzheimer" s disease patients had a two-fold

higher blood-mercury level than the control group and that early onset Alzheimer" s disease patients, blood-mercury levels where three-fold higher than the control group. Exposure of the foetus of humans to mercury can also cause late development of speech, late walking, memory shortfall in attention and autism (Zahir *et al.*, 2005).

The general human population is primarily exposed to mercury *via* food, where fish is the major source of methyl mercury exposure (Järup, 2003).

Mercury has no necessary function in any living organism and is considered as a non-essential metal, is among the most toxic elements to man and many higher animals (Steinnes, 1995; Landner and Lindestrom, 1998). Mercury has caused more problems to the consumers of fish than any other inorganic contaminant. In extreme cases, consumption of mercury-tainted fish has led to the onset of a serious neurological disease, termed Minamata disease. Victims of the disease are diagnosed as having a degeneration of their nervous systems. Numbness occurs in their limbs and lips. Their speech becomes slurred, and their vision constrict. Some people have serious brain damage, while others lapse into unconsciousness or suffer from involuntary movements. Furthermore, some victims are thought to be crazy when they begin to uncontrollably shout. In other cases, entire fisheries have been either restricted or significantly curtailed because of mercury contamination (Moore, 1991).

2.3.2 Zinc

Zinc is widely used in modern society, most commonly to coat or galvanise iron to prevent corrosion. It is also mixed with other metals to form alloys such as brass. Particles released from vehicle tyres and brake linings are a major source of zinc in the environment (WHO, 2001).

Zinc is an essential nutrient for the human body and has an importance for health (Hotz *et al.*, 2003). Zinc acts as a catalytic or structural component in many enzymes that are involved in energy metabolism and in transcription and translation of RNA (Moolenaar, 1998). Zinc also has a prominent role in determining the outcome of pregnancies and supporting neurobehavioral development (Hotz *et al.*, 2003). However, like other metals, it can be toxic in high concentrations (ANZECC, 2000). Although uncommon, gastrointestinal distress and diarrhoea have been reported following ingestion of beverages standing in galvanized cans or from use of galvanised utensils (WHO, 2001). Other symptoms of Zn toxicity are slow reflexes, shakes, paralyzation of extremities, anaemia, metabolic disorder, terratogenic effects and increased mortality (Klaassen, 1996).

Extensive literature on the aquatic toxicity of Zn and especially its toxicity to fishes has been reviewed by Alabaster and Lloyd (1980) and by Spear (1981). Zinc is unusual in that it has low toxicity to man, but relatively high toxicity to fish (Alabaster and Lloyd, 1980).

2.3.3 Iron

Iron, one of the most abundant metals on Earth, is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health (Institute of Medicine, 2001). In humans, iron is an essential component of proteins involved in oxygen transport (Dallman, 1986). It is also essential for the regulation of cell growth and differentiation (Bothwell, 1979, Andrews, 1986). A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity (Institute of Medicine, 2001, Bhaskaram, 2001). On the other hand, excess amounts of iron in man can result in toxicity and even death (Corbett, 1995).

There is considerable potential for iron toxicity because very little iron is excreted from the body. Thus, iron can accumulate in body tissues and organs when normal storage sites are full. For example, people with hemachromatosis are at risk of developing iron toxicity because of their high iron stores. Symptoms of Alzheimer" s and Parkinson" s disease may also be iron-related (Corbett, 1995).

2.3.4 Manganese

Manganese is one of the abundant elements in the earth's crust and is widely distributed in soils, sediments, rocks, water, and biological materials. A rough estimate of the average concentration of manganese in the earth's crust is about 1000 mg/kg (NAS/NRC, 1973).

Manganese concentrations in igneous rock may range from about 400 mg/kg in low-calcium granitic rock to 1600 mg/kg in ultrabasic rock and sedimentary rocks. Deep sea sediments contain concentrations of about 1000 mg/kg (Turekian and Wedepohl, 1961). It has been reported that the manganese content of coal ranges from 6 to 100 mg/kg (Ruch *et al.*, 1973) and that of crude oil from 0.001 to 0.15 mg/kg (Bryan, 1970). The major sources of man-made environmental pollution by manganese arise in the manufacture of alloys, steel, and iron products. Over 90% of the manganese produced in the world is used in the making of steel, either as ferromanganese, silicomanganese, or

spiegeleisen. Manganese is also used in the production of non-ferrous alloys, such as manganese bronze, for machinery requiring high strength and resistance to sea water, and in alloys with copper, nickel, or both in the electrical industry. Other sources include mining operations, the production and use of fertilizers and fungicides, and the production of synthetic manganese oxide and dry-cell batteries. In dry-cell batteries, manganese is used in the form of manganese dioxide, which is also used as an oxidizing agent in the chemical industry. Many manganese chemicals, e.g., potassium permanganate, manganese (II) sulfate, manganese dichloride, and manganese dioxide are used in fertilizers, animal feeds, pharmaceutical products, dyes, paint dryers, catalysts, wood preservatives and, in small quantities, in glass and ceramics. Some of these uses contribute to environmental pollution (WHO, 1981). The emission of manganese from motor vehicles powered by petrol that does not contain manganese additives has been estimated to average 0.03-0.1 mg/km (Moran *et al.*, 1972; Gentel *et al.*, 1974; Gentel *et al.*, 1974a).

Sludge and various waste waters containing manganese are used in the production of micronutrient fertilizers and manganese slurries have been used in the production of clay blocks for road construction and these can serve as sources of manganese in the environment (Eliseeva, 1973). Manganese pollution may also arise from the incineration of refuse containing manganese (WHO, 1981).

Manganese is an essential trace element for both animals and man necessary for the formation of connective tissue and bone, and for growth, carbohydrate and lipid metabolism, the embryonic development of the inner ear, and reproductive functions. Some specific biochemical functions of manganese have been discovered such as the catalysing of the glucosamine-serine linkages in the synthesis of the mucopolysaccharides of cartilage. Terrestrial mammals however, may concentrate available manganese up to a factor of 10, whereas fish and marine plants concentrate it by factors of 100 and 100 000, respectively (Preston *et al.*, 1972). The accumulation of manganese in living organism has the potential of reaching toxic levels. Symptoms of the Manganese toxicity in man include dullness, weak muscles, headaches and insomnia. High iron concentrations affect vital organs in humans including the liver, cardiovascular system and kidneys (Alabaster and Lloyd, 1980).

2.4 Sources of Heavy Metals in the Environment

The amounts of most heavy metals deposited to the surface of the Earth by man are many times greater than depositions from natural background sources. Combustion processes are the most important sources of heavy metals, particularly, power generation, smelting, incineration and the internal combustion engine (Hutton and Symon 1986; Battarbee *et al.*, 1988; Nriagu and Pacyna 1988; Nriagu 1989).

The functioning of natural biological systems is increasingly affected by human activities and it is difficult to find a river or other water body whose natural regime has not been modified by man" s activities. An increase in urbanisation and industrial activities and higher exploitation of cultivable land has brought about a huge increase in the quantity of discharges and wide diversification in types of pollutants that reach rivers and other aquatic environments. Many African countries depend on agriculture to boost their economy, thus pesticides are likely to represent an important source of xenobiotics in contaminated rivers. The ultimate sink for many of these contaminants is the aquatic environment due to discharges or to hydrologic and atmospheric processes (Lagadic *et al.*, 2000).

2.5 Pollution of the Aquatic Environment with Heavy Metals

The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both man and animal (Rashed, 2004). Nowadays, the increasing use of the waste chemical and agricultural drainage systems represents the most dangerous form of chemical pollution particularly heavy metal pollution. The most important heavy metals from the point of view of water pollution are Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd), Mercury (Hg), Nickel (Ni) and Chromium (Cr) (Rashed, 2004).

When heavy metals enter the aquatic environment, the metal ions can react with constituents of the water or settle to the bottom and react with the sediments. Heavy metals have a greater chance of remaining in solution when complexed to chelating ligands such as specific anions whose concentrations are described by the pH of the surrounding environment. Metals precipitate as oxides/hydroxides at different pH regions and the amphoteric elements return to solution at higher pH. The hydroxide concentration (or pH) is therefore of great importance for the mobility of metals. Other factors also affect the fate of the metal ions like redox conditions and the presence of adsorbent sediments (Alloway and Ayres 1998).

Water pollution is most commonly associated with the discharge of effluents from sewers or sewage treatment plants, drains and factories to the water body of rivers, seas and marines. The accumulation of metals in an aquatic environment has direct consequences to man and to the ecosystem. Metals have many sources from which they can flow into the water body, these sources are: (i) Natural Sources: Metals are found throughout the earth, in rocks, soil and are introduced into the water body through natural processes, weathering and erosion.

(ii) Industrial Sources: Industrial processes, particularly those concerned with the mining and processing of metal ores, the finishing and plating of metals and the manufacture of metal objects. Metallic compounds which are widely used in other industries as pigments in paint and dye manufacture; in the manufacture of leather, rubber, textiles , paint, paper and chromium factories which are built close to water for shipping.

(iii) Domestic Wastewater: Domestic wastewater contains substantial quantities of metals. The prevalence of heavy metals in domestic formulations, such as cosmetic or cleansing agents, is frequently overlooked.

(iv) Agricultural Sources: Agricultural discharge contains residual of pesticides and fertilizers which contains metals.

(v) Mine runoff and solid waste disposal areas.

(vi) Atmospheric pollution: Acid rains containing trace metals as well as suspended particulate matter (SPM) input to the water body will cause the pollution of water with metals.

(Source: Rashed, 2004)

2.6 Biomonitoring of Ecosystem Pollutants

Initially, most monitoring programmes were based on the chemical analysis of major contaminants within the environment, until a number of difficulties became apparent (Jamile, 2001). Many authors found that by simply monitoring contaminants in natural waters, they were unable to integrate the overall environmental conditions and their impacts on aquatic life and further found difficulty in quantifying very low contaminant concentrations commonly found in natural waters (Phillips and Rainbow, 1994; Narbonne, 2000).

Monitoring chemical contamination in an ecosystem does not enable us to assess its impact on the organisms, populations and communities. In terms of sub lethal levels the response of organisms to contamination can only be evaluated by measurement of biological, physiological and biochemical parameters according to an approach similar to that used in medical diagnostics in human or veterinary clinical toxicology (Lagadic *et al.*, 2000).

Biomonitoring is a regular systematic use of living organisms to evaluate changes in environmental or water quality in laboratory or field conditions, by assessing either bioaccumulation, biological effect, health (occurrence of disease) and/or ecosystem integrity (Van Der Oost *et al.*, 2003). It is a process in which the plant and animal organisms or their fragments used, provide continuous, realtime analytical information (Radecki and Radecka, 1995 and Namiesnik and Wardencki, 2000).

2.7 Limitations of Biomonitoring

Although the integrated weight of evidence approaches can suggest a relationship between stressors and ecological responses, they do not demonstrate causation. Descriptive approaches such as biomonitoring studies provide support for hypotheses rather than direct tests of hypotheses (Newman and Clements, 2008). Results of biomonitoring studies are often equivocal because of the lack of adequate controls, non-random assignment of treatments, and lack of replication (Hurlbert 1984). Suter (1993) discusses the "ecological fallacy" of presuming that differences between polluted and unpolluted sites are a result of anthropogenic factors when alternative hypotheses have not been tested experimentally.

2.8 Bioavailability of Heavy Metals

Bioavailability is the extent to which a contaminant in a source is free for uptake. In many definitions, especially those associated with pharmacology or mammalian toxicology, bioavailability of a contaminant implies the degree to which the contaminant is free to be taken up and to cause an effect at the site of action (Newman and Unger 2003). Whether or not the organism is exposed to the contaminant concentration will depend on whether it comes within close proximity of the media containing the contaminant. The organism must be in appropriate contact in order to absorb, ingest, imbibe, or inhale the contaminated material. Whether or not this contact results in a realized dose in

the organism will depend on a wide range of factors that collectively determine bioavailability (Newman and Clements, 2008).

2.9 Monitoring Bioavailable Metals in Aquatic Environments

Metals occur in the environment both as a result of natural processes and as pollutants from anthropogenic activities (Franca *et al.*, 2005). They are distributed between various environmental phases (including atmosphere, water and sediment) depending on the nature of the phase and the nature of the compound (Connell *et al.*, 1999). However, mere observations of the total metal concentrations in either of these phases are rarely a good predictor of impacts on organisms. For example, in an aquatic environment, determination of the metal concentrations in solution or associated with particles may not always indicate the metals that are biologically available (bioavailable) in aquatic environments. Instead, bioavailability is dependent on the chemical and physical (dissolved or particulate) forms of metals in the water column and sediments, which are controlled by several physicochemical parameters such as temperature and salinity (Wang and Fisher, 1999; Ansari, 2004).

In the attempt to define and measure the presence and effects of pollutants on aquatic systems, bioindicators have attracted a great deal of interest. The principle behind the bioindicator approach is the analysis of an organism for their metal contents in order to monitor the metal excesses in their tissues. Various aquatic organisms that occur in rivers, lakes and seas, including fish, oyster, mussels, clams, aquatic animals and aquatic plants and algae are potentially useful as bioindicators of metal pollutants (Rashed, 2004).

2.10 Bioindicators

Bioindicators are biological indicators of environmental quality that characterize environmental conditions (Gadzała-Kopciuch *et al.*, 2004) and reflect changes in the condition of an organism resulting from exposure to a toxicant (Chambers *et al.*, 2002). Their tolerance is usually limited, so their presence or absence, and health state enable the determination some physical and chemical components of the environment without complicated measurements and laboratory analyses (Gadzała-Kopciuch *et al.*, 2004) and they are indicators of normal status or changes in individuals of a study population.

The use of bioindicators for environmental safety implies a thorough knowledge of their biological function in order to avoid misinterpretation, which could lead to conclusions that abnormalities were caused by environmental parameters when in fact they were normal variations (Lagadic *et al.*, 2000).

Bioindicators may be divided into those responding to environmental changes in a visible way (morphological and physiological changes) and those whose reactions are invisible, but which accumulate different substances (pollutants) whose concentrations may be determined (Lagadic *et al.*, 2000).

There are various advantages of bioindicators in pollution monitoring. They are useful as „early warning" tools of potentially adverse effects. Furthermore, responses may provide a temporally and spatially integrated measure of bioavailable pollutants. For example biomarkers can detect intermittent pollution events that routine monitoring may miss. Specific responses can be used to attribute exposure to pollutants; bioindicators can provide information on the relative toxicities of specific chemicals; and bioindicators are applicable in both the laboratory and the field (Amiard *et al.*, 2000; Moolman, 2004).

Despite these advantages, there are also a number of limitations. According to Amiard *et al.* (2000), the major handicap in the use of bioindicators in field conditions is the interference from natural abiotic and biotic factors, as it is almost impossible to distinguish between signals of disturbance caused by pollutants and the „background noise" due to natural fluctuations. Another disadvantage is that chemicals may interact within their environment and therefore the combined action of these chemicals can complicate the interpretation of bioindicators responses (Lagadic *et al.*, 2000).

2.10.1 Selection of Bioindicators

Many species of plants and animals have been utilised in aquatic biomonitoring surveys. However, only a few species can fulfill the prerequisites of an ideal organism (bioindicator). Specifically bioindicators employed in biomonitoring surveys should possess most of the following attributes:

- Contaminants should be accumulated without lethal impacts.
- Bioindicators should be sedentary in order to represent the area in which they grow.
- Bioindicators should be abundant throughout the area.

- Bioindicators should be relatively long-lived.
- Bioindicators used should be easy to sample, hardy to survive under laboratory conditions and should provide sufficient tissue for contaminant analysis.
- Bioindicators should tolerate brackish waters, which are often the most contaminated areas in coastal waters.
- A simple correlation should exist between contaminant concentration in the bioindicator and the ambient environment.

(Phillips and Rainbow, 1994; Connell *et al.*, 1999).

2.11 Bivalves as Indicators of Water Pollution

Of all the possible biomonitors available for monitoring aquatic environments, bivalves fulfil most of the above-mentioned characteristics. This is because they are widely distributed globally, easy to handle, sessile, filter feeders that have the ability to accumulate high metal concentrations without metabolising the metals appreciably (Gunther *et al.*, 1999; Nasci *et al.*, 1999; Olivier *et al.*, 2002), provide a time-integrated indication of environmental contamination (Regoli, 1998), can concentrate pollutants in their tissues at concentrations greater than the ambient water (El-Shenawy, 2002) and provide a solution to the problem of not being able to estimate the biologically available quantity of heavy metals using water or sediment samples (Butler *et al.*, 1971).

Bivalves have been shown to be valuable sentinel organisms (Farrington *et al.*, 1982, 1983; Livingstone, 1991) because they greatly concentrate many chemical elements from seawater and sediment, making analysis easier. At the same time they integrate pollutant levels over time, thereby giving a more realistic indication of the pollution status of the environment (Huanxin *et al.*, 1999), and the knowledge of the concentration factors of metals in bivalves is useful for recognizing the relative ability of the organisms to bioaccumulate selected metals from their environment (Szefer *et al.*, 1998).

2.12 Uptake of Heavy Metals by Bivalves

Uptake of heavy metals occurs mainly through uptake in dissolved forms in the water. Apart from metal uptake from the dissolved phase in solution, uptake can occur *via* ingestion of food such as phytoplankton and suspended particulate material containing sorbed metals (Tovar Sánchez *et al.*, 2004). Only recently has the relative importance of food ingestion as a major source for metal accumulation and toxicity been identified (Wang and Fisher, 1999). According to Phillips and Rainbow (1994), who reviewed the uptake of metals from particulates, there are two distinct ways in which metals can be taken up; namely through direct ingestion of particles with a subsequent uptake from digestive gland and/or uptake *via* pinocytosis in the gills of bivalves.

The uptake of metals into the bivalves" cells is largely dependent on their ability to pass through the cell membrane, irrespective of the metals" route of entry (Connell *et al.*, 1999). Uptake can occur through a number of transport pathways, which have been well documented, since first proposed by Simkiss and Taylor (1989). These transport pathways include the passive diffusion of neutral metal species across the membrane, facilitated diffusion of metals, active transport through major ion channels and endocytosis (Wang and Rainbow, 2005). However, the relative uptake and utilisation of these routes vary between different sites on body surfaces (i.e. uptake particularly prominent at the highly permeable gills), organisms (i.e. different species utilise different routes) and environmental conditions (i.e. physicochemical changes control the uptake) (Phillips and Rainbow, 1994).

After the bioavailable metals have been taken up into the biological system, an induction of a number of processes that play an important role in controlling the level of toxicity occurs. Some of these detoxifying processes include the transportation, transformation, sequestration and/or excretion of excess metals (Connell *et al.*, 1999). According to Amiard *et al.*, 1987; Durou *et al.*, 2005), toxicity will

only occur when the rate of metal uptake exceeds the combined rate of excretion and detoxification of the bioavailable metal.

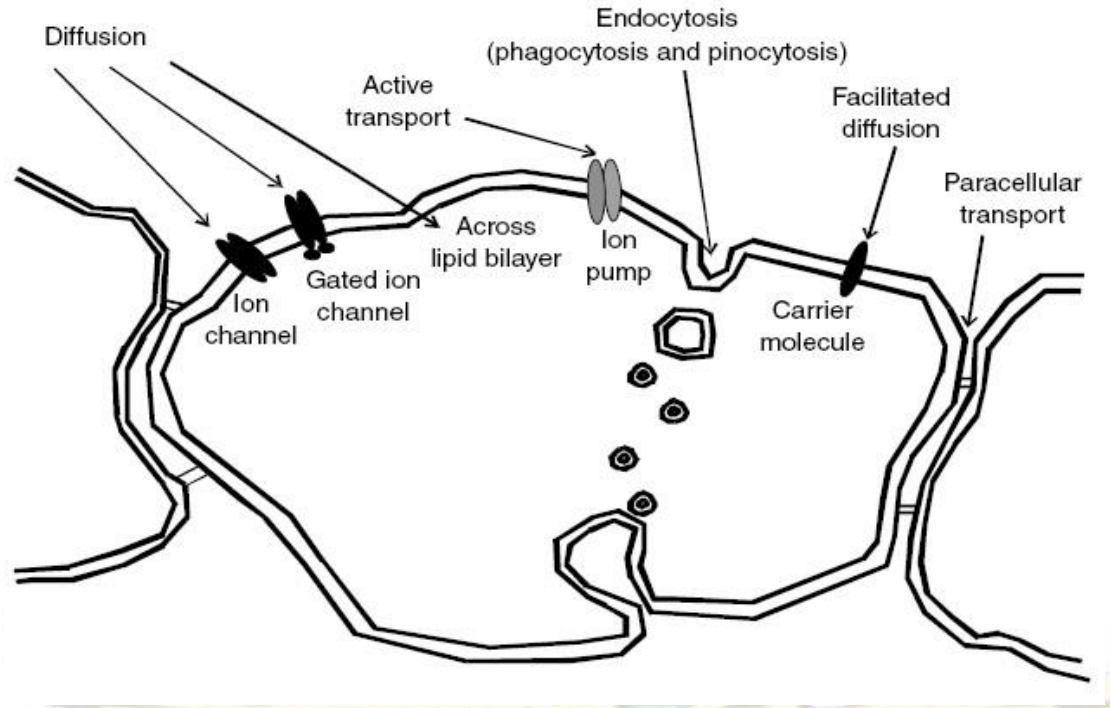

Figure 2.2 *Diagram of routes of chemical uptake into cells and the paracellular route. (Newman and Clements, 2008)*

2.13 Heavy Metals Accumulation in Bivalves

Heavy metals can be taken into the tissue of bivalves in a number of ways. They can be absorbed directly from the water across the surface of the gills (Lorenzo *et al.*, 2003). Heavy metals tend to adhere to sediment particles and if bivalves take in these sediment particles as part of their normal feeding process, the heavy metals are also ingested (Cruz-Rodriguez and Chu, 2003). Phytoplankton can take up high levels of heavy metals from contaminated waters and when bivalves consume phytoplankton containing elevated levels of heavy metals, the heavy metals become concentrated in them (Janssen and Scholz, 1979).

Different bivalve species use and hold on to heavy metals differently. The risk of heavy metal contamination must therefore be considered on a species by species basis. For example, oysters can have highly elevated levels of copper and zinc in their tissues compared to mussels grown in the same

general area. The concentration of heavy metals in some shellfish may also vary seasonally with the stage of gonadal development (Cooper *et al.*, 1982).

2.14 Factors Influencing Heavy Metal Accumulation in Bivalves

Metal accumulation in bivalves is influenced by several abiotic and biotic parameters (Phillips and Rainbow, 1994). Some of these include: season (Regoli, 1998), location (Blackmore and Wang, 2003) salinity (Chong and Wang, 2001), organic matter (Pan and Wang, 2004), sex (Sokolowski *et al.*, 2003), food acquisition capability (Saavedra *et al.*, 2004), stage of gonadal development (Bryan *et al.*, 1980) and size/weight (Phillips, 1976, Riget *et al.*, 1996). These natural variables may influence observed variations in bioaccumulated metals (Phillips and Rainbow 1994).

2.15 Persistence of Heavy Metals in Bivalves

The rate of cleansing of shellfish from heavy metals is generally considered in terms of the heavy metal "half-life" in the shellfish tissue. The half-life is the length of time taken for the level of contaminant in the tissue to reduce to half its level as a result of biological processes. Heavy metals tend to persist for long periods of time in bivalve shellfish. For example, the half-life for most heavy metals in Pacific oyster tissue has been calculated as 23-60 days (Okazaki and Panietz, 1981), although half-lives of heavy metals in bivalve shellfish may exceed 200 days (Cunningham and Tripp, 1973, Greig and Wenzloff, 1979, Roesijadi, 1996). The length of time that heavy metals persist in shellfish varies with the type of metal, shellfish species, shellfish size, environmental conditions and season (Schulz-Baldes, 1974, Denton and Burdon-Jones, 1981, Okazaki and Panietz, 1981 and Latouche and Mix, 1982)

2.16 Aquatic Sediment Contamination

Contaminated sediments, in both freshwater and marine systems, are a significant issue worldwide. Contaminants can persist for many years in sediments, where they have the potential to adversely affect human health and the environment. Some chemicals continue to be released to surface waters from industrial and municipal sources and polluted run-off streams from urban agricultural areas and

build up harmful levels of contamination in sediments (Mackevičiene *et al.*, 2002). The enrichment of metals in sediments is influenced by allocthonous influence which is made up of natural and anthropogenic effects and autochthonous influences comprising of precipitation, sorption, enrichment of organism and organometallic completion during sedimentation as well as the post depositional effects of digeneses (Forstner and Witlmann, 1979).

The analysis of sediment is a useful method of studying aquatic pollution with heavy metals (Batley, 1989). There are basically three reservoirs of metals in the aquatic environment: water, sediment and biota. Metal levels in each of these three reservoirs are dominated by a complex dynamic equilibrium governed by various physical, chemical and biological factors (Murray and Murray, 1973). Among these three reservoirs, sediment is the major repository for metals, in some cases, holding over 99% of the total amount of metal present in the system (Renfro, 1973).

The occurrence of elevated concentrations of trace metals in sediments found at the bottom of the water column can be a good indicator of man-induced pollution rather than natural enrichment of the sediment by geological weathering (Davies *et al.* 1991, Chang *et al.* 1998) and it is well known that an important proportion of metals are associated with suspended or bottom sediments dependant of sorption processes (Irion 1991; Wang *et al.* 1997).

Metals may be present in the estuarine system as dissolved species, as free ions or forming organic complexes with humic and fulvic acids. Additionally, many metals e.g. Pb associate readily with particulates and become adsorbed or co-precipitated with carbonates, oxyhydroxides, sulphides and clay minerals. Consequently, sediments accumulate contaminants and may act as long-term stores for metals in the environment (Spencer and MacLeod, 2002) and exposure of sediment-dwelling organisms to these metals may then occur via uptake of interstitial waters, ingestion of sediment particles and via the food chain (Luoma, 1989).

In a comprehensive sediments assessment approach, five basic components should be considered: (1) benthic community structure, (2) laboratory bioassays for evaluating the toxicity of in-place pollutants, (3) bioaccumulation information, (4) knowledge of site stability, and (5) physicochemical properties (Ulrich, 2001). Concentrations of bioavailable contaminants in sediment are needed to evaluate food chain transfer and the potential toxicity of sediment contaminants. The bioavailable fraction can be measured directly by collecting and analyzing benthic invertebrates or it can be estimated. Direct measurement is the preferred approach because it contributes the least uncertainty to exposure estimates. That is, it provides information on the actual contaminant loading in on-site

biota. However, direct measurement of contaminant concentrations in biota may not be feasible because of a lack time, personnel, or finances to support field sampling. When direct measurement of contaminants in biota is not possible, estimation is the only alternative (United States Department of Energy, 1998).

Contaminant concentrations in biota may be estimated using a variety of methods, ranging from complex mechanistic process models to simple accumulation factors. While mechanistic process models for the estimation of contaminant concentrations in biota may give more accurate estimates, they require information which is not generally available for a risk assessment. The simplest method for estimation of contaminant loads in biota is the use of accumulation factors (AFs). AFs consist of ratios of the concentration of a given contaminant in biota to that in an abiotic medium. For the evaluation of sediments this is commonly presented as the biota sediment accumulation factor (BSAF). The concentration in biota may be estimated by multiplying the sediment concentration by the BSAF. This method is particularly useful for ecological risk assessments because ambient media concentrations are usually available; ambient media data are needed for the site characterization and human health assessments typically conducted in conjunction with ecological assessments. Concentrations in most biota are used only for the ecological risk assessment and are frequently not available, especially for screening level assessments. Separate BSAFs are required for each chemical because they are empirically derived, rather than being based on generalizable physico-chemical parameters. Bioavailability of contaminants for uptake can be influenced by sediment conditions including the pH, the amount of acid-volatile-sulfide (AVS) that is available for complexing with divalent metals (i.e., Cd, Cu, Pb, Ni, and Zn) (United States Department of Energy, 1998), anthropogenic input, the type and concentration of organic and inorganic ligands, hydraulic processes within the water and the available surface area for adsorption caused by the variation in grain size distribution (Axtmann and Luoma 1991; Davies *et al.* 1991; Sondi *et al.* 1994).

The use of uptake factors, including BSAFs, depends on the assumption that the concentration of chemicals in organisms is a linear no threshold function of the concentration in sediment. This will not be the case if uptake or depuration of the chemical in question is well-regulated by the organism, either because it is an essential nutrient or because it is a toxicant for which the organism has inducible mechanisms for metabolism or excretion. Well-regulated chemicals will have nearly constant concentrations regardless of sediment concentrations, at least within the effective concentration range for the regulating mechanism. Various complex patterns also are possible due to lack of induction at

low concentrations, saturation kinetics at high concentrations, toxicity at high concentrations, or other processes. Despite these conditions that lead to violation of the assumptions, accumulation factors are commonly used in risk assessments (United States Department of Energy, 1998).

The assessment of sediment enrichment with elements can be carried out in using the index of geoccumulation first described by Muller in 1979 and enrichment factors. The index of geoaccumulation (Igeo) has been used as a measure of bottom sediment contamination since the 1970s (Miko *et al.*, 2000). It determines contamination by comparing current metal contents with pre-industrial levels. The content accepted as background is multiplied each time by the constant 1.5 in order to take into account natural fluctuations of a given substance in the environment as well as very small anthropogenic influences. The value of the geoaccumulation index is described by the following equation:

Igeo= $\text{Log}^2 \left[\frac{Cn}{1.5Bn}\right]$(Muller, 1979).

2.17 Review of Heavy Metals in the African Aquatic Environment

Unlike other pollutants like petroleum hydrocarbons and litter which may visibly build up in the environment, trace metals may accumulate, unnoticed, to toxic levels. Thus problems associated with trace metal contamination were first highlighted in the industrially advanced countries because of their larger industrial discharges and especially by incidents of mercury and cadmium pollution in Sweden and Japan (Kurland *et al.*, 1960; Nitta, 1972; Goldberg, 1976). In spite of the relatively low level of industrial activity in less developed regions such as Africa, there is nevertheless growing awareness of the need for rational management of aquatic resources including control of waste discharges into the environment. This becomes even more important in view of the expected increases in industrial and urban activities in all parts of the continent (Biney *et al.*, 1994).

For effective water pollution control and management there is a need for a clear understanding of the inputs (loads), distribution and fate of contaminants, including trace metals from land-based sources into aquatic ecosystems. In particular, the quantities and qualities need to be considered together with the distribution pathways and fate and the effects on biota.

The need to make an assessment of the level of heavy metal contamination in the African environment has led to the initiation of several pollution monitoring programmes and research work in various universities and scientific institutions in the region. The most relevant programmes are the Mediterranean Pollution Monitoring Programme (MEDPOL) covering also North Africa, the West and Central Africa Marine Pollution and Research programme (WACAF 2) and the Eastern Africa Marine Pollution and Research Programme (EAF/6) (Biney *et al.*, 1994).

2.18 Selected African Experiences

In the following section, selected African experiences have been summarized according to regional zonations and refers to researches conducted on distribution of metals in various environmental compartments and illustrate situations in which water bodies are influenced by metal loads.

2.18.1 Northern Africa

Heavy metals in Northern African waters have been studied more than other chemical parameters. The water and sediments compartments, as well as selected biota of inland water bodies and coastal marine areas, have been investigated and of the different matrices, sediments have been more analyzed because they can present a clearer indication of metal inputs and accumulation in aquatic environments.

Studies of heavy metals in Northern Africa have been concentrating on Egyptian inland waters and coastal zones, particularly on the River Nile and its two branches, Rosetta and Damietta, as well as on the delta lagoons. However, many studies have been conducted within the framework of the 1975 Action Plan for the protection of the Mediterranean and have therefore focused on the coastal zones. Advanced investigations on the dynamics and speciations of trace metals are also being conducted in different Egyptian inland and coastal marine waters.

Bernhard and Renzoni (1977) differentiated between natural and anthropogenic sources of mercury pollution in the Mediterranean by reviewing concentrations in pelagic fishes and benthic organisms, as well as sediments.

Toma (1980) investigated the distribution of adsorbed metals on the fine fraction of the sediments of the western part of the Nile continental shelf (offshore, near shore and river environments). The authors concluded that abundance of metals occurred in the order Fe > Mn > Zn > Cu, and their distribution was identical with the pattern of sediment transport. High concentrations of metals were recorded at certain localities and some of them, reaching potentially toxic levels for aquatic organisms, were attributed to contaminated drainage waters. The occurrence of some heavy metals in the sediments of Abu-Kir Bay of the Mediterranean Sea was studied by Saad *et al.*, (1981). The metals (Cu, Cd, Zn, Fe and Mn) showed a pattern of distribution similar to that of the mud and organic matter content of the sediments. The effects of industrial effluents were found to be restricted to sediments in the vicinity of their discharge.

The occurrence and distribution of metals in the water of the heavily polluted Lake Mariut in Egypt, and their accumulation in the different parts of a *Tilapia* species in this lake were investigated by Saad *et al.*, (1981a). Variations in the concentrations of metals (Zn, Cu, Fe, Mn and Cd) in the lake water were mostly attributed to variations in the discharge rates of the dumped wastes. The levels of these metals in fish were much higher than those in water.

Studies on the surface sediments of El-Mex region of the Mediterranean in front of Alexandria (Saad *et al.*, 1981b) revealed two zones, one of which showed high concentrations of Mn, Cu, Cd, Zn and Fe, as a result of discharges of industrial effluents. Their findings also suggested incorporation of similar proportions of Fe and Mn into the sediments and the co-precipitation of Cu and Zn by iron oxides.

The seasonal distribution of dissolved and particulate heavy metals in the water column of the Damietta branch of the river Nile was studied by Fahmy (1981). El-Rayis and Saad (1985) estimated the contribution of trace metals from the River Nile to the eastern Mediterranean by determining the concentrations of dissolved metals in the surface and subsurface water along the Rosetta branch. The relative abundance was Zn > Fe > Cu > Mn > Cd.

Saad and Fahmy (1985) studied the occurrence of trace metals in surficial sediments from the Damietta estuary of the Nile and concluded that the eastern side of the estuary was exposed to more pollution than the western side. Also, areas of maximum averages of Cu, Zn and Cd coincided with the discharge sites of sewage wastes.

Metal enrichment in surficial sediments of three shallow Nile Delta lakes (Lake Mariut, Nozha Hydrodrome and Lake Manzalah) was evaluated using Fe/metal ratios by Saad *et al.* (1985). A further study on heavy metals in water, sediments and fish of Lake Mariut was made by Saad (1985). The results revealed the existence of a direct relationship between the levels of metals in the lake water and in the different fish parts. The metal concentrations in the lake water were markedly lower than those in fish and the levels, excluding cadmium, in the lake sediments were considerably higher than those in fish. Analyses of sediments, aquatic plants and water from the river Nile and its branches at selected sites characterized by heavy industrialization and dense populations (Fayed and Abd El-Shafy, 1985) showed higher concentration factors in sediments than in plants.

El-Rayis and Saad (1986) studied the levels of heavy metals in a big land-based source of contaminated drainage water contributing six million m^2 /day to the coastal Mediterranean region in front of Alexandria. Another study by El-Rayis and Saad (1986a) divided Lake Mariut into two zones, septic and non-septic, after metal analysis of suspended matter and water. According to ElRayis *et al.*, (1986), copper and zinc were found to be concentrated in the sandy sediments of the shallow sides of the Eastern Harbour of Alexandria, whereas iron and manganese occurred in the deeper sediments.

El Rafei *et al.*, (1987) quantified the levels of trace metals in waste waters discharged into the river Nile from some industries near Cairo. El-Nabawi *et al.*, (1987) determined metal concentrations in fish from Lake Mariut, Lake Edku and Abu-Kir Bay and found the highest levels in *Sphyraena sphyraena* from this bay. Moharram, (1987) estimated the levels of total, organic, inorganic mercury, total selenium and the interaction between both metals in *Mugil cephalus*. A strong correlation was reported between fish length and each of these variables.

Heavy metal pollution in Lake Mariut has been further investigated by El-Rayis and Saad (1990), based on the distribution of Cu, Zn, Fe and Mn in water, suspended matter and sediments. The contribution of metals from this lagoon to the Mediterranean Sea via Umum Drain (contaminated land-based source) was also estimated.

Madkour (2005) analysed selected samples of the giant clam shells and the associated surface sediments for Fe, Mn, Zn, Cu, Pb, Ni and Cd and realised significant spatial differences in metal concentrations in *Tridacna maxima* and sediments.

El-Moselhy, (2006) devoted a research to determine the levels of total mercury in the different tissues of fish *Mugil seheli*, crab *Portunus pelagicus*, shrimp *Metapenaeus stebbingi*, and bivalves *Paphia undulata* and *Gafrarium pectinatum* collected from Lake Timsah and Bitter Lakes. Levels of Hg in the edible parts of the investigated organisms showed the ranges 2.62 – 25.45 and 0.94 – 7.94 ng/g wet wt. in fish, 16.02 – 117.26 and 9.86 – 64.18 ng/g wet wt. in crab, 4.55 – 14.67 and 5.76 – 15.58 ng/g wet wt. In shrimp, and 1.06 – 36.31 and 5.38 – 69.59 ng/g wet wt. in bivalves from Lake Timsah and Bitter Lakes, respectively.

Beldi *et al*., (2006) studied the seasonal variations in the concentrations of four trace heavy metals (cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn)) in *Donax trunculus* (Mollusca, Bivalvia) at two contaminated sites in the gulf of Annaba (East of Algeria): El Battah and Sidi Salem. The average concentrations of the metals exhibited the following order: Zn>Cu> Pb>Cd for the two sites.
In Tunisia, Chouba *et al*., (2007) conducted a study to investigate the toxic contaminants cadmium (Cd), lead (Pb) and mercury (Hg) in surface sediments and in the fish species *Mugil cephalus* of the lagoon of Ghar El Melh (GEM), and realised that levels of Cd and Pb varied in sediment from 0.4 to 0.9µgg-1dw and 25 to 70µgg-1dw, respectively. Mercury concentrations in sediment were generally below 1µgg-1dw. The highest level was observed in the northeast of lagoon. In fish muscle, concentrations of Cd, Pb and Hg varied between 0.013 to 0.025µgg-1dw, 0.048 to 0.422µgg1dw and 0.222 to 0.409µgg-1dw, respectively. Results of heavy metal analyses in sediment and fish indicated that there were relatively metal contamination problems in GEM lagoon near the harbour due to the anthropogenic activities, notably from the Medjerda River and wastewater from the coastal towns around the lagoon.

2.18.2 West and Central Africa

Studies on the occurrence and distribution of metals in the West and Central African Regions, especially Nigeria have been conducted on all the major environmental matrices (water, sediment, fauna and flora) but again with more emphasis on sediments.

Statistical treatment of the result of metal analyses of 176 stream sediment samples from the Ifellesha area (1800 km^2) of southern Nigeria (Ajayi, 1981) showed that all the elements have density

distribution close to natural background levels. Ojo (1988) also used various statistical methods for the interpretation of the geochemical data obtained from analyses of Cu, Pb, Zn, Co, Ni, Fe, Mg, Mn and Ca in 374 stream sediment samples collected over an area of 700 km^2 within the upper Benue Trough (Nigeria) and concluded that these elements exhibit various patterns of association depending on their nature and prevailing environmental conditions. Other studies in the area (Kakulu and Osibanjo, 1988, 1991) revealed elevated levels of Pb, Cr, Ni, V and Zn in Port Harcourt and Warri sediments which suggest that effluents from petroleum refineries located in these cities have contributed significantly to the heavy metal pollution of the respective aquatic ecosystems.

Okoye *et al.*, (1991) reported anthropogenic heavy metal enrichment of Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb and Zn in the Lagos lagoon and implicated land based urban and industrial wastes sources.
Pollution studies on 26 rivers in some southern and northern states of Nigeria (Ajayi and Osibanjo, 1981), on rivers in the Niger Delta (Kakulu and Osibanjo, 1991), on the cocoa growing area of Ondo State in South West Nigeria (Ogunlowo, 1991) and the Lagos waters (Okoye, 1991a) showed that, with the exception of iron, the concentrations of most trace metals in the surface waters are generally lower than the global average levels for surface waters and the international drinking water standards.

Ndiokwere and Guinn (1983) determined As, Cd, Cr, Hg, Mn, Mo, Ni, Se and Sb in two Nigerian rivers and two harbours and attributed high metal concentrations to local pollution sources. In their studies of streams and lakes around Ibadan, Mombeshora *et al.*, (1983) reported much higher levels of lead in sediments than in water. The highest levels of lead coincided with areas of high traffic density.

Analyses of sediments and fish from the Niger Delta area of Nigeria (Kakulu and Osibanjo, 1986) revealed that the area was relatively unpolluted with mercury compared to some European areas (Mediterranean, Baltic Sea and North-East Atlantic). Report from the same area (Kakulu *et al.*, 1987) indicated that the levels of Cd, Cu, Fe, Mn, Pb, and Zn were higher in shellfish than in finfish. With the exception of the lead levels in some shellfish, levels of these metals were generally lower than the WHO recommended limits in foods. Concern about the high levels of lead in Lagos lagoon fish has also been expressed (Okoye, 1991).

Other Nigerian studies include that of Sridhar (1988) who analyzed the aquatic plant *Pistia stratiotes* and showed that the shoot system accumulated more K, Ca, and Mg, whereas the root system accumulated significantly more Cd, Cr, Co, Fe, Pb, Hg, Na, and Zn.

Onwumere and Oladimeji (1990) reported that *Oreochromis nilotica* exposed to treated petroleum refinery effluents accumulated trace metals in the order Pb > Cu > Zn > Mn > Cr > Ni > Cd.

Davies *et al*., (2006) studied the accumulation of three heavy metals; chromium (Cr), cadmium (Cd) and lead (Pb) in periwinkle (*Tympanotonus fuscatus var radula*; shell and soft tissues), water and sediment collected from four stations along Elechi Creek course in Nigeria and realised that the sediment concentrated more heavy metals than the water while the periwinkles accumulated more of these metals than the sediment. Cr was the highest concentrated heavy metals in both the normal and depurated periwinkles. The biological concentration factor (BCF) revealed that these periwinkles have high potential to concentrate heavy metals in their shells and soft tissues, and it is directly proportionate to their sizes. However, the observed heavy metals concentrations in these animals are below the recommended limits for human consumption.

In Ghana, one of the earliest studies (Amasa, 1975) examined various matrices, including drinking water, from the Obuasi gold mining area and found that arsenic concentrations occurred above normal values. A study (Akoto-Bamford, 1990) in which heavy metal pollution from gold mining activities was assessed by analyzing gold ore, tailings, sediments and water for Cr, Mn, Fe, Cu, Zn, As, Pb, Rb, Sr, Y, Zr and Nb, revealed the presence of all the elements in sediments within a concentration range of 0.08 to 49 000 μg g^{-1}, whereas only iron and zinc were detected in water at levels of 0.08–2.4 (μg ml^{-1}).

Total mercury concentrations in commercial fish from different coastal sites of Ghana have been determined by Ntow and Khwaja (1989) who concluded that all values were well below the 0.5 μg g_1 action level adopted in many countries. Biney and Beeko (1991) conducted a survey of metals in fish and sediments from the River Wiwi in Kumasi and found a positive correlation between mercury concentration and body weight of fish. They also reported higher levels of cadmium and mercury in fish than in sediment. Studies on the distribution of Hg, Cd, Pb, Cu, Zn and Fe in water, finfish and shellfish, macrophytes and sediments from Kpong headpond and lower Volta River (Biney, 1991) showed the highest concentration of iron and lead in sediments and of manganese and cadmium in macrophytes. Finfish had the lowest concentrations of the metals, except for lead.

Pelig Ba *et al.*, (1991) assessed the level of contamination of drinkable ground-water from the Accra plains and upper regions of Ghana and found that in some areas Pb, Cr and Fe concentrations exceeded the WHO guideline limits for drinking water.

Seasonal sampling of the bivalves: *Anadara (Senilia) senilis* (n = 260), *Crassostrea tulipa* (n = 220), from two „open" lagoons (Benya and Ningo) and a „closed" lagoon (Sakumo), and *Perna perna* (n = 170), from rocky shores adjacent to Benya and Sakumo, were analyzed for their total Cu, Zn, Fe, Mn, Cd and Hg concentrations and total body burden (that is concentration multiply by total flesh weight) by Otchere in 2003. Median concentrations for cockles were: 5, 38, 710, 10, 0.22 and 0.20 μg/g dw respectively whiles median total body burden in cockles were: 3.3, 30.5, 370, 5.2, 0.28 and 0.13 μg respectively.

In Cote d'Ivoire Marchand and Martin (1985) and Kouadio and Trefry (1987) have studied sediments of the Ebrié Lagoon and reported metal concentrations in excess of background levels, this was attributed to the disposal of untreated sewage and industrial effluents.

A comparative study by Metongo (1991) of Cd, Cu, Hg and Zn in samples of oysters (*Crassostrea gasar*) from urban and rural lagoon areas of Côte d'Ivoire revealed higher but background levels of the metals in the urban area. Likewise, other studies of heavy metals in *Callinectes amnicola* (Metongo and Sankaré, 1990) and in *Thunnus albacares* (Metongo and Kouamenan, 1991) gave concentrations lower than internationally acceptable limits for seafood.

In Senegal, analyses by Gras and Mondain (1978) of fish and crustaceans from coastal waters revealed lower mercury concentrations than the generally acceptable limits (0.5 μg g^{-1}), except in swordfish and sharks weighing more than 5 kg.

In other parts of West Africa, the concentrations of major and minor ions, including Cu, Mn and Fe in river Jong, Sierra Leone, was determined by Wright (1982), who found a clear relationship between metal concentrations and seasonal variations in rainfall.

Other studies on the occurrence of trace metals have been conducted as part of the Joint FAO/IOC/WHO/IAEA/UNEP Project on monitoring of pollution in the marine environment of the West and Central African region. Within this framework, concentrations in marine biota have been reported for Cameroon (Mbome, 1985; 1988), Ghana (Biney, 1985; Biney and Ameyibor, 1989) Côte

d'Ivoire (Metongo, 1985, 1988) and Senegal (Ba, 1985; Ba, 1988). On the basis of these studies, Portmann, (1989) reviewed the levels of contaminants in the marine environment of the region and concluded that there was little input of mercury and other metals into the coastal zone from land.

2.18.3 Eastern Africa

Early studies in this region focused on Lake Nakuru in Kenya, one of a number of Soda lakes in the Great Rift Valley which was made a national park in 1986 because of its world-famous flamingo population (Biney *et al.*, 1994).

In an attempt to produce baseline information for monitoring pollution, Koeman *et al.*, (1972) determined As, Sb, Cu, Zn, Cd and Hg in muscle, liver and kidney of birds and fish. They concluded that the metal concentrations did not constitute a hazard to the biota of Lake Nakuru. Six years later, Greichus *et al.*, (1978) studied water sediment, benthos and fish, and reported slightly elevated concentrations as compared to values found by Koeman *et al.*, (1972).

The effect of copper ions on the photosynthetic oxygen production of phytoplankton, on the growth rate of blue-green algae (*Spirulina platensis*) and on populations of rotifers (*Brachionus* sp.) in water from Lake Nakuru was experimentally investigated by Kallqvist and Meadows (1978). The rotifers were less sensitive to copper than algae. Other studies by Lewin (1976) showed that Lake Nakuru water contained 0.08 mg/l Cu mainly from pesticide containing run-off from the surrounding agricultural lands. This value was thus higher than the critical value of 0.02 mg/l Cu which may significantly reduce algal growth (Kallqvist and Meadows, 1978).

Earlier studies on sediment, water and biota of the second largest natural lake in the world, Lake Victoria (Alala, 1981; Onyari, 1985; Ochieng, 1987) showed no significant heavy metal pollution. However, further studies in the same area revealed increased lead levels largely due to increased shipping traffic and associated problems, car washing and discharge from local industries (Wandiga and Onyari, 1987; Onyari and Wandiga, 1989). Ochumba (1987) studied physico-chemical parameters, dissolved oxygen and heavy metal concentrations in Lake Victoria as the possible causes of periodic fish kills. The author attributed the fish kills to dissolved oxygen depletion.

In other East African areas, copper ion distribution in the surface waters of Lakes George and

Edward (ldi Amin) in Uganda was studied alongside other chemo-limnological parameters (Bugenyi, 1979). Concentrations ranged from 0.07 to 0.13 μg ml^{-1} in Lake George and from 0.006 to 0.02 μg ml^{-1} in Lake Edward. A direct relationship was established between copper, water hardness, alkalinity and total dissolved solids. Bugenyi (1982) studied the occurrence of Cd, Cu, and Fe in sediments of the same lakes and concluded that the concentrations, although distinct in the different water bodies, did not show much variation within each of the lakes.

Effluent, air and soil samples near a battery factory in Dar-es-Salaam, Tanzania, were analyzed for mercury by Semu *et al.*, (1986). The highest levels of contamination were associated with the disposal of defective batteries. A preliminary investigation of the extent of metal pollution of the Msimbazi River in Dar-es-Salaam, which receives industrial, urban and agricultural waste waters, was conducted by analyzing sediments and biological indicators (Akhabuhaya and Lodenius, 1988). Metal concentrations were in general low but some of the results indicated localised industrial pollution.

Studies of dissolved metals in the marine environment were conducted by Norconsult (1977) concluding that the concentrations for Tudor Creek fell within the normal range of unpolluted natural sea water. Oteko (1987) studied the Mombasa Creek and suggested crustal sources to be responsible for copper concentrations and increased anthropogenic sources from automobile exhausts for cadmium and lead concentrations.

According to Bryceson, (1990) available data on marine contaminants was scarce however, localised hot spots of metal pollution are found in the vicinity of cities and industrial centres that may constitute a danger to the public health. Wandiga and Onyari (1987) found slightly higher metal concentrations in marine fishes from Mombasa when compared to fish from Lake Victoria. The reported concentrations did not pose an immediate danger to the fish industry.

Matthews (1981) found evidence of surprisingly high mercury levels in fish (1.0 – 2.0 μg g^{-1}) and in hair and blood of inhabitants of Seychelles, where fish consumption is very high. The sources and pathways of such high mercury levels are a mystery.

2.18.4 Southern Africa

The concentrations and distributions of metals amongst other chemical contaminants were investigated by Greichus *et al.*, (1977) in two South African lakes; Hartbeespoort Dam, which receives industrial and municipal waters from Johannesburg and Voelvlei Dam, situated in mainly agricultural area. Water, sediment, aquatic plants and insects, fish, fish-eating birds and their eggs were analyzed for As, Cd, Cu, Mn, Pb, Zn and Hg. The results indicated higher levels in Hartbeespoort dam than in Voelvlei for all metals in sediments and birds, except for copper in bird carcasses. Mercury levels in birds were 2 to 5-fold greater than in fish, whereas lead values were 2 to 10-fold greater.

Greichus *et al.*, (1978) investigated metals among other contaminants in Lake Mcllwaine, a eutrophic water body near Harare, Zimbabwe. Water, sediment, plankton, bottom fauna and fish were analyzed. The data gave intermediate levels of metals between those found in Hartbeespoort Dam and Voelvlei Dam.

Watling and Emmerson (1981) identified areas of metal input to the River Papenkuils which was considered to be a serious source of pollution to the marine environment around Port Elizabeth. In contrast, the estuary of River Swartkops was found generally unpolluted on the basis of metal concentration in water, surface sediments and sediment cores (Watling and Watling, 1982). Similar studies also showed that the estuary of River Knysna as well as the Bushmans, Kariega, Kowie and Greatfish Rivers were unpolluted (Watling and Watling, 1982a, 1983).

Concentrations of dissolved trace metals, Fe, Mn, Al, Cu, Zn, Pb and Cd were determined in the Umtata River in the plateau region of the Eastern Cape province of South Africa, approximately midway between the Drakensburg escarpment and the Indian Ocean by Fatoki *et al.*, (2002). High levels of Al, Cd, Pb, Zn and Cu were observed, which may affect the health of the aquatic ecosystem. Generally the sources of the metals in the River appeared to be diffuse, which included rural, urban and agricultural runoff sources in the catchment, although there may be contributions from natural and point sources.

The review of heavy metals in the African aquatic environment has shown that available data originate from only a few areas of the continent are scattered and may be inconsistent in some cases. Besides, depending on the area, more information may exist on coastal than on inland areas or *vice versa*. It is also not possible to establish a trend in heavy metal accumulation since data cover only a narrow

period of time. There is, therefore, a need to generate more data covering the different environmental compartments in all the African sub-regions.

Despite this inadequacy, some conclusions may be drawn from this review. Generally, lower concentrations of heavy metals occur in African aquatic systems compared to other areas of the world and comparison of the levels of some heavy metals in the edible tissues of fish and shellfish with the WHO recommended maximum permissible levels in food, indicates that most African fisheries resources are only slightly contaminated and are presently safe for human consumption with respect to heavy metals. Concentrations in inland and coastal environments exhibit no significant differences and on a continental level, the four geographical areas-Northern, Western, Eastern and Southern Africa - have similar low levels (Table 2.1).

Table 2.1 *Overview of trace metal concentrations in the whole soft tissue of Bivalve species collected in African waters (All values are expressed in μg/g dw).*

			HEAVY METAL CONCENTRATION (μg /g dw)			
AREA	REFERENCE	SPECIES	Zn	THg	Mn	Fe
Volta River, Ada, Ghana	Present work	*G. paradoxa*	71-316	13-43	0.028-0.056	49-867
Volta River Aveglo, Ghana	Present work	*G. paradoxa*	123-539	16-49	0.037-0.074	73-206
Volta River,Ghana	Tay *et. al.*, 2004	*G. paradoxa*	174.96	89.4		116.7
Nigeria	Okoye, 1991	*G. paradoxa*		1440		
Lake Timsah, Egypt	El-Shenawy, 2004	*R. decussatus*	2243.4			139.8
Cross River, Nigeria	Etim, *et. al.*, 1991	*G. paradoxa*		117		
Benya, Ningo and Sakumo Lagoons, Ghana	Otchere, 2003	*C. tulipa*	280-700	380-2780		11-20
Benya, Ningo and Sakumo Lagoons, Ghana	Otchere, 2003	*P. perna*	900-1130	12-16		12-15
Benya, Ningo and Sakumo Lagoons, Ghana	Otchere, 2003	*A. senelis*	210-1170	6-104		5-19
Matola River, Mozambique	Böhlmark, 2003	*M. meretrix*	131-4275	7.7-69.7		5.8-76.3

2.19 Distribution of the Freshwater Clam, Galatea paradoxa (Born 1778)

The freshwater bivalve mollusc, *Galatea paradoxa* (Born, 1778) (=*Egeria radiata* (Lamarck, 1804)) is stenotopic, being restricted in its mega-scale occurrence to few large West African rivers namely: Volta River in Ghana, Nun and Cross Rivers in Nigeria, and Sanaga River in Cameroon (King and Udoidiong, 1991). It is edible and widely distributed. An FAO Species Identification sheet shows that its range extends from the Gulf of Guinea to the Congo. Despite its wide range, it is only in few countries like Ghana and Cameroon that literature is available to show its occurrence in terms of commercial use.

Before the construction the Akosombo and Kpong Dams on the Volta River in 1964 and 1981 respectively, seasonal floods flushed out sandbars, which might have started during the dry season, thus the estuary was largely kept free of sandbars. However, after the construction of the dams and the subsequent absence of annual floods, sandbars have gradually formed at the estuary and with the passage of time virtually blocked it. The effect of this was that saline water, which during high tide flowed upstream into the river channel, completely ceased (UNEP, 2002). The changes in the flow regime led to physico-chemical changes in the water and consequently, there was a gradual shift in the habitat of *Galatea paradoxa* from the upper and mid-section of the lower Volta towards the estuary with a decline in abundance of the clam. By the late 1980s, the clam was in danger of extinction. The dredging of the Volta estuary, initiated in 1990 and recently carried out in April 2009 by the Volta River Authority (VRA) is aimed at breaking down "islands" built by heaps of sand at the estuary. The dredging process has allowed intrusion of some sea water into the river creating suitable conditions for breeding of *Galatea paradoxa* hence the resurgence of the fishery some 4 to 10 km above the estuary. This rejuvenation in the clam industry does not, however, compare with what it used to be with respect

to size of fishing grounds, the number of people involved and the present catches are just a fraction of the pre-dam periods (Amador, 1997) when the clam industry stretched between Akuse and Sogakope (Lawson, 1963).

The distribution of the clam is currently restricted to a very narrow stretch of the South Volta River, between Agave-Afedume (15 km from the Volta estuary) and Ada-Foah (10 km from the estuary). It is interesting to note that the clam is not found in any other estuary or river in Ghana apart from the Volta. Throughout its geographic distribution, *Galatea paradoxa* supports a thriving artisanal clam fishery. Its exploitation in most rivers is largely devoid of management and conservation strategies and these have resulted in over-exploitation leading to a decline in abundance and sizes of clams caught (Amador, 1997).

The clam which is locally referred to as „afane" (Ewe) and „adode" (Twi) is a highly priced delicacy especially among travelers along the Accra-Lome and Accra-Ho routes. It constitutes an important and affordable protein source to the riparian human communities of the rivers of its occurrence and those around the Volta are of no exception (Amador, 1997). The flesh of the clam is a good source of animal protein. Calculated on a dry matter basis, the average protein content of smoked clam fish is 46.5% (Kwei, 1965). The shell of the clam has various uses notably as the main source of calcium in the poultry feed and lime manufacturing industries. One interesting use to which the clam shells have been put in the South Volta is in the construction industry. The shells are used as an alternative to stone chippings in concrete. Additionally, it is used as a pavement material to overcome muddy conditions in village compounds.

CHAPTER 3

MATERIALS AND METHODS 3.1 Study Area

The study was carried out at Ada and Aveglo, both at the Volta Estuary, Ghana, over an 18-month period, from March 2008 to August 2009. Ada (Latitude 05°49' 18.6" N and 000°38.46' 1"E) and Aveglo (05°53 28.2" N and 000° 38' 24.7"E) represent the southern and northern limits of the most active clam fishing grounds at the Volta Estuary (Fig.3.1).

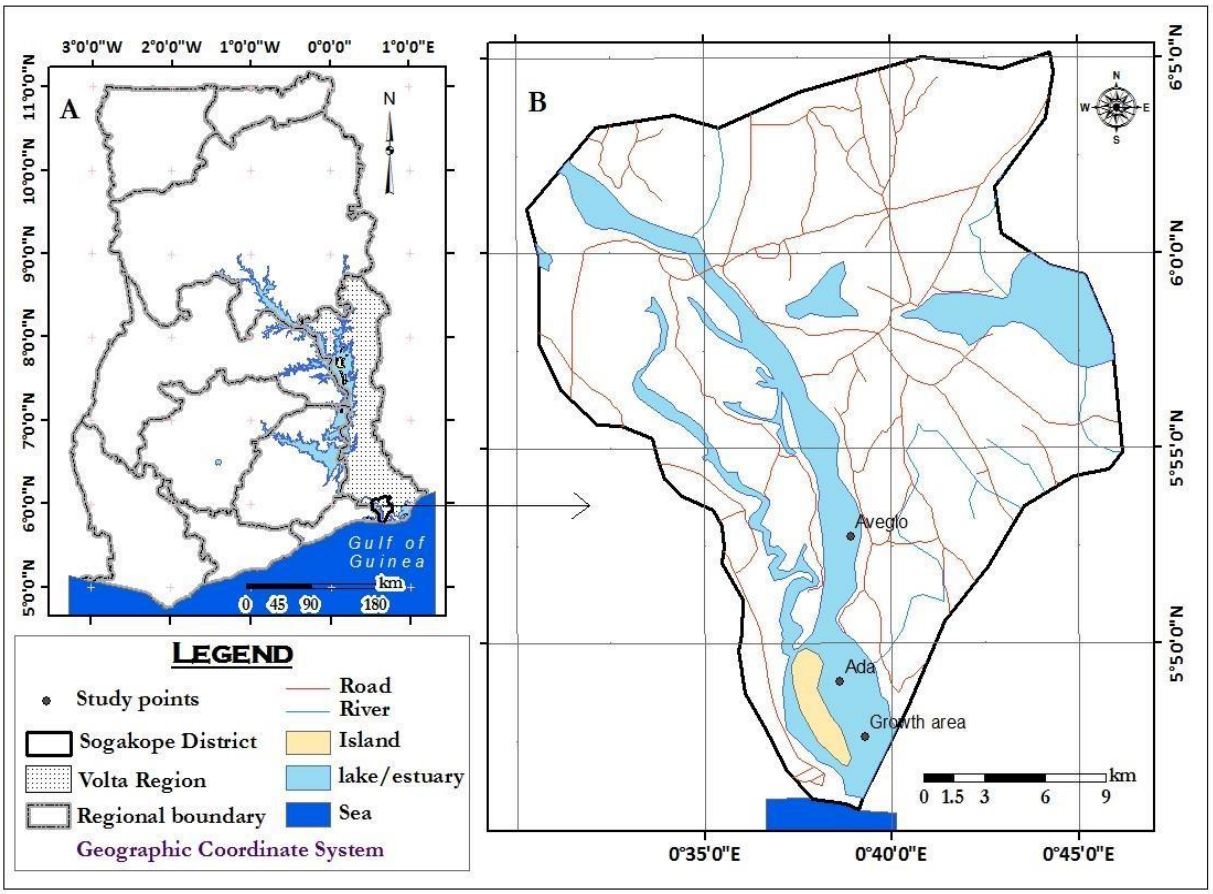

Figure 3.1 *Clam sampling locations at Ada and Aveglo in the Volta estuary in Ghana*

The Volta River in Ghana has been known to support substantial local clam fishery. In addition to over 60 commercially important food fishes, the river supports prawn and clam fisheries at its lower reaches (Attipoe and Amoah, 1989).

Before the construction of the Akosombo and Kpong Dams on the Volta River in 1964 and 1981 respectively, areas within Senchi and Atimpoku were noted for their prawn industry while the area between Akuse and Sogakope was considered the centre for the clam industry (Amador, 1997).

3.2 The Meteorology and Hydrology of the Study Area

The meteorology and hydrology of the study areas based on previous researches are presented below.

3.2.1 Climatic Conditions

The climate of the study area lies within the dry Equatorial climatic region of Ghana, which also covers the entire coastal belt of the country. This region is the driest in the country and is referred to as the central and southeastern coastal plains. The coastal lands of Ghana have two clearly defined seasons, the Dry season and the Rainy season. The Rainy season exhibits double maxima, the main one occurring between April and June and the minor one between September and October. June is normally the wettest month in the area. The annual isohyetal pattern of the coastal belt has the minimum in the west outside Accra up and close to Songor lagoon in the east. The prevailing wind direction is from the southwest (the southwest monsoons). This is a characteristic feature for the entire coastal belt of the country. Mean monthly averages of daily wind speed range between 21.1 and 29.0 km h^{-1}. However, high velocity winds (110 km h^{-1}) of short duration have been recorded in the climatic region. The north east trade winds rarely reach the coast.

3.2.2 Daylight and Sunshine (hrs) at the Study Area

The day length varies between 11.8 h and 12.5 h in the study area. It reaches its maximum in June and minimum in January. Daily sunshine duration is least in June (4.8 h) when there is maximum cloud cover and maximum in November (8.4 h) with a mean of 6.9 h. The values in table 2 below give an idea of the general variation in the hours of sunshine within the study area.

Table 3.1 *Day length (hours) and hours of sunshine within the study area*

Day Length												
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	**Mean**
11.8	11.9	12.1	12.3	12.4	12.5	12.4	12.3	12.2	12.0	11.9	11.8	**12.1**
Hours of Sunshine												
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	**Mean**
7.1	7.2	7.2	7.0	6.6	4.8	5.4	6.3	6.7	7.8	8.4	7.8	**6.9**

3.2.3 Relative Humidity

Relative humidity data for the study areas are estimated using data at Ada. Since local variation in relative humidity is not appreciable especially within the same climatic belt, humidity values at Ada are considered representative of relative humidity for the Ada and Aveglo sites. Generally, relative humidity is high in the mornings and at night, but is at a minimum in the afternoon (Table 3.2).

Table 3.2 *Percent relative humidity at Ada (5-year average)*

Time	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Mean
00:00	89	90	90	88	89	91	94	96	94	91	89	91	**81**
06:00	90	92	89	91	93	93	95	97	95	93	92	92	**93**
12:00	71	74	74	76	77	82	81	80	78	75	74	71	**76**
18:00	83	86	85	84	85	88	90	91	90	88	86	87	**87**

3.2.4 Temperature

Long-term temperature records are available at the Ada Synoptic Station. Records at this station give minimum average temperatures between 23ºC and 26ºC whereas the maximum lies between 27ºC and 32ºC. August is normally the coldest month in the area. Records from the Synoptic Station indicate that the minimum average temperature is 24ºC, whereas the maximum average is 31ºC.

3.2.5 Rainfall

The study area experiences two rainfall maxima with the annual average for different periods ranging from 688 to 855 mm. Rainfall occurs between March/April to July (the major rainy season) and September–October (the secondary rainy season) (Fig 3.2). The low rainfall gives rise to stream flow mainly in the Rainy season only. Between November and April, many small streams that drain the area dry up.

Rainfall records in the study area were reliable up to the 1980s. However, after this period, there are a lot of gaps in the data; only the station at Ada has consistent data. Extensive analyses of rainfall in the study area by researchers have revealed that the variation in annual totals was small. Hence, for the southern section where the annual rainfall was about 900 mm, isohyets of the average monthly rainfall were not necessary.

Long records of rainfall data are available for the Ada Synoptic Station. From the data, the following pattern of rainfall is observed. The maximum rainfall occurs in June with the major season itself beginning from March/April. There is also a minor season between September and October. The mean monthly variation is depicted in figure 3.2. Whereas the long-term mean annual rainfall is 891.6 mm, the mean between the mid-1970s and the early 1990s falls below this long-term mean by over 23%.

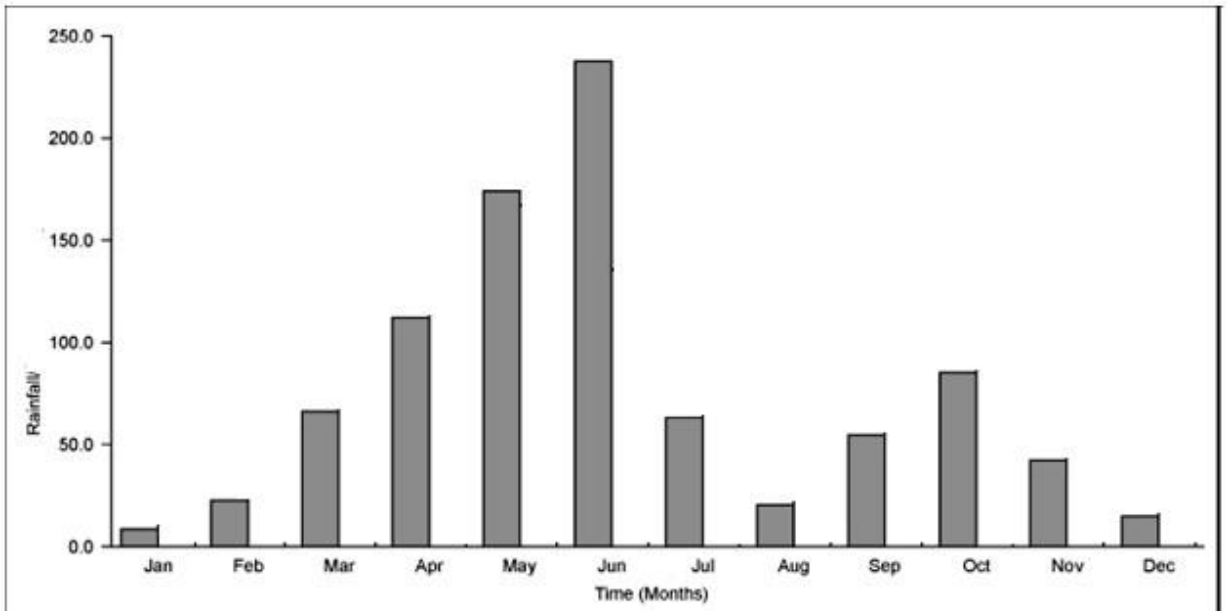

Figure 3.2 *General monthly rainfall distribution at the Volta Estuary, Ada, 1991*

3.2.6 Variations in Water Level of the Volta River

Most of the hydrological data available for the study area after 1968 are water levels at a few gauging stations within the basin. On the Volta River, mainly water releases through the penstocks for power production are available. There are gaps in the hydro-meteorological data, especially after 1980.

However, some water level measurements were carried out at selected stations in the Lower Volta Basin, including the study area. Before 1964, records on the Volta at Sogakope (about 15km from Aveglo) showed that water levels increased from 1.4 m in the Dry season to about 6.6 m in September or October. After the construction of the Akosombo Dam, however, water level records were uniform at this station, the slight fluctuations resulting from the operation of the hydropower station and rainfall downstream of the dam. Between 1990 and 1992, variation in water levels at the study area had a maximum value of 0.5 m within a year.

3.2.7 Changes in Flow Regime

The regulated flow in the Volta River began when Akosombo and Kpong hydro-power plants were commissioned in 1965 and 1984 respectively. This has created a new flow regime between Kpong and Ada, resulting in a progressive growth of a sandbar at Ada, which restricts flood discharge (into the sea) and tidal movement into the River. The resulting change in fauna and flora encouraged the growth of disease vectors such as schistosomiasis-carrying snails, and created changes in the flow regime between the interconnecting creeks and streams between the Lower Volta River and the Avu–Keta Basin, including Avu, Keta and Angaw Lagoons. In the early 1990s and more recently in April 2009, the Volta River Authority dredged the estuary. Whilst the dredging has controlled vector snails by admitting some amount of saline water into the river, salinity levels have been slightly altered in the lower reaches of the River. This trend of decreased pH levels was observed after the April 2009 dredging (see Appendix 2). Salinity studies carried out under a feasibility study indicated a decreasing trend in salinity from the estuary at Ada Foah, with the water at Sogakope being almost unaffected by the incursion of saline water from the estuary.

3.2.8 Physicochemical Water Parameters

Physicochemical parameters of the Volta River used as reference data for this work dates back to the late 1970s because of difficulty in accessing more current data. A summary of physicochemical water quality of the Lower Volta River at (1977 to 1978)

Table 3.3 *Summary of Physicochemical Water Quality of the Lower Volta River at (1977 to 1978)*

Parameter	**Mean**	**Standard Deviation**

pH	7.1	0.3
Conductivity	520	245
Dissolved Oxygen	8.0	2.0
BOD	2.8	2.2
Alkalinity	44.8	6.4
Chloride	4.6	2.5
Calcium	5.3	1.4
Total Hardness	17.4	7.3
Magnesium	2.5	1.9
Ammonia-N	0.4	0.5
Phosphate	0.2	0.3
Nitrate	6.6	10.9
Nitrite	0.6	1.3
Sulphate	1.2	0.8

Source: Andah *et al.*, (2003)

3.2.9 Hydrogeological Setting

Quaternary coastal marine sands and gravels are the main hydrogeological features of the Volta River Estuary.

3.3 Clam Fishing Methods at the Sampling Locations

Clam fishing methods vary at the two sampling locations in the estuary because of varying water depths. Fishing at the Ada sampling station, which is usually about one (1) metre deep is done by women who wade through the water and feel for the clam at the bottom with their toes (Plate 3.1). Clam harvesters at the Aveglo sampling station, (usually more than 6 meters deep), employ scubadiving techniques for harvesting the clams (Plate 3.2). They are supplied with atmospheric air through tubes fitted from a petrol-powered air compressor that sits in the canoe (Plate 3.3).

Plate 3.1 *Clam harvesting at the Ada sampling*

Plate 3.2 *Diving for clams at Aveglo Station*

Plate 3.3 *A motorized air compressor*

3.4 Study Species

Galatea paradoxa (Born, 1778) is a commercially important bivalve species exploited for food mainly at the Volta Estuary at Ada. *Galatea paradoxa* was chosen as the indicator species of heavy metal pollution because of the following reasons. They are filter feeders and can significantly concentrate many chemical elements from water and sediment. They are sedentary and represent the area in which they grow. They are abundant in the sampling stations and are relatively long lived. They are easy to sample, hardy enough to survive under laboratory conditions and provide sufficient tissue for contaminant analyses.

Plate 3.4 The Volta Clam, *Galatea paradoxa*

3.5 Collection and Processing of Samples

Collection and processing of *G. paradoxa* and sediment samples involved a series of steps as presented in the steps below.

3.5.1 Surface Sediments

Riverbed sediment samples were collected on a monthly interval for 18 months using an Ekman grab at the two locations from March 2008 to August 2009. The samples were collected at each sampling sites according to the standard procedures described in USEPA" s sediment sampling guide (USEPA, 1994) and were kept in LDPE bottles pre-washed with 10% HC1 and stored in insulated iced chests for analysis in the laboratory.

In the laboratory the sediment subsamples of 500g from each sampling location were placed in ceramic mortars for drying at 80ºC for 48hrs to a constant weight (Phillips and Yim, 1981). The dried samples were then gently disaggregated and 250g of each sample stored in 250 ml acid-washed LDPE bottles and kept at 4ºC in a refrigerator for heavy metal and granulometric analyses (USEPA, 1994).

3.5.2 Biota- G. paradoxa

Clam samples were obtained from the two sampling locations on a monthly basis from fishermen" s catch for 18 months and transported to the laboratory, submerged in river water, in insulated chests

within 12 hours for processing and storage for heavy metal analyses. The samples were obtained from March 2008 to August 2009.

In the laboratory, clam samples were cleansed to remove the mud and any debris and then washed with double distilled water. The clams were categorized into three groups each with 10 individuals for each sampling station based on shell length as follows: small (25mm-40mm), medium (41mm55mm), and large (above 55mm). The groupings were done based on the three dominant size groups in the natural populations to give a broad and fairly representative range of metal concentration in the clams. The various clam size classes were purged of ingested organic and inorganic particles before being analyzed for heavy metal accumulation by keeping each size class in distilled water for a 24- hour depuration. After the depuration process, a sterile stainless steel knife was used to dislodge and remove the soft tissue of each clam from the shell (Chiu *et al*., 2000).

The removed flesh of each subsample was oven-dried to a constant weight at 60ºC for 72 hours (Rebelo *et al*., 2005). Each dry clam sample was weighed on a Sartorius BP 210 S micro balance to the nearest 0.0001 g. Individuals of each size class were ground together into fine powder using a porcelain pestle and mortar. Homogenized subsamples were then stored in air-tight, acid-washed (0.1 M HCl) snap-top glass vials with plastic caps for heavy metals analyses (United Kingdom Environmental Agency, 2008).

3.6 Measurements and Analytical Methods

The categorization of the clam samples into the three size classes was done by measuring the shell length and width of the individual clams using a Powerfix digital caliper to the nearest 0.01mm. The total weights of the clams, including shell were also taken as well as wet and dry flesh weights.

3.7 Digestion of the Samples

About 0.5g of the homogenized clam subsamples and the sediment samples were weighed into a 50 ml digestion tube and 1ml of distilled water, 2.0 ml perchloric acid (HNO_3-$HClO_4$) (1:1 vv) and 5.0 ml sulphuric acid (H_2SO_4) were added. Each mixture was refluxed at 200ºC for 30 minutes in a clean fume chamber. The completely digested subsamples were allowed to cool at room temperature, and the undigested portion of the sediments filtered off through a Whatmann Glass Microfibre filter paper (GF/C) to obtain a clear solution and diluted to 50 ml in volumetric flasks with double

distilled water (Jin *et al.*, 1999; Otchere, 2003).

3.8 Determination of Zn, Fe and Mn

Concentrations of Zinc, Iron and Manganese were determined at the Soil Research Institute, Kwadaso, Kumasi using a Buck Scientific Model VGP flame Atomic Absorption Spectrophotometer (AAS). The results were expressed as total concentrations (µg/g dry weight (*dw*). Wavelengths and detection limits of the AAS for the analysed metals are shown in the table below.

Table 3.4 *Wavelengths and detection limits for the studied heavy metals*

Element	Slit	Wavelength	Detection Limit
Manganese	0.7	385.2	0.001
Zinc	0.7	213.9	0.005
Iron	0.7	248.3	0.03

3.9 Determination of Total Mercury (THg)

The Automatic Mercury Analyzer (Model HG 6000) at the Chemistry Department of Kwame Nkrumah University of Science and Technology (KNUST), equipped with a mercury lamp at a wavelength 253.7 nm was used for the determination of total mercury in the clam and sediment subsamples. During the determination, a known volume (5 ml) of the sample solution was introduced into a reaction vessel using a micropipette and immediately stoppered. 0.5 ml of the 10% (w/v) stannous chloride ($SnCl_2.2H_2O$) in 1 ml HCl was added from a dispenser to aid the reduction reaction. The stannous chloride solution (10% w/v) was prepared by dissolving 10 g of the salt in 100 ml of 1M HCl. The solution was aerated with nitrogen gas at 50 ml per minute for 30 minutes to expel any elemental mercury from it.

Responses were recorded on strip chart recorders as sharp peaks. The peak heights were used for the computation of the total mercury concentrations in the clam and sediment subsamples which were expressed as microgram per gram dry weight (µg/g dw).

Plate 3.5 *The Automatic Mercury Analyzer (Model HG 6000)*

3.10 Quality Control and Assurance

All tissue and sediment analytical batches for the determination of Mn, Zn and Fe concentrations were accompanied by blanks at a minimum rate of one blank per 20 samples. Replicate analyses were conducted on 10% of the samples to assess precision of the analytical techniques. The preparation of the blank solutions were according to the following procedure: Approximately 1ml of distilled water was poured into a digestion tube and 2ml of nitric acid and perchloric acid (HNO_3- $HClO_4$) mixture in the ratio of 1:1 was added and swirled to mix. 5ml of sulphuric acid was subsequently added and the mixture was shaken well to mix and heated at 200ºC for 30 minutes. The blank solution (1ppm) was prepared by diluting an appropriate aliquot of the stock solution to 50 ml with distilled water.

To validate the results realized for the total mercury, 0.5g of the homogenized clam and sediment samples were weighed into two (2) 50ml digestion tubes. The 25ng/g and the 50ng/g standard mercury solutions were added to each of the weighed samples in the digestion tubes and digested using the same digestion method described above. The digested samples were then analysed for the total mercury concentration using the Automatic Mercury Analyzer (Model HG 5000). Each determination was carried out at three (3) times and compared to the results realized from the clam and sediment samples for precision and accuracy.

To minimize contamination, all glassware for the digestion process were first cleaned under running tap water and soaked in 10% (v/v) Nitric acid (HNO_3) for 24 hours. They were then rinsed with distilled water followed by 0.5% (w/v) potassium permanganate ($KMnO_4$). Distilled water was used to finally rinse the glassware which were subsequently dried using an electric drier.

3.11 Physicochemical Water Parameters

Monthly measurement of temperature, salinity, pH, pressure, total dissolved solids (TDS), conductivity and dissolved oxygen (DO) of the Volta River were taken at both sites for the period using a Hanna (HI 9028) multi-parameter probe.

3.12 Sediment Pollution Analysis

The concentrations of the four metals in the sediment samples were subjected to various calculations to ascertain the extent of metal pollution at the Estuary as far as sediments are concerned.

3.12.1 Biota-Sediment Accumulation Factor (BSAF)

BSAFs were calculated for the two sampling stations and the different clam size classes (treated as one unit) to evaluate the efficiency of metal bioaccumulation in the tissues of the organisms. BSAFs were calculated for each analyte for each month using the equation:

$$\mathbf{BSAF} = \frac{\textbf{Concentration of heavy metal in the organism}}{\textbf{Concentration of the heavy metal in sediment}}$$ (Thomann *et al.*, 1995)

This method was to help in ecological risk assessments because ambient media concentrations were readily available; ambient media data were needed for the site characterization and human health assessments typically conducted in conjunction with ecological assessments.

3.12.2 Index of Geoaccumulation and Contamination Factor

Results of the heavy metal concentrations in the sediments were compared to sediment standards set by GESAMP (1982) to ascertain the extent of heavy metal pollution in the sediments at the two sampling stations

Müller" s geochemical index (Igeo) was further used to measure the pollution intensities in the study areas (Muller, 1979). The Igeo is associated with a qualitative scale of pollution intensity and samples were classified as unpolluted (<0), unpolluted to moderately polluted ($0 \leq I_{geo} \leq 1$), moderately polluted ($1 \leq I_{geo} \leq 2$), moderately to strongly polluted ($2 \leq I_{geo} \leq 3$), strongly polluted ($3 \leq I_{geo} \leq 4$), strongly to extremely polluted ($4 \leq I_{geo} \leq 5$) and extremely polluted ($I_{geo} \geq 5$).

The formula used for the calculation of Igeo is: $I_{geo} = \mathrm{Log}_2 \left[\frac{Cn}{1.5Bn}\right]$ (Muller, 1979).

Cn is the measured content of element "n", and Bn the element" s content in "average shale" (background concentration) (Turekian and Wedepohl, 1961). The geoaccumulation index (I_{geo}) was originally defined by Müller (1979) for a quantitative measure of the metal pollution in aquatic sediments. The content accepted as background is multiplied each time by the constant 1.5 in order to take into account natural fluctuations of a given substance in the environment as well as very small anthropogenic influences. The calculated Igeo values were compared to description of sediment quality Igeo classification table (Table 3.5) (Müller, 1979) to ascertain the pollution intensities of the two sampling sites.

Table 3.5 *Description of sediment quality Igeo classification (Müller, 1979)*

Geoaccumulation index (Igeo)	**Class**	**Pollution Intensity**
<0	0	Unpolluted
0-1	1	Unpolluted to moderately polluted
1-2	2	Moderately poluted
2-3	3	Moderately to strongly polluted
3-4	4	Strongly polluted
4-5	5	Strongly to extremely strongly polluted
>5	6	Extremely contaminated

Contamination factors (CFs) were calculated for each metal using the equation:

$$CF = \frac{Cmetal}{Cbackgound\ value}$$

This calculation was done to get a fair idea of the extent of anthropogenic pollution and accumulation of heavy metals in the sediments at the two sampling locations.

3.13 Granulometric Analysis of Sediments

The granulometric analysis of the sediments from the two sampling sites was carried out at the Soil Research Institute, Kwadaso, Kumasi. The granulometric analysis of the sediments was carried out following the procedures described in Cardoso *et al*., (2008). Grain size analysis was performed based on a series of sieves of different mesh sizes. Sediments were divided into the following fractions; clay (<0.002mm), silt (0.002-0.02mm) and sand (0.02-2mm). The sand component was further broken down into further fractions; very fine sand (0.02-0.06mm), fine sand (0.06-0.2mm), medium sand (0.2-0.6mm), and coarse sand (0.6-2mm). Each fraction retained in each sieve was weighed and expressed as a percentage of the total sediment weight.

3.14 Assessment of the Health Risk Associated with the Consumption of Clams from Ada and Aveglo

Comparable to Fung *et al.,* (2004), this work assessed the human health risk associated with the consumption of *G. paradoxa* by making a comparison between environmental status (represented by the concentrations of heavy metals in the clam) and threshold values which may cause adverse effects in human consumers.

Risk quotient (RQ) was calculated as the ratio between concentration of heavy metal in the clam and the level of concern (LOC) for that metal (Fung *et al.*, 2004). A level of concern (LOC), which is a threshold concentration of a chemical above which a hazard to human health may exist, was calculated as the ratio of Tolerable Daily Intake (TDI) and the Rate of Shellfish Consumption (RSC) (Fung *et al.*, 2004). For the purpose of this calculation, it was assumed that total trace metal exposure was derived solely from shellfish consumption.

$$\text{Level of Concern (LOC)} = \frac{\text{Tolerable Daily Intake (TDI)}}{\text{Rate of Shellfish Consumption (RSC)}}$$

$$\text{Risk quotient (RQ)} = \frac{\text{concentration of heavy metal in the clam}}{\text{Level of Concern (LOC)}}$$

Data on average national rate of shellfish consumption (RSC) of Ghana was calculated from the Daily Food Supply per capita from Fish and Fishery Products of the FAO (FAOSTAT 2004; http://apps.fao.org) which estimates the daily food supply from fish and fishery products in Ghana to be 62.6 g/person/day for the year 2002. The total fishery production for the same year was approximately 380,000 metric tons, of which 5,794 metric tons constituted mollusks (Directorate of Fisheries 2005). The daily rate of shellfish consumption for the Ghanaian population was calculated to be 0.95 g/person/day using simple proportion. This value however reflects the national and not local shellfish consumption levels as there is no documented data on the shellfish consumption levels of the riparian communities where the study was conducted. This was used to calculate the levels of concern (LOCs) for the average shellfish consumption group.

In the absence of a health criteria in Ghana, the Tolerable Levels of Intake (TDI) and Estimated Safe and Adequate range of Daily Dietary Intake Levels (ESAADI) for the studied heavy metals were provided either by the US Food and Drug Administration (http://vm.cfsan.fda.gov), FAO/WHO, or the National Research Council (NRC) of the US National Academy of Sciences (NAS) for the calculation of the relevant level of concern for each metal.

In the absence of health criteria in Ghana, the Tolerable Levels of Intake (TDI) and Estimated Safe and Adequate range of Daily Dietary Intake Levels (ESAADI) for heavy metals provided either by the US Food and Drug Administration (http://vm.cfsan.fda.gov), FAO/WHO or the National

Research Council (NRC) of the US National Academy of Sciences (NAS) to calculate the relevant level of concern for each metal. Results of the evaluation of the risks to human health associated with consumption of the clams containing trace metals are summarized in Table 4.3.
For cases where RQ<1, the heavy metals involved are unlikely to cause harm to human consumers (Fung *et al.*, 2004).

3.15 Statistical Analysis

Results of the heavy metal analyses were subjected to a one-way Analysis of variance (ANOVA) to test for significant differences ($p<0.05$) in the concentrations of the heavy metals in the tissues of the different class sizes of the clams. The whole tissue concentration for each metal was further subjected to a post-test; the Bonferroni's Multiple Comparison Test to compare all the possible pairs of columns; i.e. Small *vs* Medium, Small *vs* Large and Medium *vs* Large for significant differences between the compared classes

The Mann-Whitney non-parametric test ($p<0.05$) was used to test for differences in heavy metal concentrations between each clam size group and sediment samples from the two sampling stations over the 18-month period; It was also used to test for spatial variations in metal concentrations in the sediments from the two sampling stations.

Column statistics ($p<0.05$) was used to test for temporal variations in the concentrations of the heavy metals in the clam and sediment samples over the sampling period.

All descriptive statistics and graphs were executed using the GraphPad Prism 5 Software.

CHAPTER 4

RESULTS

4.1 Physicochemical Parameters of the Volta Estuary

pH for the Ada sampling site ranged from 6.18 in October 2008 to 8.50 in January 2009 and values were fairly constant from March to December 2008. Temperature values over the 18-month period varied between a narrow range of 27.28°C and 29.59°C in September 2008 and June 2009 respectively. Dissolved oxygen (DO) values ranged between a low of 1.52mg/l in September 2008 to 8.76mg/l in March 2008. The values dropped steadily from March to October 2008 after which there was a progressive increase to the end of the sampling period, although there were periodic drops during certain months. Salinity was constant at 0.03 throughout the periods of March 2008 to February 2009. Salinity values dropped to 0.02 and remained constant from March to August 2009 although April 2009 recorded a salinity value of 0.03. The sudden drop in salinity could be attributed to the dredging of the Volta estuary, initiated and recently carried out in April 2009 by the Volta River Authority (VRA) is aimed at breaking down "islands" built by heaps of sand at the estuary. The dredging process has allowed intrusion of more of the River water into the sea shortening the retention period of the sea water during high tides. Concentrations of total dissolved solids (TDS) were fairly constant with values ranging from 27 to 35mg/l over the sampling period. Conductivity values ranged from 52μs/cm in May and August 2009 to 70μs/cm in July 2008 (Fig 4.1).

At the Aveglo sampling site pH values were similar to the values recorded at Ada over the sampling period although values were generally slightly lower during most of the sampling period. The pH values ranged between 6.23 in October 2008 to 7.28 in August 2009. Temperature values ranged between a very narrow range of 27.19°C and 29.62°C in September 2008 and June 2009 respectively. DO values at the Aveglo sampling station exhibited a trend similar to that of the Ada sampling station with values dropping steadily from March to October 2008 indicating a similar underlying factor responsible for the decline. Values ranged from 1.58 to 6.79mg/l. TDS values ranged from a low 27mg/l and a high of 42mg/l during the sampling period. Conductivity values were between 54 and 84μs/cm during the sampling period similar to the values recorded at the Ada sampling station. Salinity was fairly constant at 0.03 for all the months from March to September 2008 except July of that same year, which recorded a slightly higher value of 0.04. The sudden drop in salinity at the Aveglo sampling station could also be attributed to the dredging of the Volta estuary initiated and recently carried out in April 2009 by the Volta River Authority (VRA).

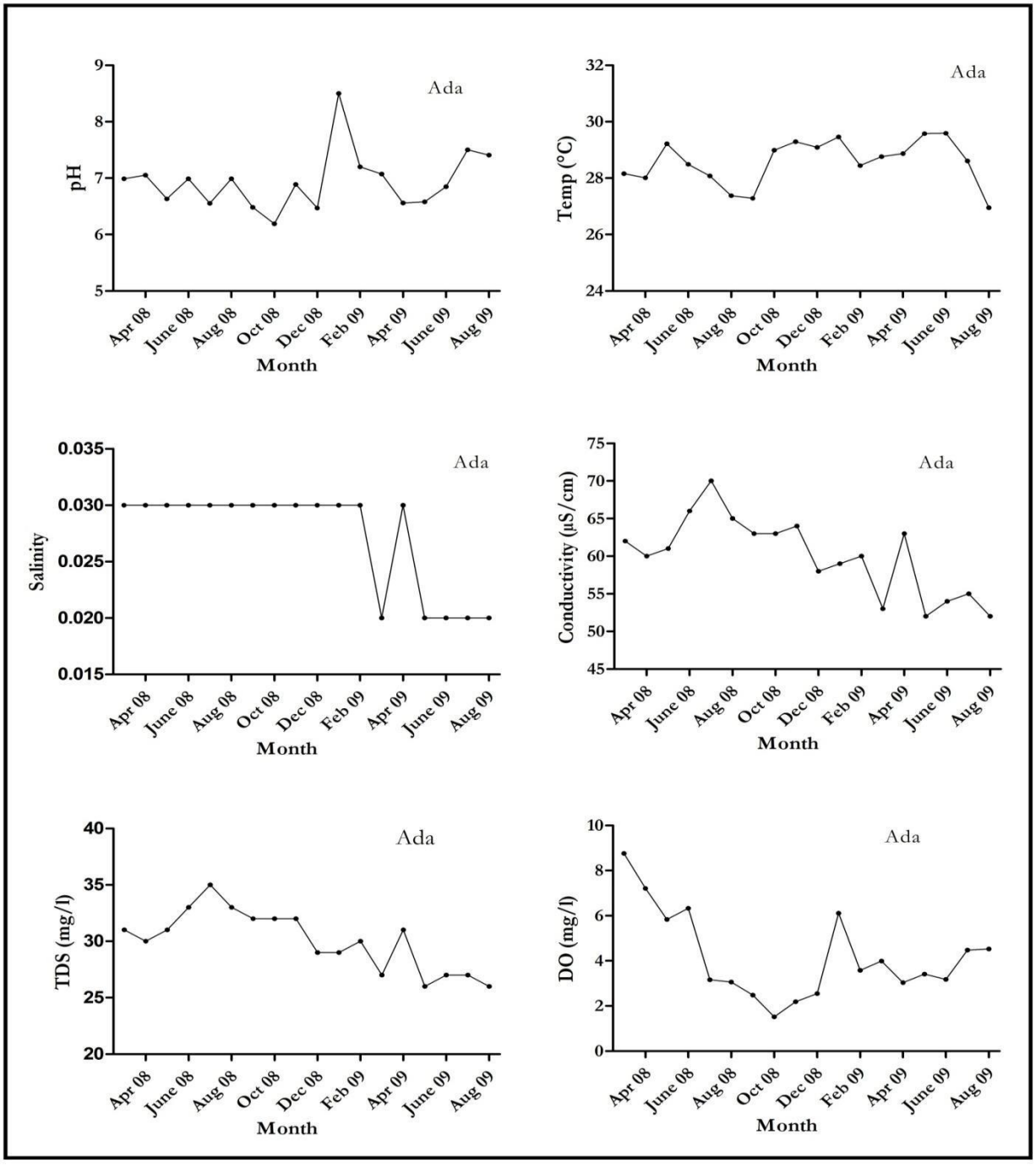

Figure 4.1 *Trends in Physicochemical water parameters of the Volta Estuary at Ada over the 18-month period*

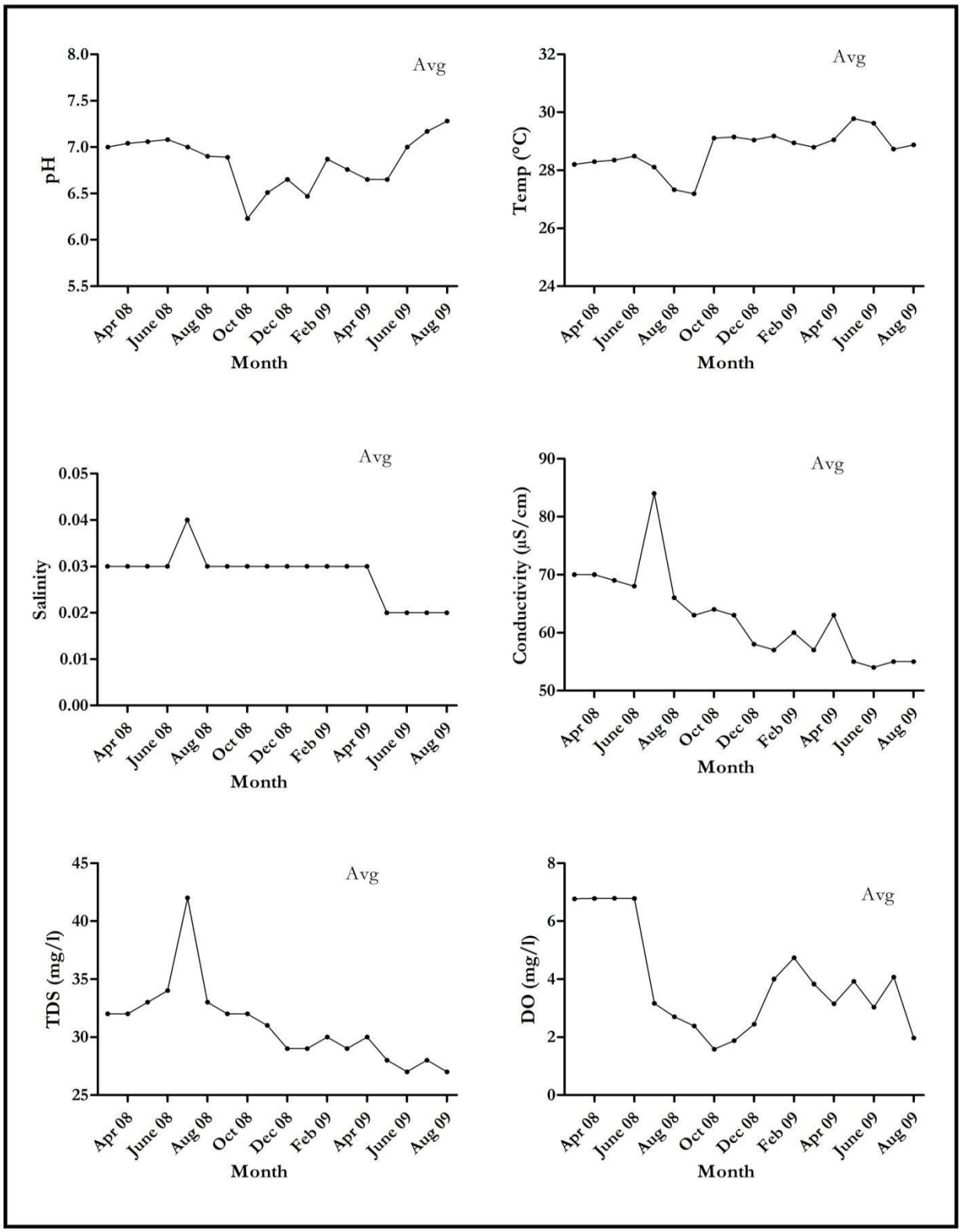

Figure 4.2 *Trends in Physicochemical water parameters of the Volta Estuary at Aveglo over the 18-month period*

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4.2 Grain Size Composition of the Sediment Samples

Results from the composition analyses of the sediments from both sampling stations revealed that the sampled surface sediment from the riverbed used for the heavy metal determination was predominantly sand (between 98.18% and 99.48% sand). Silt and clay jointly constituted less than 2% of the sediment. Further analyses of the sand component of the sediment revealed that it was predominantly coarse sand (between 63.36% and 98.71%) (Appendix 3).

4.3 Heavy Metal Concentrations in the Clam and Sediment Samples

The results of heavy metal concentrations (Manganese, Zinc, Iron and Total Mercury) in the whole tissue of the different clam size classes and in the sediment samples from the Ada and Aveglo sampling stations are presented in Table 4.1. All concentrations are recorded on a dry weight basis (µg/g dw).

4.3.1 Heavy Metal Concentrations in Clams Samples

Heavy metal concentrations in the clams at Ada and Aveglo are presented below

Ada Sampling Station

Manganese

Manganese (Mn) concentration in the whole soft tissue of the small-sized clams (shell lengths of 25mm-40mm) at the Ada sampling station varied from 73µg/g in June 2008 to 867µg/g in July that same year depicting a relatively wider variation in Manganese concentration. The medium-sized clams (shell lengths of 41mm-55mm) recorded manganese values of between 68µg/g in May 2008 and 336µg/g in August 2008. Mn concentration in the tissues of the large-sized clams (shell length over 55mm) ranged from 49µg/g in June 2008 to 316µg/g in February, 2009.

Zinc

The highest concentrations of zinc in the tissue of the small-sized clams (59µg/g) at the Ada sampling station were recorded December 2008 with the lowest concentrations of 19µg/g being recorded in the month of August of the same year. Concentrations of 13µg/g and 57µg/g were recorded as the lowest and highest concentration of Zinc for the medium-sized clams in the months of March and

December, 2008 respectively. In the large-sized clams, Zinc concentrations varied between 16μg/g in March 2008 and 49μg/g in December, 2008.

Iron

The results obtained for Iron concentrations in the tissues of the small-sized clams indicated a highest value of 484μg/g in January 2009 and a lowest value of 103μg/g in November, 2008. The medium-sized clams recorded Iron concentrations ranging between 79μg/g and 340μg/g in April 2009 and February 2009 respectively. The large-sized clams recorded values ranging between a low of 96μg/g in June 2009 and a high of 313μg/g in January 2009.

Total Mercury

Total Mercury (THg) concentrations for the small-sized clams ranged between 0.028μg/g in April 2008 and 0.042μg/g in August 2008. The medium-sized clams recorded a highest THg value of 0.049μg/g in March and September 2008 and a low value of 0.035μg/g in April 2008. THg concentrations ranged between a low of 0.044μg/g and high of 0.059μg/g in July 2008 and September 2008 in the large-sized clams.

Aveglo Sampling Station

Manganese

The highest concentration of Manganese in the small-sized clams at the Aveglo sampling station was observed in February 2009 (201μg/g) and lowest in June 2008 (79μg/g). The medium-sized clams recorded concentrations varying between a low of 73μg/g in May and June 2008 and 206μg/g in March of the same year. The large-sized clams recorded values ranging between 72μg/g in November 2008 and 228μg/g in August 2008.

Zinc

Zinc concentrations for the small-sized clams ranged from a lowest value of 25μg/g in April 2008 to a highest value of 59μg/g in December of the same year. Values of 16μg/g and 54μg/g in June and

December 2008 respectively were recorded as the lowest and highest values for the medium-sized clams. The large-sized clams recorded Zinc concentrations of between 16μg/g in June 2008 and 48μg/g in September and December 2008.

Iron

Results from the analysis of Iron in the tissues of the small-sized clams indicated a lowest value of 119μg/g in October 2008 and a highest value of 427μg/g in January 2009. The medium-sized clams had iron concentrations ranging between a low of 72μg/g and a high of 539μg/g in November 2008 and March 2008 respectively. 79μg/g and 304μg/g were the lowest and highest values recorded in the months of November 2008 and January 2009 respectively for the large-sized clams.

Total Mercury

THg concentrations in the tissues of the small-sized clams at the Aveglo sampling station ranged between 0.037μg/g and 0.055μg/g in May and March 2008 respectively. Sampled medium-sized clams recorded values ranging from 0.042μg/g in July 2008 to 0.056μg/g in August 2008. The largesized clams had a lowest THg concentration of 0.037μg/g in March 2008 and a highest concentration of 0.074μg/g in June 2008.

Table 4.1

Heavy Metal Concentrations (µg/g dw) of Mn, Zn, Fe, and Hg in the different clam size classes from Ada and Aveglo (March 2008 to August 2009)

PERIOD	n	SIZE CLASS	Mn	Zn	Fe	Hg
March 2008	10	Ada Small	109	23	194	0.029
	10	Ada Medium	102	13	187	0.049
	10	Ada Large	118	16	166	0.049
	10	AVG Small	120	29	139	0.055
	10	AVG Medium	206	41	539	0.047
	10	AVG Large	116	41	136	0.037
April 2008	10	Ada Small	129	36	179	0.028
	10	Ada Medium	72	21	79	0.035
	10	Ada Large	103	30	133	0.051
	10	AVG Small	95	25	143	0.042
	10	AVG Medium	123	43	161	0.045
	10	AVG Large	101	32	152	0.047
May 2008	10	Ada Small	123	42	197	0.043
	10	Ada Medium	68	26	102	0.040
	10	Ada Large	91	30	121	0.049
	10	AVG Small	108	31	187	0.037
	10	AVG Medium	73	49	157	0.054
	10	AVG Large	115	34	178	0.040
June 2008	10	Ada Small	73	42	139	0.049
	10	Ada Medium	97	27	233	0.045
	10	Ada Large	49	26	118	0.048
	10	AVG Small				
	10	AVG Medium				
	10	AVG Large				

Table 4.1 Cont" d

	10	AVG Small	79	28	149	0.046
	10	AVG Medium	73	16	170	0.047
	10	AVG Large	140	32	230	0.074
July 2008	10	Ada Small	867	26	197	0.039
	10	Ada Medium	120	22	113	0.042
	10	Ada Large	212	43	142	0.044
			164	35	195	0.045
			96	28	123	0.042
			103	16	160	0.042

Heavy Metal Concentrations (µg/g dw) of Mn, Zn, Fe, and Hg in the different clam size classes from Ada and Aveglo (March 2008 to August 2009)

PERIOD	n	SIZE CLASS	Mn	Zn	Fe	Hg
August 2008	10	Ada Small	629	19	209	0.042
	10	Ada Medium	336	29	156	0.041
	10	Ada Large	120	33	121	0.049
	10	AVG Small	95	30	172	0.053
	10	AVG Medium	125	32	307	0.056
	10	AVG Large	190	31	252	0.064
Sept. 2008	10	Ada Small	98	23	160	0.040
	10	Ada Medium	197	19	316	0.049
	10	Ada Large	145	31	154	0.056
	10	AVG Small	154	42	214	0.047
	10	AVG Medium	87	24	142	0.051
	10	AVG Small				
	10	AVG Medium				
	10	AVG Large				

Table 4.1 Cont" d

	10	AVG Large	130	48	151	0.045
Oct. 2008	10	Ada Small	119	47	124	0.044
	10	Ada Medium	95	40	119	0.044
	10	Ada Large	82	42	106	0.054
	10	AVG Small	115	49	119	0.049
	10	AVG Medium	105	43	112	0.045
	10	AVG Large	98	38	113	0.040
Nov. 2008	10	Ada Small	102	48	103	0.039
	10	Ada Medium	122	47	128	0.039
	10	Ada Large	75	39	255	0.045
	10	AVG Small	110	46	207	0.041
	10	AVG Medium	82	28	72	0.047
	10	AVG Large	72	47	79	0.039
Dec. 2008	10	Ada Small	160	59	209	0.033
	10	Ada Medium	106	57	117	0.037
	10	Ada Large	99	49	120	0.055
			137	59	153	0.038
			136	54	185	0.055
			98	48	137	0.043

Heavy Metal Concentrations (µg/g dw) of Mn, Zn, Fe, and Hg in the different clam size classes from Ada and Aveglo (March 2008 to August 2009)

PERIOD	n	SIZE CLASS	Mn	Zn	Fe	Hg
Jan. 2009	10	Ada Small	100	54	484	0.026
	10	AVG Small				
	10	AVG Medium				
	10	AVG Large				

Table 4.1 Cont" d

	10	Ada Medium	150	40	225	0.033
	10	Ada Large	99	34	313	0.048
	10	AVG Small	152	48	427	0.046
	10	AVG Medium	165	36	282	0.042
	10	AVG Large	127	32	304	0.037
Feb. 2009	10	Ada Small	204	47	370	0.038
	10	Ada Medium	180	41	340	0.037
	10	Ada Large	316	31	295	0.039
	10	AVG Small	201	44	239	0.039
	10	AVG Medium	162	40	142	0.043
	10	AVG Large	228	41	160	0.039
March 2009	10	Ada Small	200	34	175	0.034
	10	Ada Medium	173	33	197	0.036
	10	Ada Large	119	35	155	0.045
	10	AVG Small	172	35	166	0.049
	10	AVG Medium	166	36	174	0.049
	10	AVG Large	205	35	211	0.041
April 2009	10	Ada Small	140	37	134	0.041
	10	Ada Medium	147	35	94	0.044
	10	Ada Large	163	38	213	0.043
	10	AVG Small	175	32	186	0.044
	10	AVG Medium	190	51	215	0.047
	10	AVG Large	163	36	211	0.040
May 2009	10	Ada Small	132	38	143	0.039
	10	Ada Medium	171	37	165	0.045
	10	Ada Large	187	32	105	0.049
	10	AVG Small				
	10	AVG Medium				
	10	AVG Large				

Table 4.1 Cont" d

142	32	181	0.043
119	39	125	0.050
97	40	125	0.047

10	AVG Small
10	AVG Medium
10	AVG Large

Table 4.1 Cont" d

Heavy Metal Concentrations (µg/g dw) of Mn, Zn, Fe, and Hg in the different clam size classes from Ada and Aveglo (March 2008 to August 2009)

PERIOD	n	SIZE CLASS	Mn	Zn	Fe	Hg
June 2009	10	Ada Small	138	31	169	0.049
	10	Ada Medium	154	38	148	0.041
	10	Ada Large	78	29	96	0.051
	10	AVG Small	117	35	139	0.041
	10	AVG Medium	112	37	136	0.048
	10	AVG Large	130	35	150	0.049
July 2009	10	Ada Small	154	35	179	0.047
	10	Ada Medium	102	35	196	0.049
	10	Ada Large	107	39	140	0.050
	10	AVG Small	154	36	247	0.050
	10	AVG Medium	102	39	269	0.044
	10	AVG Large	107	36	208	0.048
August 2009	10	Ada Small	78	33	138	0.044
	10	Ada Medium	120	34	141	0.050
	10	Ada Large	70	33	130	0.048
	10	AVG Small	114	35	193	0.048
	10	AVG Medium	157	37	205	0.048
	10	AVG Large	101	35	169	0.045
WHO STANDARD (WHO, 2000)			*****	**1000**	*****	**0.5**

* *No health-based guideline values were found*

4.3.2 Heavy Metal Concentrations in the Sediment Samples

Ada Sampling Station

The concentrations of Manganese in the sediments sampled from the Ada sampling station did not vary considerably over the 18-month period and were generally similar. The lowest Manganese concentration of 110μg/g in the sediments from the Ada sampling station was recorded in November 2008 whiles the highest of 393μg/g was recorded in March 2009.

Zinc concentrations were generally very low in the Ada sediments with very small variations in concentrations over the sampling period. Concentrations were observed to be lowest in September 2008 where concentrations were in trace amounts below the detection limit of the AAS. A concentration of 9μg/g was recorded in the month of July 2009 as the highest Zn concentrations during the sampling period.

The monthly Iron concentrations over the sampling period were generally very high and followed no particular pattern. Concentrations varied from a lowest value of 696μg/g in August 2008 to 2758 μg/g in March of the same year.

Concentrations of total mercury ranged between 0.0069μg/g and 0.0240μg/g as the lowest and highest recorded concentrations in the months of April and August 2008.

Aveglo Sampling Station

Manganese concentrations in the Aveglo sediments varied between a low of 100μg/g in May 2008 and 290μg/g in August 2008.

Concentrations of Zinc in the sediments from the Aveglo sampling station were very low, similar to the concentrations recorded at Ada between the months of May and September of 2008. Zn values

ranged from the lowest value 1μg/g in the months of October and November 2008 to the highest of 8μg/g in the months of May and June 2009.

Iron concentrations were relatively very high with the lowest concentration of 1114 μg/g recorded in June 2008 and the highest value of 3476μg/g in August of 2008.

Total mercury concentrations in the sediments from the Aveglo sampling station were far lower than the concentrations observed in the tissues of the clams of all the three size classes. The lowest concentration of 0.0078μg/g was observed in May 2008 and the highest of 0.0230μg/g in June 2008.

Heavy metal content of the sediment samples from the two sampling stations were compared to the Unpolluted Sediment Standard (GESAMP, 1982) to ascertain the level of pollution (Table 4.2)

On the average the heavy metal concentrations in the sediment were well below the Sediment Standard values indicating that the local metal fabrication industries and the surrounding agricultural lands have not severely impacted negatively on the Estuary.

Table 4.2 *Heavy Metal Concentrations (µg/g dw) of Mn, Zn, Fe, and Hg in Sediment Samples from Ada and Aveglo (March 2008 to August 2009)*

PERIOD	SAMPLING SITE	Mn	Zn	Fe	Hg
March 2008	Ada	180	3	2758	0.0079
	Aveglo	160	3	1564	0.0210
April 2008	Ada	192	3	2532	0.0082
	Aveglo	187	2	1214	0.0240
May 2008	Ada	201	3	2540	0.0079
	Aveglo	100	2	1158	0.0099
June 2008	Ada	189	3	2541	0.0080
	Aveglo	106	2	1114	0.0230
July 2008	Ada	154	1	1749	0.0078
	Aveglo	139	3	1683	0.0080
Aug. 2008	Ada	157	3	696	0.0140
	Aveglo	290	5	3476	0.0100
Sept. 2008	Ada	297	ND	978	0.0069
	Aveglo	245	6	3244	0.0119
Oct. 2008	Ada	150	2	960	0.0088
	Aveglo	163	1	1728	0.0095
Nov. 2008	Ada	110	1	1650	0.0076
	Aveglo	197	1	1836	0.0088
Dec. 2008	Ada	198	2	2160	0.0100

	Aveglo	187	2	1140	0.0094

Table 4.2 Cont" d *Heavy Metal Concentrations (µg/g dw) of Mn, Zn, Fe, and Hg in Sediment Samples from Ada and Aveglo (March 2008 to August 2009)*

PERIOD	SAMPLING SITE	Mn	Zn	Fe	Hg
Jan. 2009	Ada	139	3	1266	0.0094
	Aveglo	130	2	1620	0.0106
Feb. 2009	Ada	117	2	1362	0.0083
	Aveglo	199	3	1206	0.0097
March 2009	Ada	393	3	2010	0.0083
	Aveglo	140	3	1542	0.0095
April 2009	Ada	147	3	1630	0.0079
	Aveglo	139	3	1452	0.0081
May 2009	Ada	198	2	1548	0.0082
	Aveglo	124	8	1560	0.0080
June 2009	Ada	173	8	1578	0.0081
	Aveglo	156	8	1656	0.0084
July 2009	Ada	178	9	1872	0.0095
	Aveglo	245	7	2862	0.0092

Aug. 2009	Ada	175	7	2034	0.0089
	Aveglo	188	6	1598	0.0095
Unpolluted Sediment Standards*		**770**	**95**	**4100**	**1**

* GESAMP, 1982

4.4 Temporal Variations and Trends in Heavy Metal Concentrations in the Clam and Sediment Samples

Heavy metal concentrations of the four studied metals in the tissues of clam and samples from the two sampling locations were observed over the 18-month period for peculiar temporal trends and variations and whether or not the monthly concentrations varied significantly over the 18-month period. The trends were observed in the tissues of the three clam size classes on a monthly basis over the 18-month sampling period. The results for the temporal trends and variations of the metal concentrations in the tissue of each clam size class and in the sediments are presented below for both sampling locations.

4.4.1 Temporal Trends in the Ada Clams

The temporal variations in the monthly concentrations of Manganese, Zinc, Iron and Total Mercury at the Ada sampling station are presented in Figs. 4.3 and 4.4

Manganese

The concentrations of Manganese in the small-sized clams at the Ada sampling station did not vary significantly ($p>0.05$) during the sampling period. Temporal trend was non-uniform with peak values of 867μg/g and 629μg/g registered in July and August 2008 (Fig. 4.3). These values coincide with the spawning period of *G. paradoxa* which starts in June (when the mean dry tissue weight maximum occurs) and completed between the end of October and the beginning of November (when the mean dry tissue weight minimum) (Etim *et al.*, 1991). Mn concentrations however dropped to 98μg/g September 2008 with a gradual rise to 200μg/g in March 2009 indicating a gradual build-up of Mn in the tissue of the small-size clams towards the onset of the spawning season.

Manganese concentrations in the tissues of the medium-sized clams rose steadily from April (72μg/g) to August 2008 (336μg/g) (Fig. 4.3). The peak Manganese concentration registered in August also coincided with the spawning period indicating a build-up of Manganese (which is an essential heavy metal) towards and into the spawning period. Concentrations however dropped steadily from August to December 2008 although there was another steady build-up from December 2008 to May 2008 indicating the peculiar trend of metal accumulation towards the beginning of the clam spawning season. Significant temporal variations ($p<0.05$) were registered for Manganese in the medium-sized clams during the sampling period.

The peak value of manganese concentration in the tissues of the large-sized clams was recorded in July 2008 (212μg/g). This also coincided with the spawning period as was observed in the small and medium-sized clams. Temporal trend showed a steady decline from March (118μg/g) to June 2008 (49μg/g) after which the concentration rose sharply to 212μg/g in July 2008 (Fig. 4.3). Mn concentration in the large-sized clams however dropped consistently on a monthly basis from July to November 2008 after which there were consistently higher concentration towards the onset of the clam spawning season. There were significant temporal variations ($p<0.05$) in the monthly concentrations of manganese in the tissue of the large- sized clams.

Zinc

The concentrations of Zinc in the small-sized clams sampled at Ada showed a significant temporal trend ($p<0.05$) rising steadily from a concentration of 23 μg/g in March to 42 μg/g in May and June coinciding the onset of the spawning season (Fig. 4.3). This trend is comparable to the one exhibited by the medium-sized clams for manganese indicating the possible accumulation of heavy metals in the

tissues of *G. paradoxa* prior to and during the spawning season. The Zn concentration in the small clams exhibited another steady rise from August to December 2008 where whole tissue concentrations rose from 19 to 59μg/g. There was however an irregular rise-and-fall in Mn concentrations from January to August 2009.

The medium-sized clams exhibited a steady rise in zinc concentrations from March to June 2008 with values of 13μg/g and 27μg/g respectively. There was however a declining trend from June (27μg/g) to September (19μg/g) (Fig. 4.3). There is an indication of a relationship between the accumulation of essential heavy metals and the reproductive cycle of *G. paradoxa* with concentrations increasing steadily up to a peak value which typically coincides with the spawning season of *G. paradoxa*. The concentration of Mn in the medium-sized clams also rose steadily from August to December 2008 similar to the one exhibited by the small-sized clam. A similar irregular rise-and-fall pattern as was observed in the small-sized clam was observed in the medium-sized clams from January to August 2009.

Zinc concentrations in the tissue of the large-sized clams showed a steady rise from 16μg/g in March to 43μg/g in July 2008 and again from August to December of the same year showing a remarkably similar temporal trend to those exhibited by the small and medium-sized clams (Fig. 4.3).

Temporal variations were significant ($p<0.05$) during the sampling period.

Iron

Iron concentrations in the small-sized clams exhibited an irregular rise-and-fall temporal trend. There were significant differences ($p<0.05$) in the monthly Fe concentrations over the sampling period with a peak value of 484 μg/g in January 2009 (Fig. 4.4).

As with the small-sized clams, the medium-sized clams also exhibited an irregular rise-and-fall temporal trend for zinc. Temporal variations were also significant ($p<0.05$) over the study period. Fe concentrations in the medium-sized clams ranged between 79μg/g in April and 316μg/g in September 2008 (Fig. 4.4).

Temporal trends for Fe exhibited by the large-sized clams were similar to the trends exhibited by the small and medium-sized clams indicating a similar regulating mechanism for Fe in the tissues of *G.*

paradoxa regardless of size. Fe concentrations in the tissues of the large-sized clams showed significant temporal variations ($p<0.05$) during the study period with the lowest and highest concentrations of 96μg/g and 313μg/g being registered in June and January 2009 respectively (Fig. 4.4).

Total Mercury

Total mercury (THg) concentrations in the tissues of the small-sized clams exhibited a steady rise from March (0.029μg/g) to June (0.049μg/g). A declining trend was however observed from June (0.049μg/g) to September (0.040μg/g) (Fig. 4.4). There were significant temporal variations ($p<0.05$) in THg concentrations in the tissues of the small-sized clams.

The medium-sized clams showed an irregular temporal trend. THg concentrations exhibited a riseand-fall pattern during the sampling period with peak concentrations of 0.049μg/g registered in the months of March and September 2008 (Fig. 4.4). Temporal variations in THg concentrations in the medium-sized clams were significant ($p<0.05$) over the sampling period.

There was an exhibition of temporal trends similar to the one observed in the medium-sized clams in the large-sized clams. Significant temporal variations ($p<0.05$) were also observed over the sampling period (Fig. 4.4).

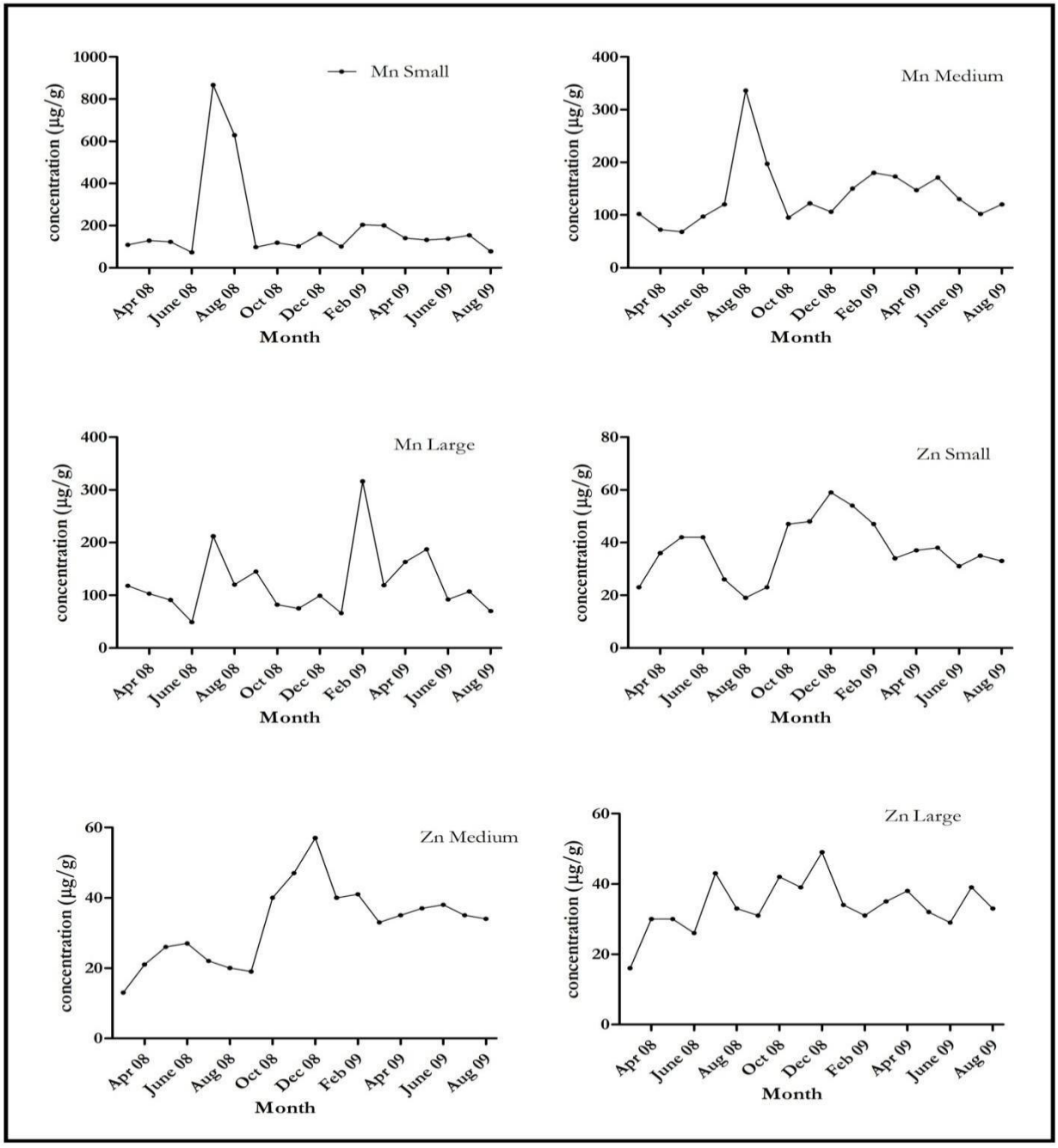

Figure 4.3 *Temporal variations in Mn and Zn concentrations in the tissues of the three clam size classes from Ada*

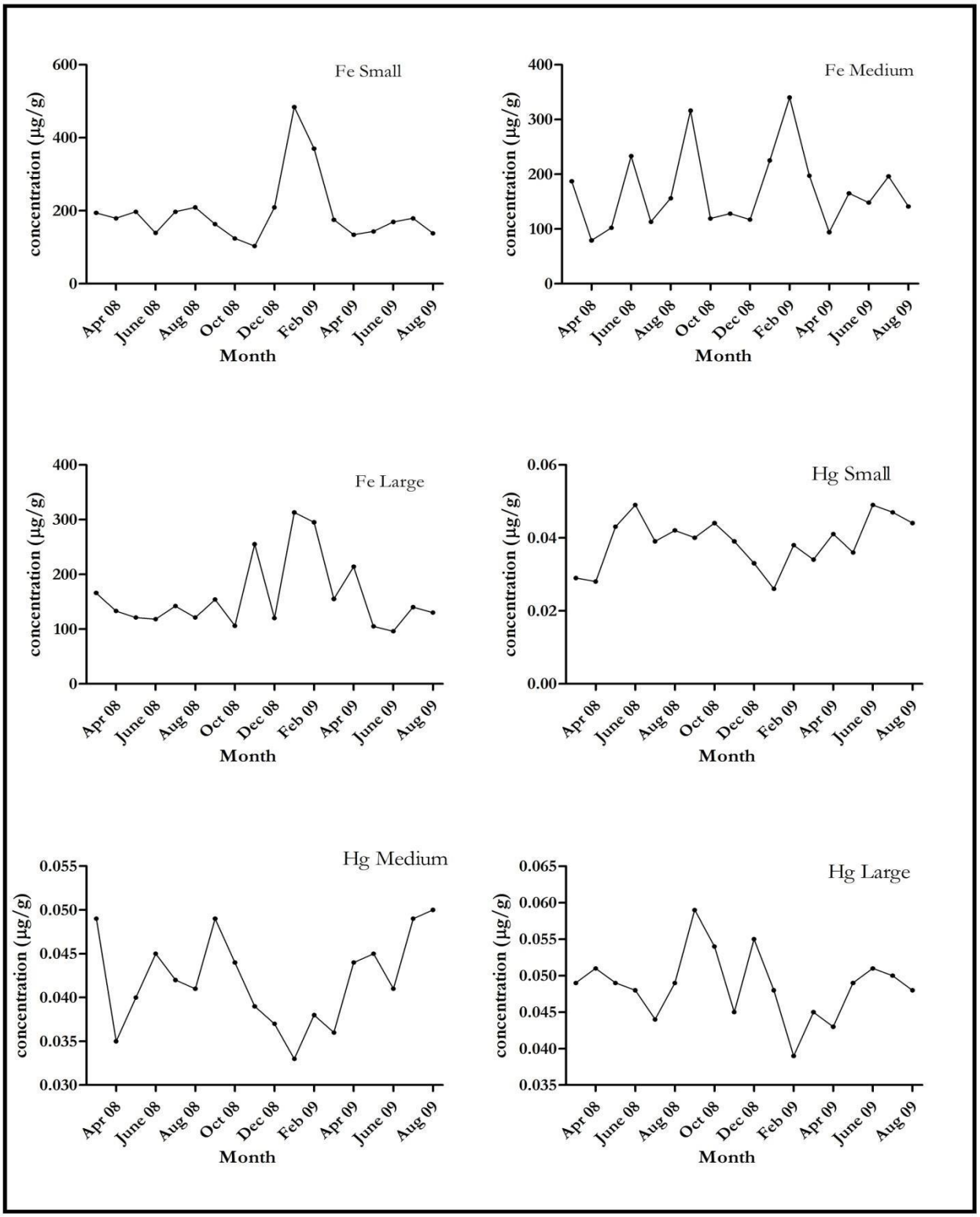

Figure 4.4 *Temporal variations in Fe and Hg concentrations in the tissues of the three clam size classes from Ada*

4.4.2 Temporal Trends in the Aveglo Clams

The temporal variations in the concentrations of Manganese, Zinc, Iron and Total Mercury at the Aveglo sampling station are presented in Figs. 4.5 and 4.6

Manganese

Manganese concentrations in the tissues of the small-sized clams showed a steady decline from 120μg/g in March to 79μg/g in May. It however peaked in June (164μg/g) coinciding with the onset of the *G. paradoxa* spawning season (Fig. 4.5). There was no regular trend displayed for Mn in the tissue of the small-sized clam over the sampling period and variations in the monthly Mn concentrations were significant variations ($p<0.05$) over the sampling period.

Manganese concentrations increased gradually from 73μg/g in May to 125μg/g in August (Fig. 4.5). The peak value (206μg/g) however was registered in March**,** coinciding with the beginning of the major rainy season in Ghana. Manganese concentrations showed significant variations ($p<0.05$) over the study period. There was a gradual build-up of Mn in the tissues of the medium-sized clams from November 2008 to April 2009 with concentrations rising from 82μg/g to 190μg/g exhibiting the familiar trend of Mn build-up towards the onset of the spawning season.

Manganese concentrations over the sampling period for the large-sized clams exhibited a trend similar to what was observed for the small and medium-sized clams, indicating similar regulation of manganese in the tissues of clams regardless of size. Manganese concentrations showed significant changes ($p<0.05$) over the sampling period (Fig. 4.5).

Zinc

Zinc concentrations in the small-sized clams over the sampling period exhibited an irregular temporal pattern. September registered the highest zinc concentration of 42μg/g with June recording the lowest value of 28μg/g (Fig. 4.5). Concentrations rose steadily from 28μg/g in June 2008 to 59μg/g in December of the same year. Concentrations however dropped thereon to 32μg/g in May 2009. Temporal variations in the monthly concentrations of the zinc in the tissues of the small-sized clams were very significant ($0<0.05$).

Temporal variations in the concentrations of zinc in the medium-sized clams followed a nonuniform trend with December 2008 registering the peak value of 54μg/g (Fig. 4.5). Variations in zinc concentration the medium-sized clams with respect to the sampling months were significant ($p<0.05$).

In the large-sized clams, zinc concentrations showed a steady decline from March to July 2008 (from 41μg/g to 16μg/g) (Fig. 4.5). The peak concentration of 48μg/g was recorded in September and December 2008. Temporal trends were however very irregular. Variations in monthly concentrations of Zinc were significant over the study period.

Iron

Iron concentrations in the small-sized clams varied significantly ($p<0.05$) over the sampling period with the peak value of 427μg/g being registered in January 2009. The temporal trend exhibited over the sampling period was non-uniform with iron concentrations following a rise-and-fall pattern (Fig. 4.6).

A concentration of 539μg/g recorded in March 2008 was the peak value of iron recorded for the medium-sized clams over the sampling period (Fig. 4.6). This peak value coincided with the onset of the major rainy season in Ghana. The temporal trend of iron concentrations in the tissues of the small-sized clams followed an irregular pattern and revealed significant variations ($p<0.05$) over the sampling period.

Iron concentrations in the large-sized clams rose steadily from 136μg/g in March to 230μg/g in June (Fig. 4.6). The peak concentration of 304μg/g was recorded in January 2009. There were very significant variations ($p<0.05$) in iron concentrations in the large-sized clams over the sampling period.

Total Mercury

The temporal trend of total mercury concentrations in the small-sized clams was non-uniform and varied significantly ($p<0.05$) over the study period.

Total mercury concentrations in the medium-sized clams showed a decline from 0.054μg/g in May to 0.042μg/g in July. The peak concentration of 0.056μg/g was recorded in May (Fig. 4.6).

Differences in THg concentrations over the sampling months varied significantly.

THg concentrations in the tissues of the large clams rose steadily from 0.037µg/g in March to 0.074µg/g in June (the onset of the spawning period and the minor rainy season in Ghana). Variations in temporal trends of total mercury concentrations over the sampling period were significant ($p<0.05$).

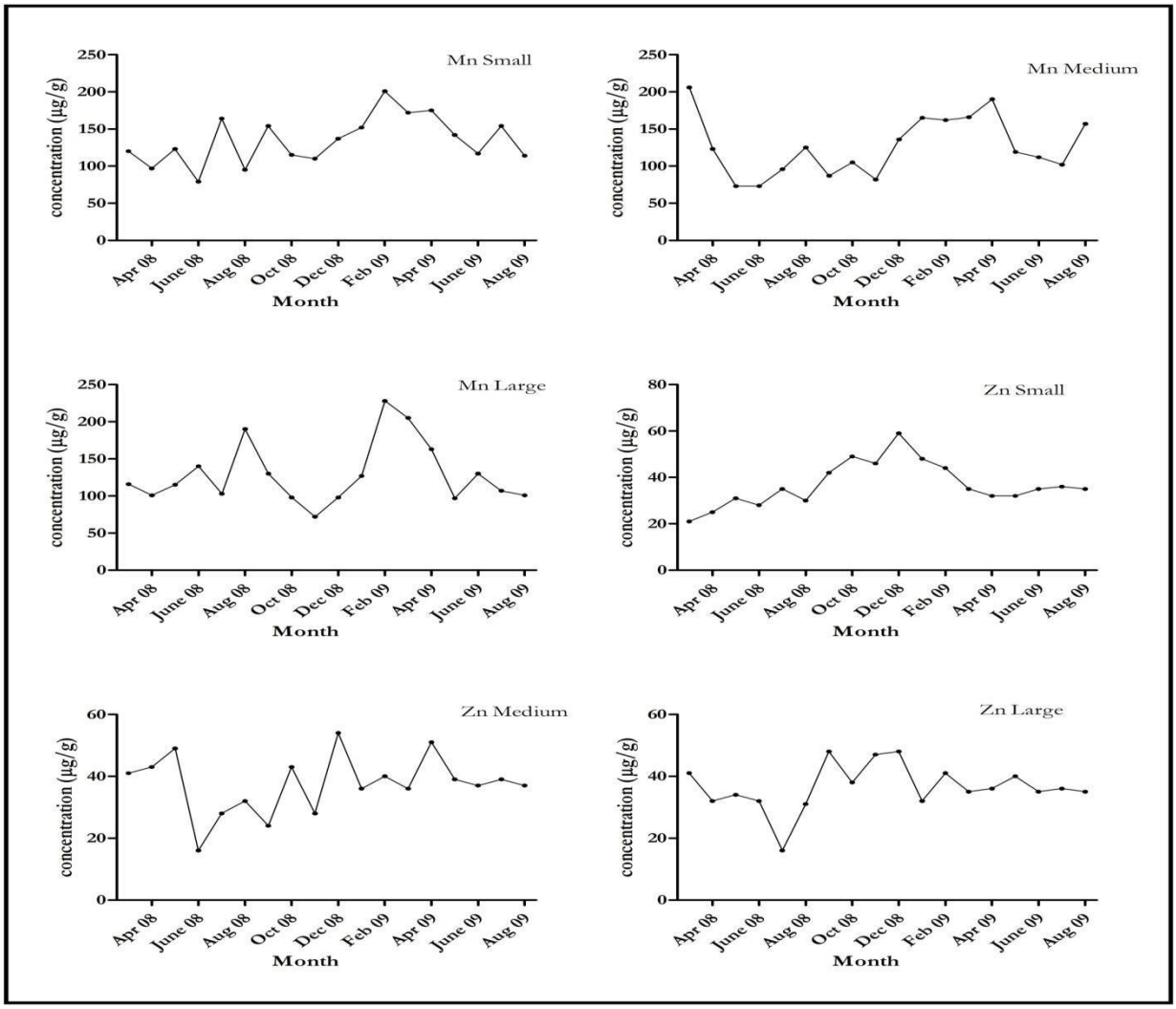

Figure 4.5 *Temporal variations in Mn and Zn concentrations in the tissues of the three clam size classes from Aveglo*

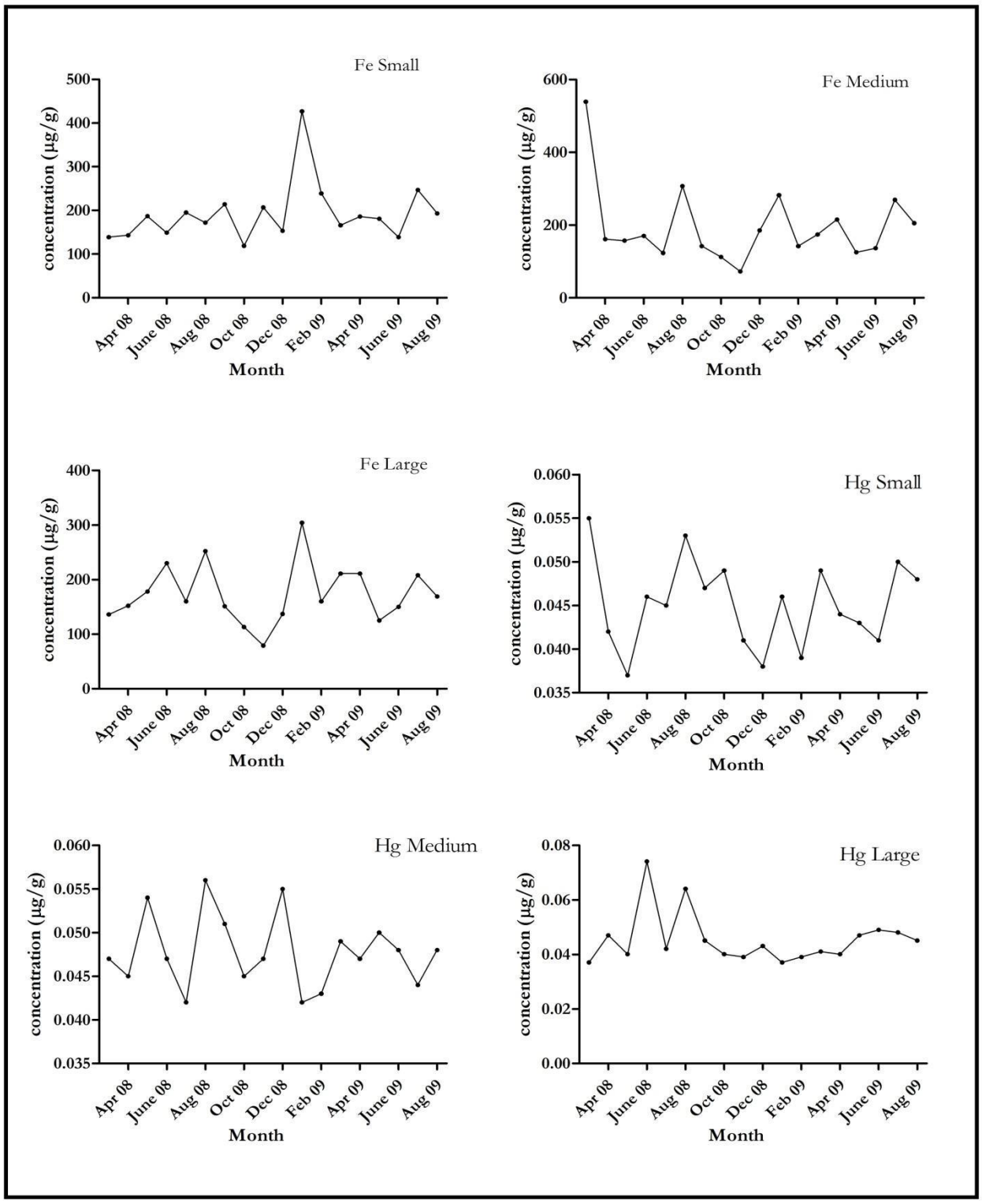

Figure 4.6 *Temporal variations in Fe and Hg concentrations in the tissues of the three clam size classes from Aveglo*

4.4.3 Temporal Trends in the Ada Sediments

In the entire sampling area Manganese levels ranged from 180μg/g in March 2008 to 393μg/g in March 2009, 2008 (Fig. 4.7). Temporal variations in manganese concentrations over the sampling period were significant ($p<0.05$).

Relatively low concentrations of Zinc were recorded at the Ada sampling station. Concentrations ranged between ND and 9μg/g during the sampling period (Fig. 4.7). Temporal variations in zinc concentrations were not significant ($p>0.05$) over the study period and followed a non-uniform trend.

Iron concentrations in the Ada sampling area ranged from 696μg/g in August 2008 to 2758μg/g in March 2008. Temporal trends were non-uniform and monthly variations in iron concentrations were significant ($p<0.05$) (Fig. 4.7).

The relatively high Iron concentrations in the Ada sediments provide evidence to the fact that areas with no known point sources of contamination may have measurably high heavy metal concentrations probably due to the processes of natural weathering in the locality.

The levels of THg varied between 0.0069μg/g and 0.0240μg/g in September and April respectively. Temporal trend was irregular over the sampling period (Fig. 4.7). Differences in THg concentrations from March to September 2008 were significant ($p<0.05$)

4.4.4 Temporal Trends in the Aveglo Sediments

Manganese concentrations ranged between 100μg/g and 290μg/g over the study period (Fig. 4.7). Temporal trend was irregular and variations in monthly concentrations were significant ($p<0.05$).

Zinc concentrations rose steadily from 1μg/g in October and November 2008 to 8μg/g in May and June 2009. Differences in monthly concentrations varied significantly ($p<0.05$) over the sampling period (Fig. 4.7).

Iron concentrations in the Aveglo sediments ranged from a low level of 1114μg/g to 3476μg/g in June and August 2008 respectively (Fig. 4.7). Significant monthly variations in concentrations ($p<0.05$) were recorded over the study period.

Significant monthly variations ($p<0.05$) were observed for THg concentrations in the Aveglo sediment samples. Concentrations ranged between 0.0080μg/g in July to 0.0230μg/g in June.

Temporal trends in monthly concentrations were irregular (Fig. 4.7).

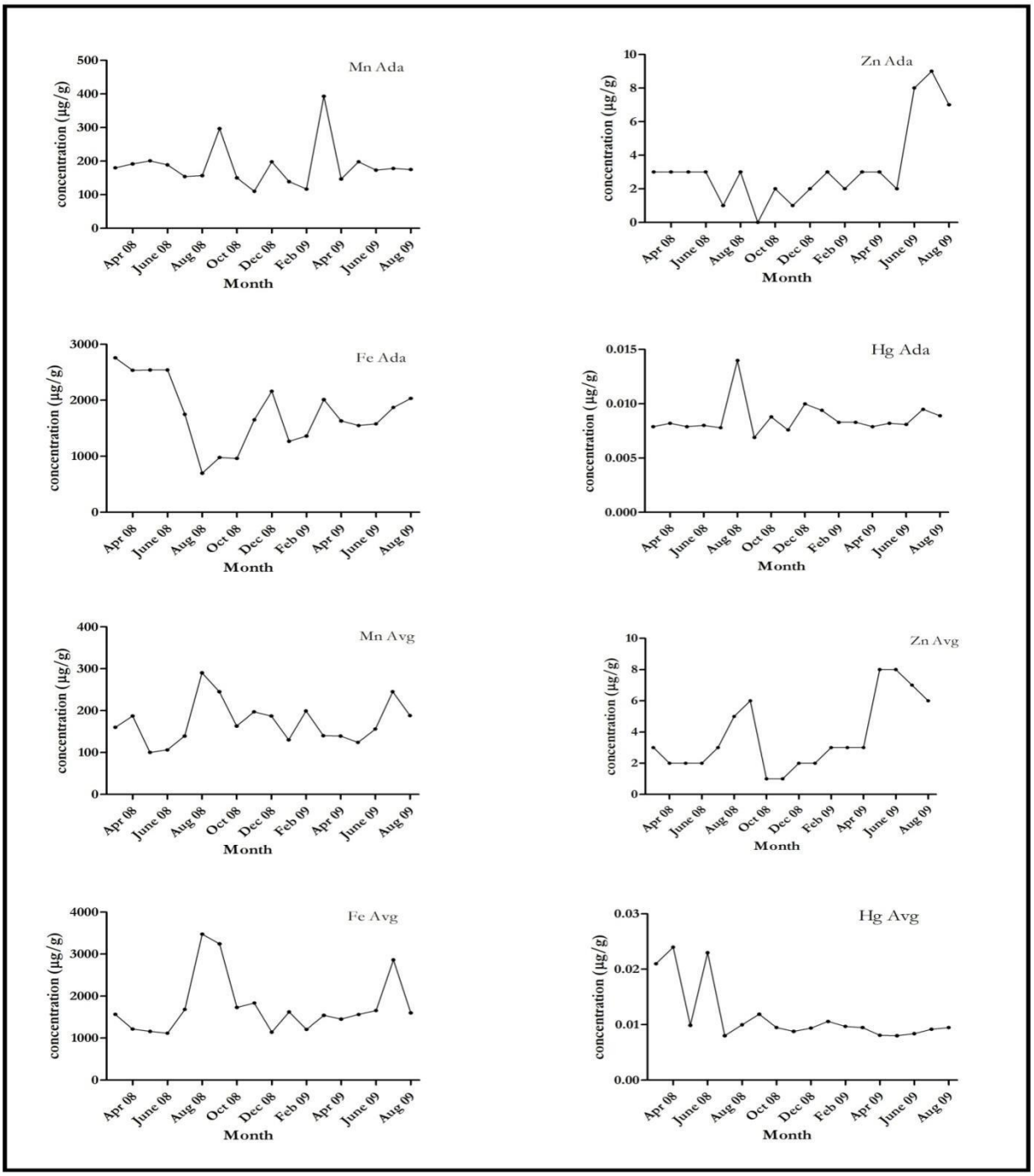

Figure 4.7 *Temporal trends in heavy metal concentrations in sediments from Ada and Aveglo*

4.5 Spatial Variations in Heavy Metal Concentrations in the Clam Samples

Comparing the different clam size classes (small vs. small, medium vs. medium and large vs. large) from the two sampling stations using the Kruskall-Wallis non-parametric test ($p<0.05$), no significant

differences ($p>0.05$) were observed for Mn, Zn, Fe and Hg concentrations in the tissues of the compared class sizes Fig 4.8 (a-f) and Fig 4.9 (a-f). The graphs are presented as means of all the monthly concentrations of each metal ± SD

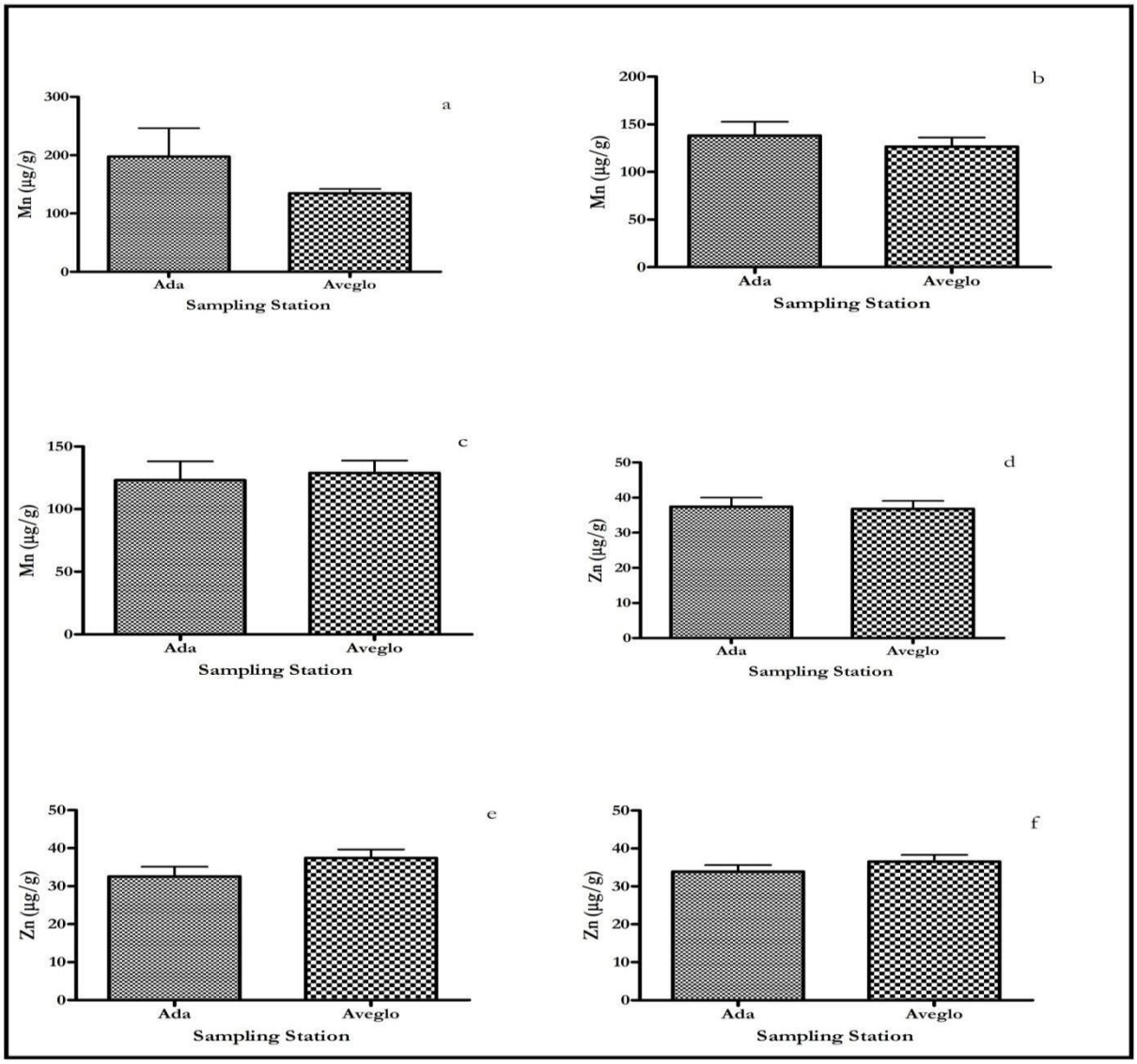

Figure 4.8 *Means ± SD of Mn and Zn concentrations in the different clam sizes from Ada and Aveglo. (a: Mn small, b: Mn medium, c: Mn large, d: Zn small, e: Zn medium, f: Zn large)*

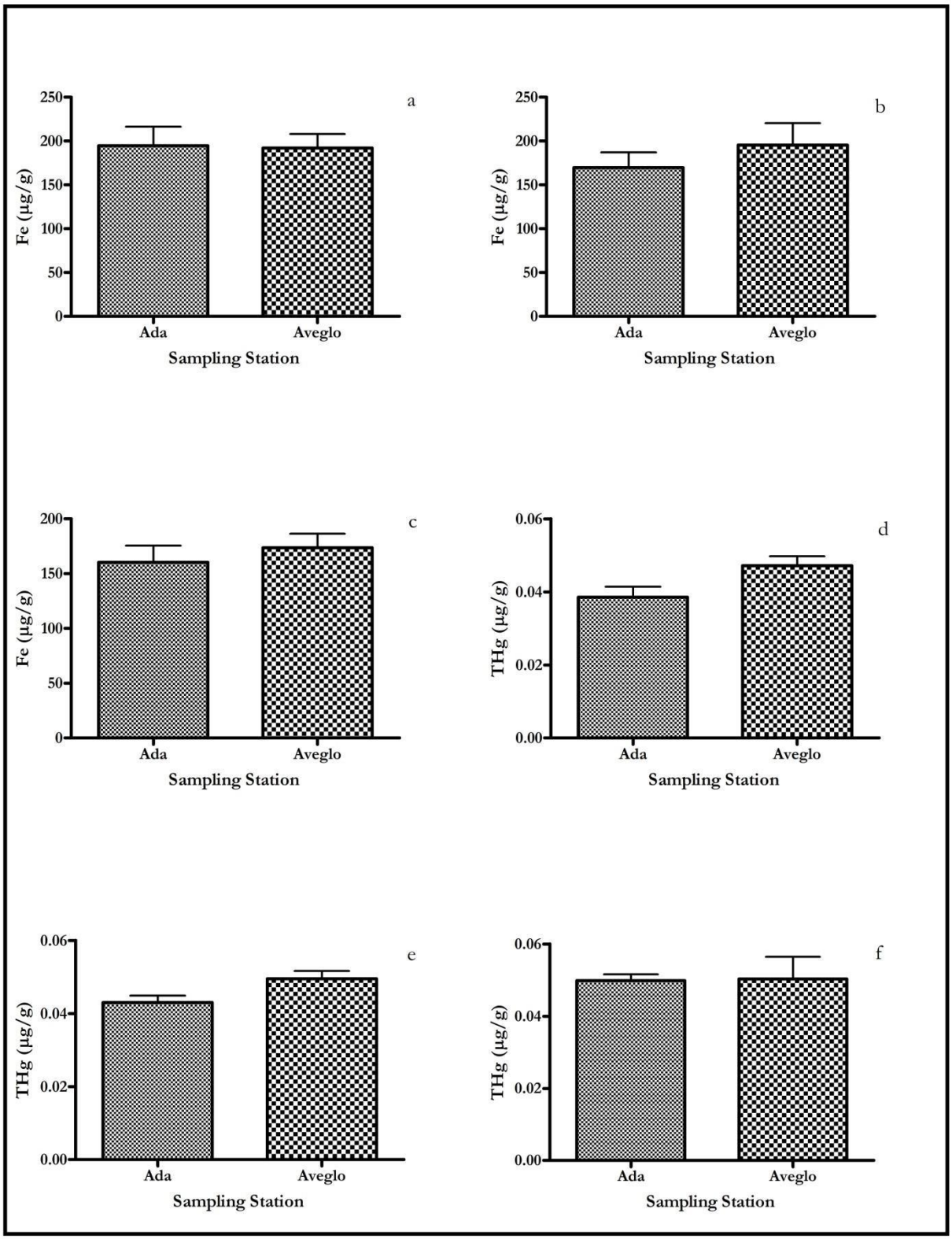

Figure 4.9 *Means ± SD of Fe and THg concentrations in the different clam sizes from Ada and Aveglo. (a: Fe small, b: Fe medium, c: Fe large, d: THg small, e: THg medium, f: THg large)*

4.6 Spatial Variations in Heavy Metal Concentrations in Sediment Samples

Results of the metal concentration in the sediment samples were subjected to the Mann-Whitney Test for Independent samples ($p<0.05$) to test for significant differences between the two sites.

Results of the statistical test revealed no significant differences ($p>0.05$) in the concentrations of Mn, Zn, Fe and THg between the two sampling sites during the study period (Fig. 4.10 (a-d). This indicates a similar bioavailability of the heavy metals in the sediments of the two sampling stations.

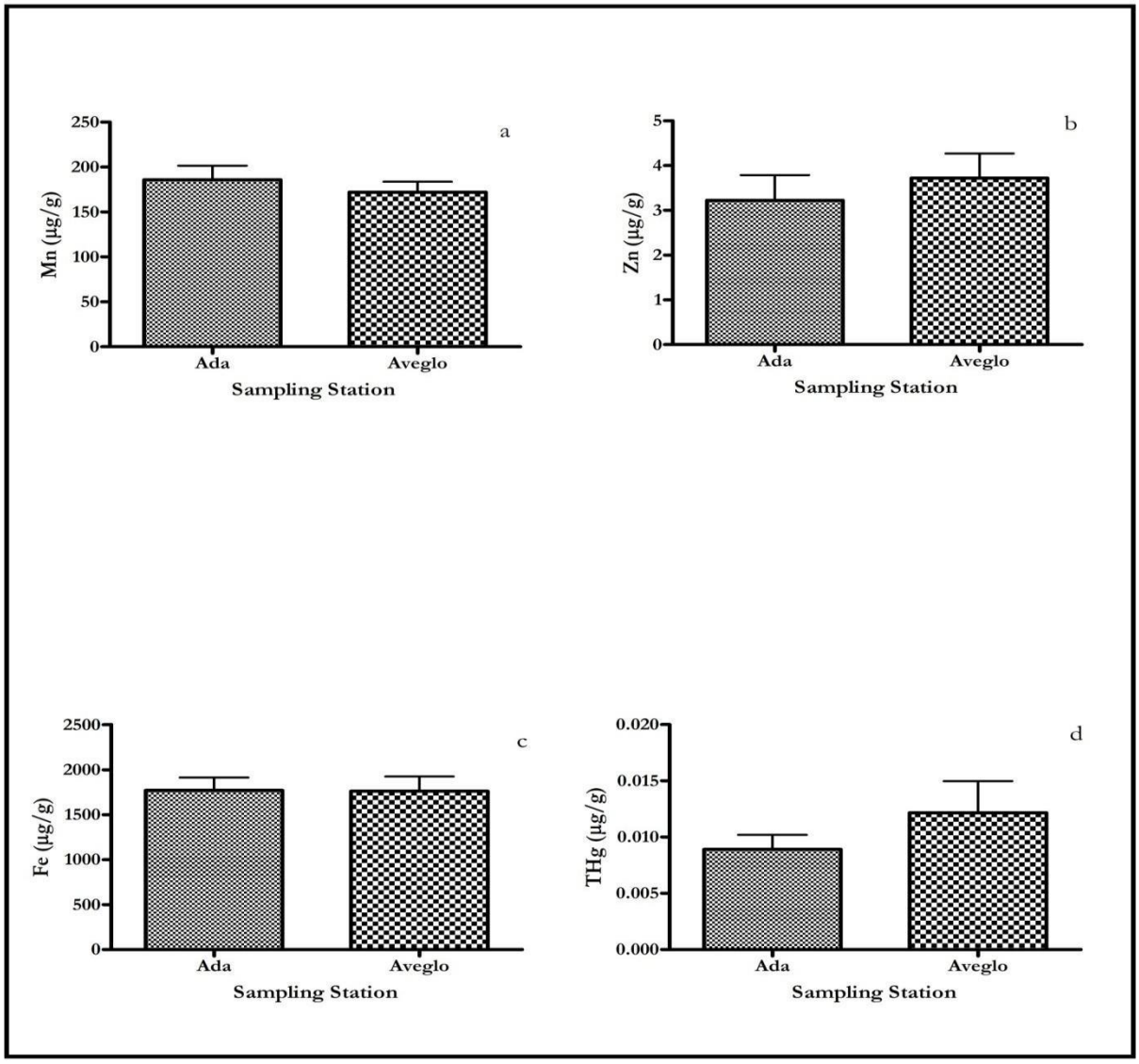

Figure 4.10 *Means ± SD of Mn, Zn, Fe and THg concentrations in the sediment samples from Ada and Aveglo (a-d).*

4.7 Comparative Evaluation of Heavy Metal Concentrations in the Clam Tissues and the Sediments

A comparative evaluation of the heavy metal concentrations in the clam tissues and sediment samples was carried out to assess the extent of heavy metal contamination and test for significant differences or otherwise of the heavy metal concentrations of the two media in relation to each other.

Differences in heavy metal concentrations between each clam size group and sediment samples from the two sampling stations were carried out using the Kruskall-Wallis non-parametric test ($p<0.05$) and the results and the resulting graphs of the analyses (Figs. 4.11 and 4.12) are shown below for each sampling station.

4.7.1 Ada Sampling Station

No significant differences ($p>0.05$) were observed in Manganese concentrations between the smallsized clams and sediment samples over the study period. Mn concentrations were higher in the sediment samples throughout the 18-month sampling period except during July and August 2008 where Mn concentrations in the small-sized clams were approximately 8 and 6 times higher in the clams than in the sediments. Significant variations were however observed in the Mn concentrations between the medium and large clams and the sediment samples. Mn concentrations in the sediments were consistently higher than in the medium and large clams over the sampling period except for periods within the clams spawning season where tissue concentrations were higher. Zinc concentrations were significantly higher ($p<0.0001$) in all the clam size classes as compared to the sediment samples. Iron concentrations in the sediment samples were as much as 10 and 18 times higher than the concentrations in the small-sized clams in certain months of the sampling periods. Similar trends were observed between the sediments and the other size classes as far as Fe concentration was concerned. Highly significant differences ($p<0.0001$) were observed in all the size-classes and the sediment samples for Iron. Total mercury concentrations showed highly significant variations (($p<0.0001$) between all the clam size classes and the sediment samples. THg concentrations were approximately five (5) times higher in the clam tissues.

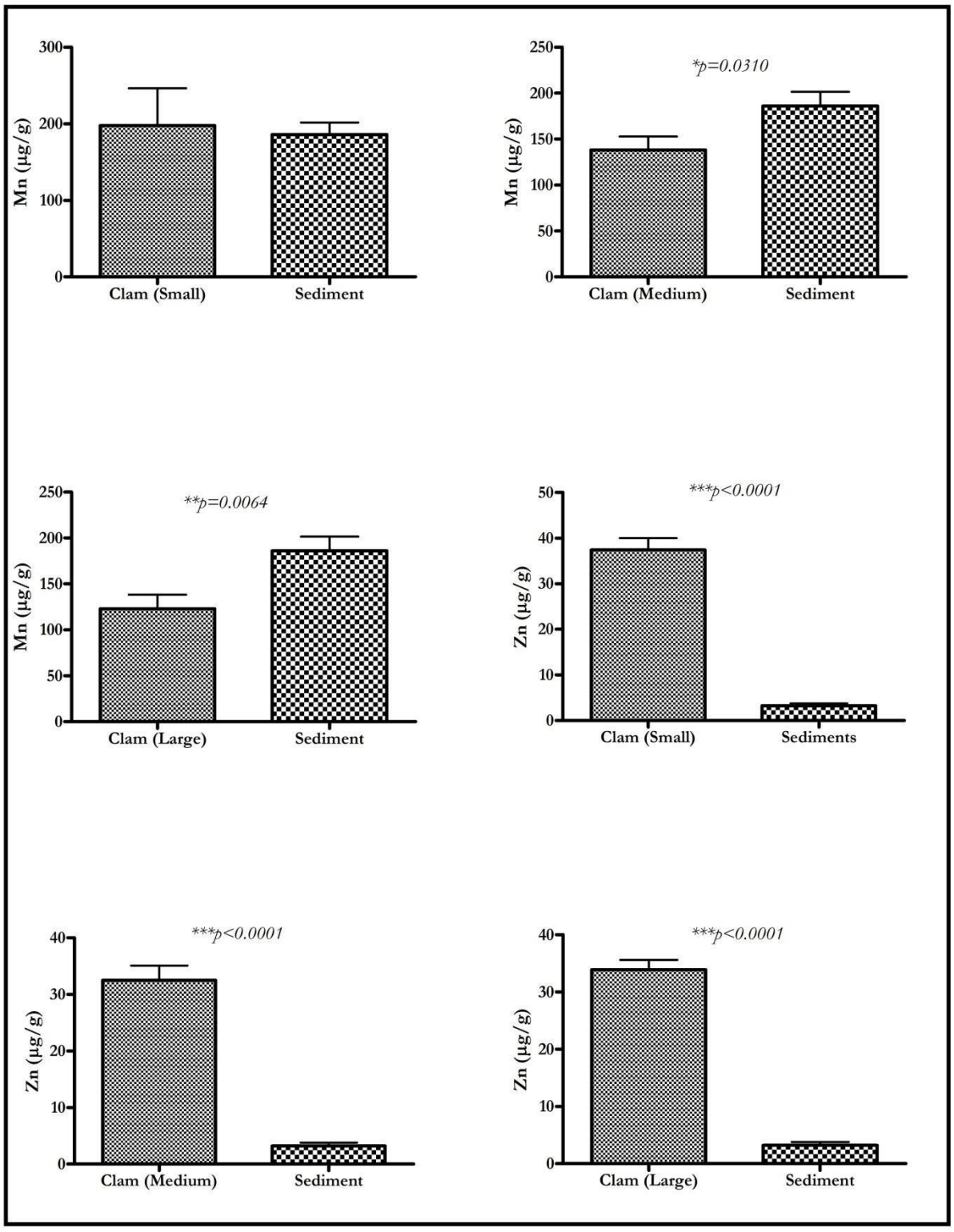

Figure 4.11 *Means ± SD of Mn and Zn concentrations in the clam and sediment samples from Ada. Significant variations: *p<0.05, **p<0.001, ***p<0.0001*

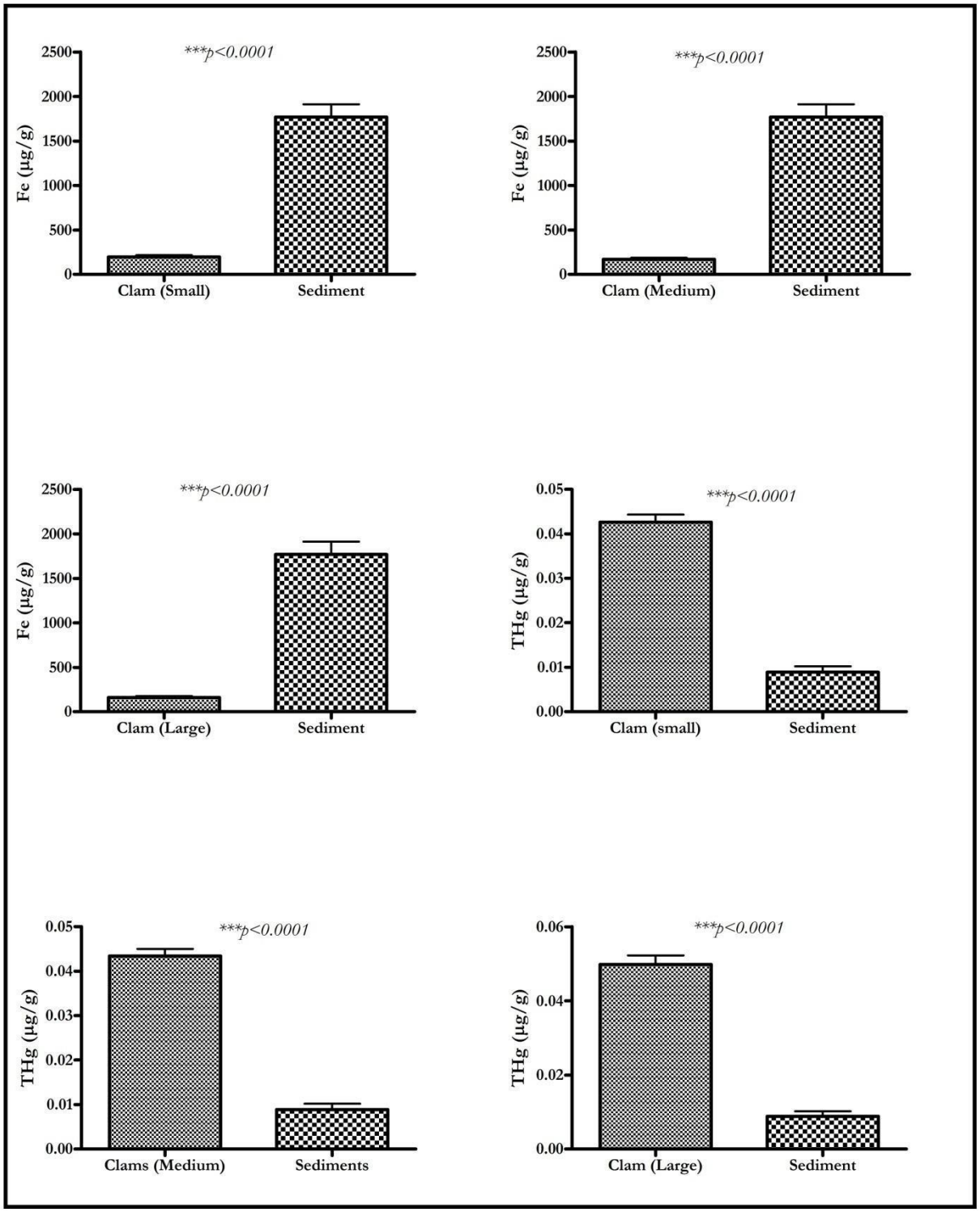

Figure 4.12 *Means ± SD of Fe and THg concentrations in the clam and sediment samples from Ada. Significant variations: ***p<0.0001*

4.7.2 Aveglo Sampling Station

Results of the comparative evaluation of the clam and sediment samples from the Aveglo sampling station portrayed a trend similar to the one observed for the Ada sampling station. Differences in Mn concentrations between the clam and sediment samples were significant ($p<0.05$) for all the clam size classes although the monthly Mn concentrations did not vary widely between the two media. Zn showed highly significant variations ($p<0.0001$) between the all the size classes and the sediment. Differences in concentration were similar to the trend observed at the Ada sampling station. Zn concentrations in the clams were as high as 26 and 47 times higher than the sediment concentration during certain months of the 18-month period indicating the possibility of uptake and accumulation of Zn by the clams and the water medium being an additional source of Zn for the clam. Highly significant differences ($p<0.001$) existed between all the clam size classes and sediment samples for Fe. Concentrations in sediments were significantly higher than in the clam samples; in March 2008, sediment concentration was approximately 14 times higher than the concentration in the small-sized clams. Highly significant differences ($p<0.0001$) were recorded for Total Mercury concentrations in the clam and sediment samples. The differences in concentrations ranged between two (2) to five (5) times more in the clam tissues.

The resulting graphs of the analyses (Figs. 4.13 and 4.14) are shown below for each sampling station.

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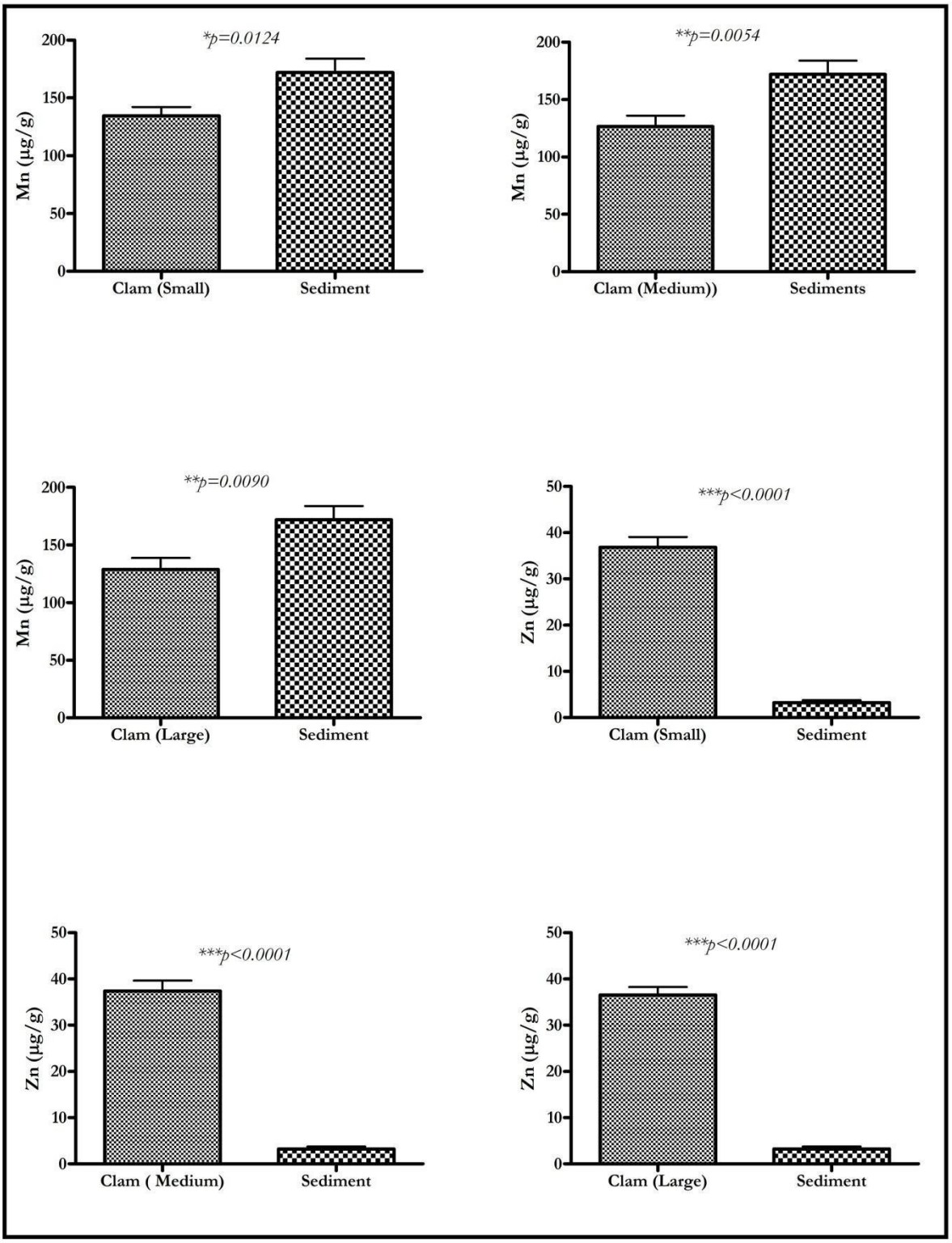

Fig.4.13 *Means ± SD of Mn and Zn concentrations in the clam and sediment samples from Aveglo. Significant variations: *p<0.05, **p<0.001, ***p<0.0001*

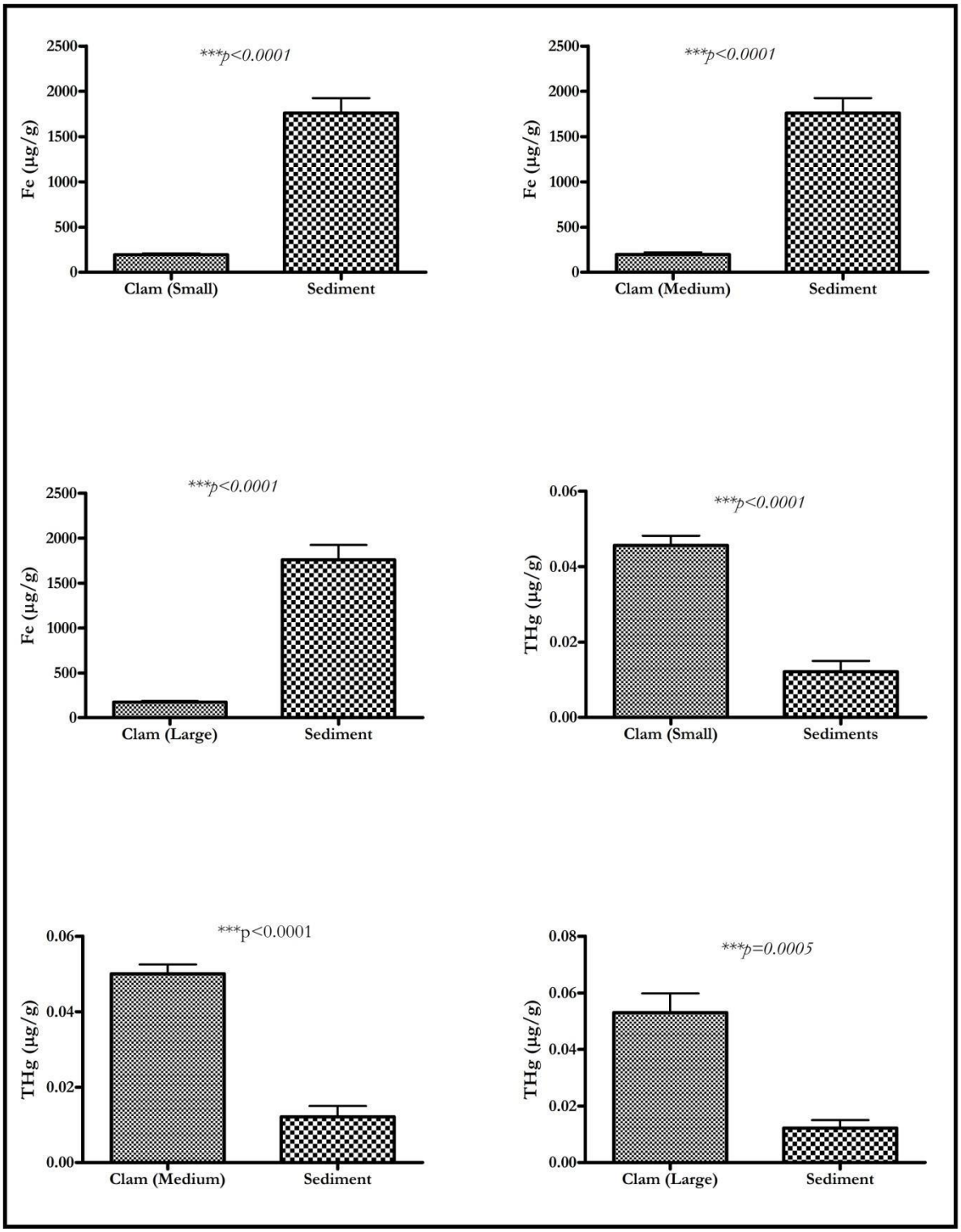

Figure 4.14 *Means ± SD of Fe and THg concentrations in the clam and sediment samples from Aveglo. Significant variations: **p<0.001, ***p<0.0001*

4.8 Relationships Between Heavy Metal Concentrations in Sediment and Accumulation in the Tissues of the Different Clam Size Classes

G. paradoxa are predominantly found at the sediment-water interface and thus metal contaminants in the sediments have a potential influence on metal concentrations in their tissues.

To understand the possible relationships between metal concentration in the sediments and accumulation in the tissues of the three clam size classes as far as Mn, Zn, Fe and Hg were concerned, the monthly concentrations of the studied metals were graphed to observe distinct metal accumulation patterns (Figs 4.15-4.18).

The graphs revealed no simple linear relationships between the concentrations of heavy metals in the clam tissues and the sediments at the two sampling stations although some distinct trends were observed. Mn concentrations in the clams and sediments from the two stations showed some distinct positive relationship patterns with increments in sediment concentrations resulting in increments in clam tissue concentrations. This relationship though, was not too clear-cut.

No defined accumulation patterns were established for Zn, Fe and Hg concentrations at the two sampling locations. Zn concentrations in the sediment samples were consistently low with values over the 18-month sampling period all falling below 10 μg/g. Fe concentrations on the other hand were clearly higher in the sediment samples and showed no distinct correlation with the concentrations in the clam tissues.

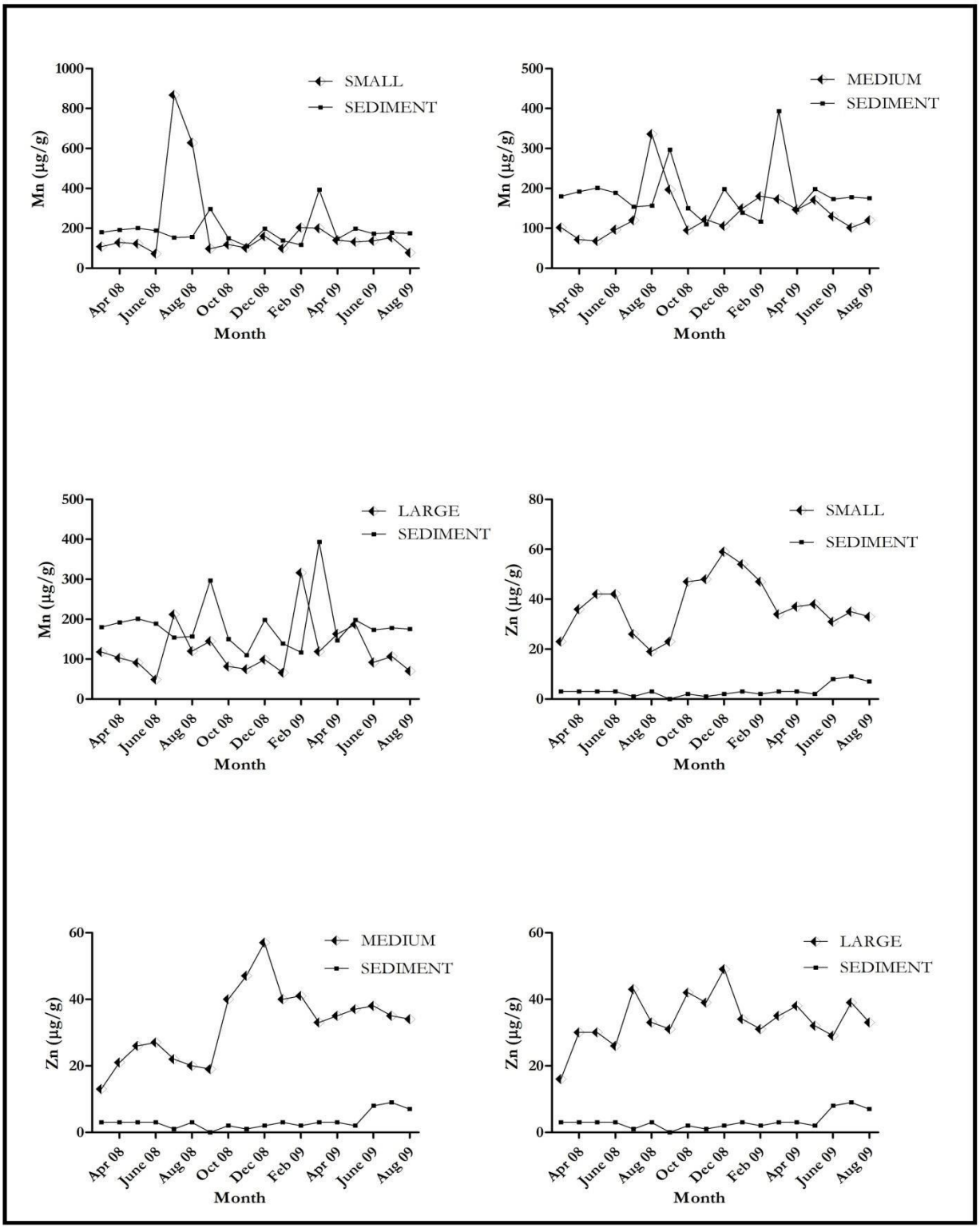

Fig 4.15 *Relationships between Mn and Zn concentrations in the tissues of the different clam sizeclasses and sediments form the Ada sampling station*

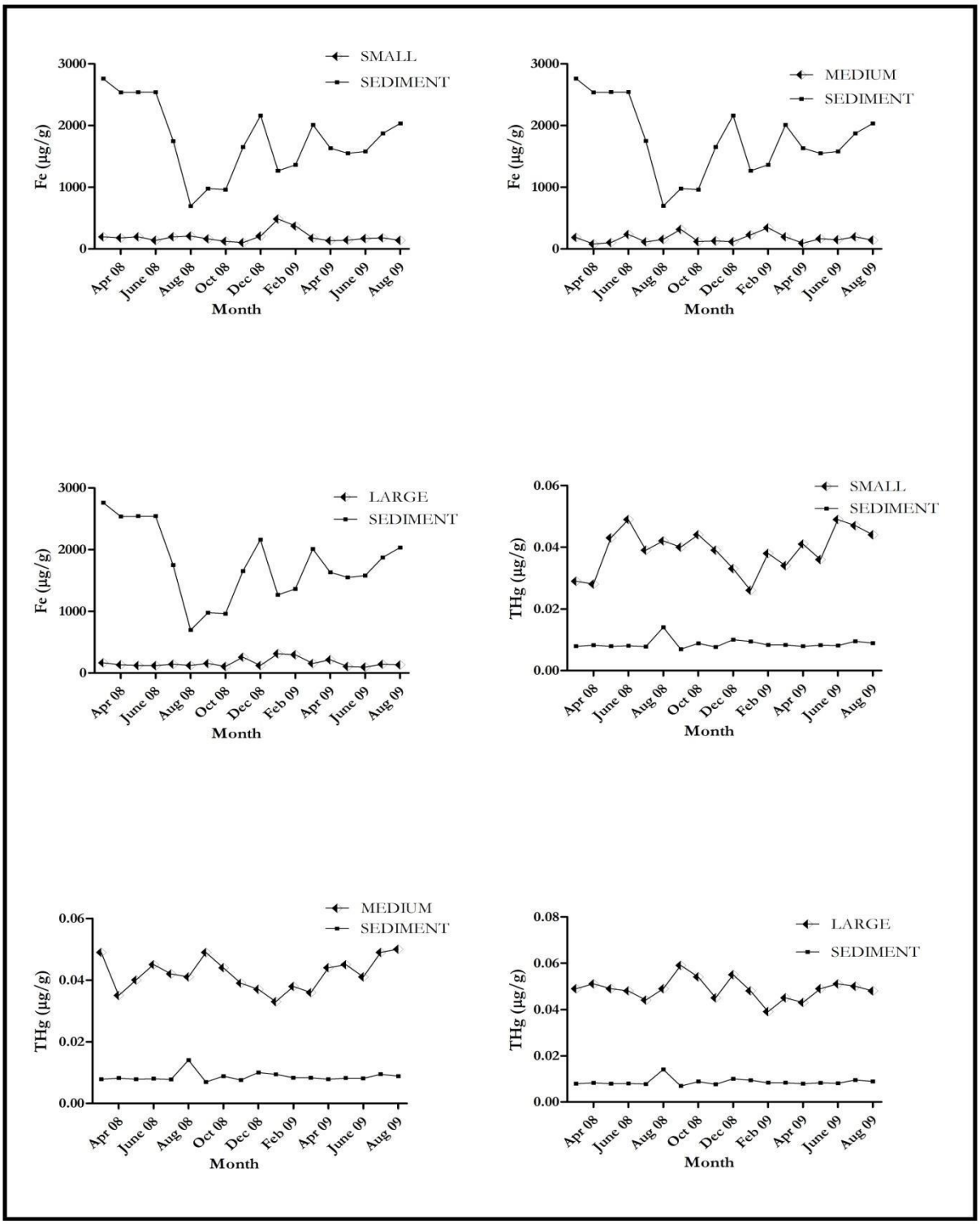

Fig 4.16 *Relationships between Fe and Hg concentrations in the tissues of the different clam sizeclasses and sediments form the Ada sampling station*

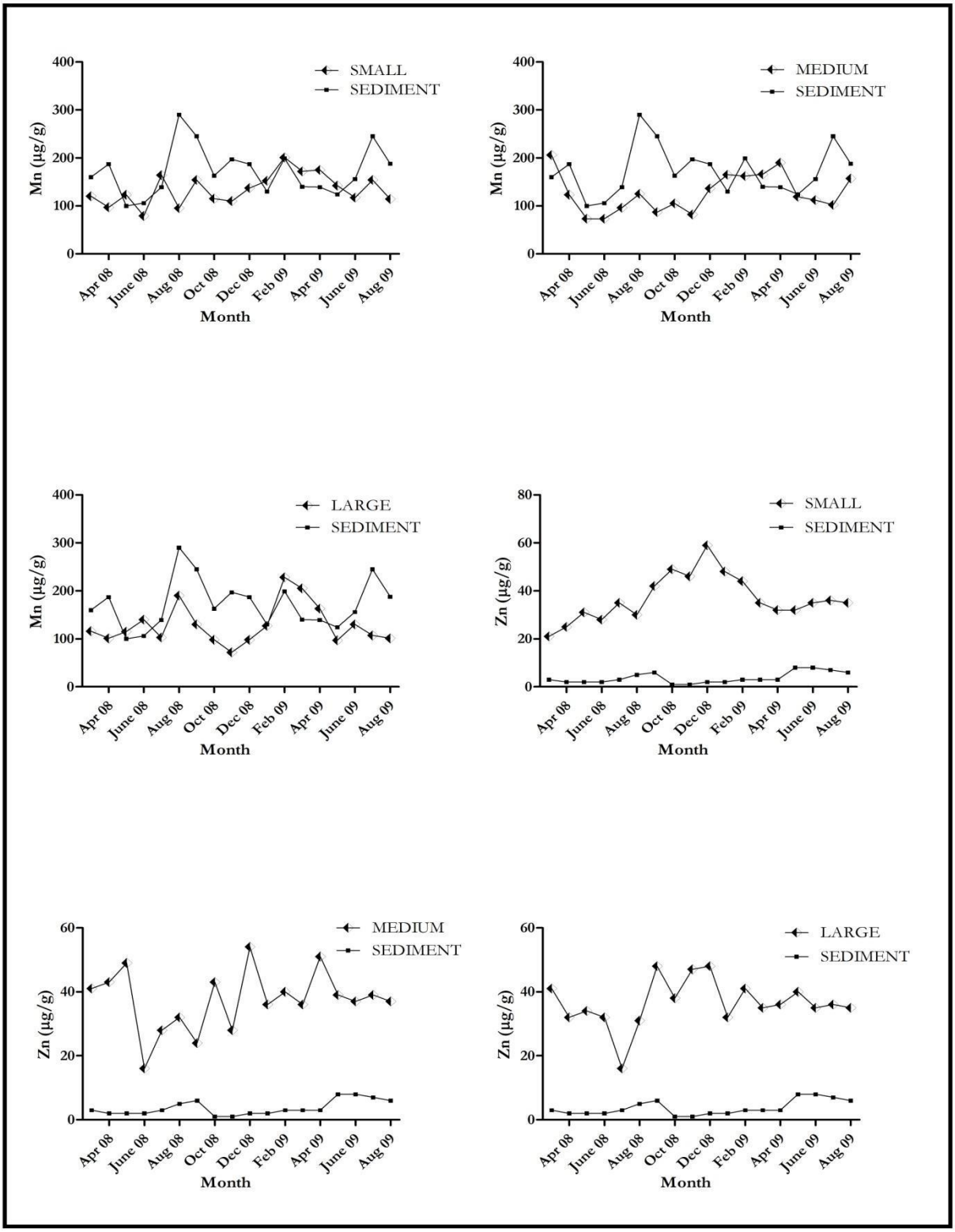

Fig 4.17 *Relationships between Mn and Zn concentrations in the tissues of the different clam size classes and sediments form the Aveglo sampling station*

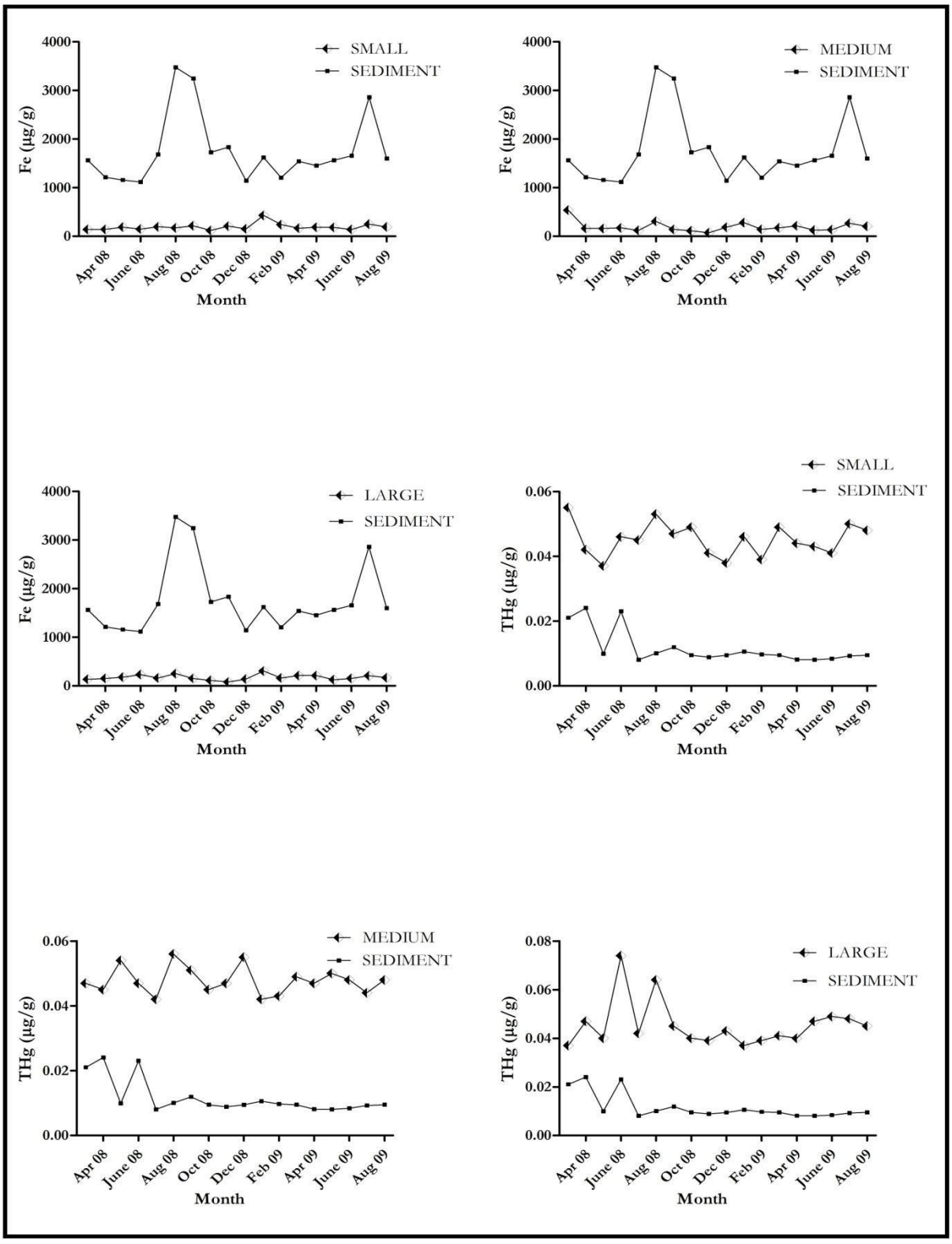

Fig 4.18 *Relationships between Fe and Hg concentrations in the tissues of the different clam sizeclasses and sediments form the Aveglo sampling station*

4.9 Variation in Heavy Metal Concentrations in Relation to Clam Size

One of the objectives of this work was to study the variations in heavy metal concentrations in the tissue of *G. paradoxa* in relation to body size and investigate whether metal uptake, storage and sequestration varied with clam sizes. The concentrations of the four studied metals in the whole soft tissues of the three clam size classes were subjected to one way ANOVA to determine whether or not there are significant differences in the concentrations of the studied heavy metals as far as clam size was concerned. The whole tissue concentration of the metals in different clam sizes was further subjected to a post-test; the Bonferroni's Multiple Comparison Test to compare the mean tissue concentrations of all the possible pairs of size classes; i.e. Small *vs.* Medium, Small *vs.* Large and Medium *vs.* Large for significant differences between the compared classes.

Variations in the mean heavy metal concentrations in the different clam size classes for both sampling stations over the sampling period were not significant ($p>0.05$) except for Total Mercury concentrations in the Ada clams (Fig 4.19 a-h)

The full results of the one way ANOVA and the Bonferroni's Multiple Comparison Test are shown in Appendix 3

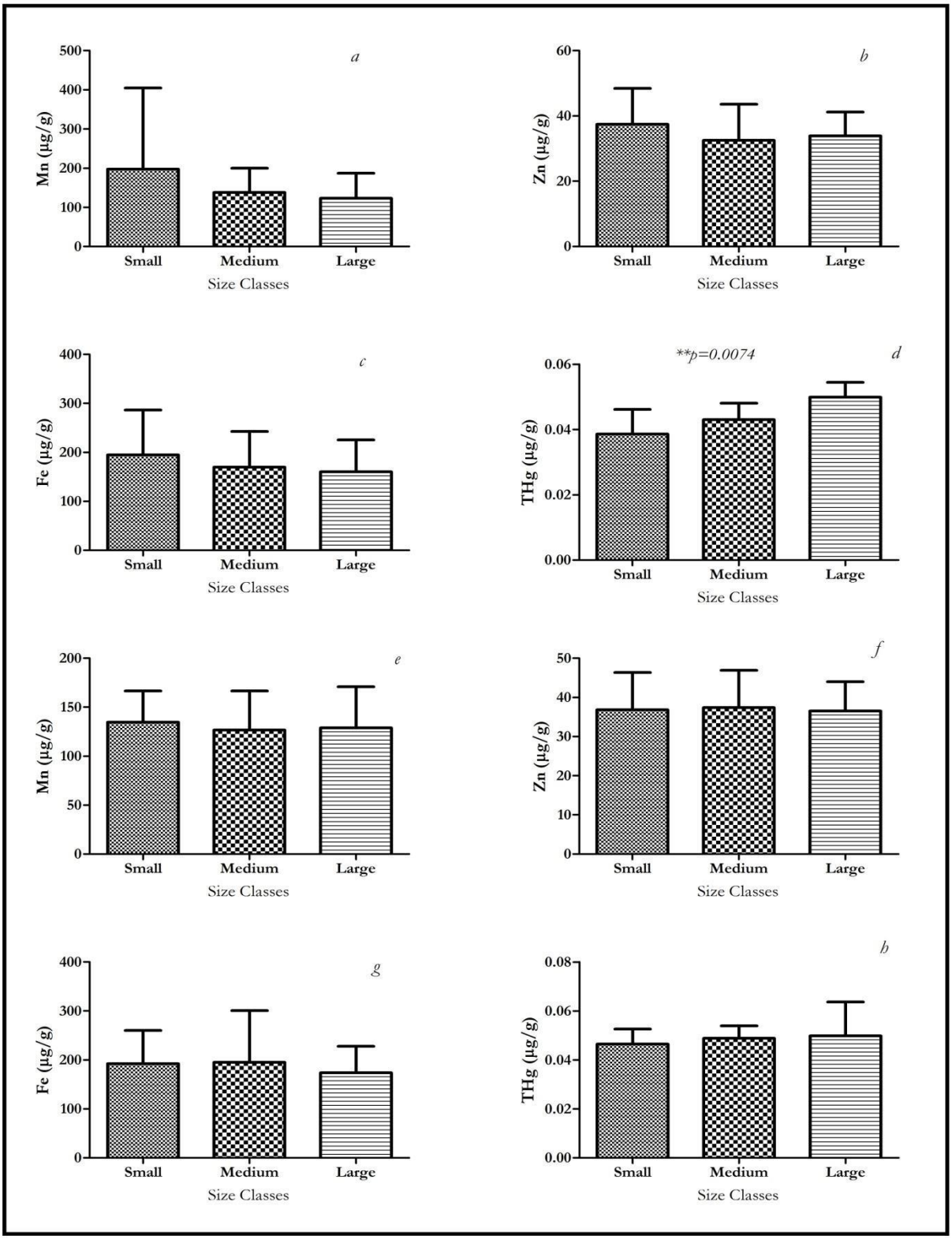

Fig .4.19 *Means ± SD of heavy metal concentrations in the clam size classes from Ada (a-d) and Aveglo (e-h). Significant variations: **p<0.001*

4.10 Health Risk Associated with the Consumption of Clams from Ada and Aveglo

In the absence of WHO Health-based Guidelines (2000) for Mn and Fe, this study evaluated the risk implications for human consumption of whole soft tissue of the Volta clam in the light of various health standards. Analysis of risks levels associated with the consumption of clams revealed that the concentration of the heavy metals found in the clam tissues were within permissible limits using various indicators such as Tolerable daily Intake (TDI), Estimated Safe and Adequate range of Daily Dietary Intake levels (ESAADI), Rate of Shellfish (RSC), Risk Quotients (RQs) and levels of concerns (LOCs) (Table 4.3). Against this background, the clams can be said to contain acceptable limits of Manganese, Zinc, Iron and Mercury for human consumption.

Based on the maximum and minimum concentrations of the heavy metals in the tissues of the clam from Ada and Aveglo (treated as one unit), all the calculated RQ" s, were 13 (Mn) to 1613 (THg) times lower than "1", suggesting that probably no health-related problems might be encountered, at least not in moderate shellfish consumers.

Table 4.3 *Risk analysis for the minimum and maximum concentrations of metals present in the clam samples from Ada and Aveglo*

Metal	Min. Conc (µg/g)	Max. Conc (µg/g)	TDI or ESADDI (µg/g/d)	RSC (g/p/d)	LOC_1 (µg/g)	LOC_2 (µg/g)	1-For LOC_1	2-For LOC_2
THg	0.028	0.074	33-43[a]	0.95	34.74	45.26	0.0021	0.0016
Zn	13	59	5600-15000[b]	0.95	5894.74	15789.47	0.0083	0.0031
Fe	79	539	8000-45000[c]	0.95	8421.05	47368.42	0.0064	0.011
Mn	49	867	2000-11000[c]	0.95	2105.26	11578.95	0.41	0.075

RQ_{WCS}

Legend:

TDI-Tolerable Daily Intake (in µg/person/day)
ESAADI-Estimated Safe and Adequate range of Daily Dietary Intake levels (in µg/person/day) for all foods set by the National Research Council of the National Academy of Sciences of the USA
RSC- Rate of Shellfish Consumption for Ghana calculated from the Daily Food Supply per capita from Fish and Fishery Products of the FAO (FAOSTAT, 2004- http:// apps.fao.org)
LOC_1-Level of Consumption (in µg/g) calculated from the lowest value of TDI or ESADDI range
LOC_2-Level of Consumption (in µg/g) calculated from the highest value of TDI or ESADDI range
RQwcs- Risk Quotient for worst-case scenario: 1-For lowest value of TDI or ESADDI range
2-For highest value of TDI or ESADDI range [a]- Provisional Tolerable
Daily Intake of total mercury; set by FAO/WHO.
[b]- Dietary reference value for zinc; WHO, 2001
[c]- Tolerable Daily Intake of Iron and Manganese; set by the Institute of Medicine of the USA, 2003

However, it should be pointed out that exposure estimates for heavy metal intake from shellfish consumption based on the national average shellfish consumption data may not be suitable for estimating exposures of particular subpopulations or individuals residing in specific regions and towns of the country, such as coastal settlements and locations of active shellfish production, where more shellfish is consumed.

4.11 Geochemical Features of the Sampling Stations

Different metal assessment indexes were used for determining the quality of the sediments from the two sampling stations. The different indexes gave diverse status of the Volta Estuary sediment quality. Evaluating the sediment contamination of the Volta Estuary using the Contamination Factor (CF) and Geoaccumulation Index (*I*geo) gave the advantage of not aggregating all the contaminants into one value and therefore treating each metal independently, giving a good picture of the extent of individual metal pollution. The estimation of natural background concentrations of each metal is to provide a precise identification of anthropogenic heavy metals and their sources.

The bioavailability of the metal pollutants in the sediments in the sampling locations was also evaluated through the calculation of the biota-sediment accumulation factor (BSAF) to ascertain the degree to which the total concentration of each metal is susceptible to uptake by the surrounding biota, with emphasis on *Galatea paradoxa*. A high bioavailability is linked to high concentrations and bioaccumulation/biomagnification within organisms which may lead to deleterious effects on biodiversity via the inability to secrete physiologically-stored pollutant concentrations, causing toxic effects that could progress through subsequent levels of the food chain.

4.11.1 Biosediment Accumulation Factors (BSAFs) for G. paradoxa in the Ada and Aveglo Sampling Stations

The BSAFs (Table 4.4) for each site were calculated to evaluate the efficiency of metal uptake by the clams and to describe the accumulation of studied metals. On the basis of the calculated BSAFs, metal enrichment in the tissues of the clams ranked in the following order; Zn>Hg>Mn>Fe. The average BSAF values reveal Zn as having the highest BSAF values although its concentration in the sediments were relatively far lower than Mn and especially Fe. Zn and Hg contamination levels were found to be higher in the clams than in the sediments, suggesting a higher rate of accumulation of the two metals by *G. paradoxa* as far as sediments were concerned. This could be as a result of the water acting as an additional source of Hg and Zn accumulation in *G. paradoxa.* Fe and Mn concentrations were generally lower in the clam tissues than in the sediments, suggesting that the levels of contamination of these metals in the estuary do not exceed the clams" capacity to regulate them. The interactions between metal geochemistry and animal physiology determine the differences in the bioavailability among heavy metals (Wang *et al*, 2002). Relationship between concentrations of the studied contaminants in the clam tissues and sediments was not clear-cut, supporting the fact that several variables control both the bioavailability and accumulation of heavy metals in individuals exposed to contamination (Ansari *et al.,* 2004).

Although Zn and Hg had reasonably high BSAFs, the build-up of these two metals in the tissues of the clams however do not exceed permissible levels for human consumption indicating trace quantities of these metals in the sediment and water media.

Table 4.4 *Average Biota-sediment accumulation factors (BSAFs) for G. paradoxa from Ada and Aveglo*

Month	Mn (µg/g)	Zn(µg/g)	Fe (µg/g)	Hg (µg/g) Ada
March 08	0.61	5.77	0.07	5.43
April 08	0.53	9.67	0.05	4.65
May 08	0.31	10.89	0.07	5.64
June 08	0.39	10.56	0.06	5.92
July 08	1.13	30.33	0.09	5.34
Aug. 08	0.97	9.00	0.14	3.14
Sept. 08	0.74	*	0.21	7.00
Oct. 08	0.64	21.50	0.12	5.34
Nov. 08	0.90	44.67	0.10	5.28
Dec. 08	0.61	27.50	0.07	4.50

	Jan. 09	0.83	15.33	0.26	3.30
	Feb. 09	1.99	21.34	0.25	4.58
	March 09	0.41	11.33	0.09	4.61
	April 09	1.02	12.22	0.09	5.41
	May 09	0.84	17.84	0.09	4.98
	June 09	0.71	4.08	0.09	5.80
	July 09	0.68	4.04	0.09	5.13
	Aug. 09	0.51	4.76	0.07	5.31
Aveglo					
	March 08	0.92	12.33	0.20	2.20
	April 08	0.57	16.67	0.13	1.86
	May 08	0.32	19.00	0.22	4.41
	June 08	1.46	12.67	0.16	2.42
	July 08	3.07	8.78	0.10	5.37
	Aug. 08	0.35	6.20	0.07	5.77
	Sept. 08	0.45	6.33	0.05	4.01
	Oct. 08	0.65	43.33	0.07	4.92
	Nov. 08	0.45	40.33	0.07	4.81
	Dec. 08	0.66	26.84	0.14	4.82
	Jan. 09	1.14	19.34	0.21	3.93
	Feb. 09	0.99	13.34	0.15	4.15
	March 09	1.29	11.78	0.12	4.87
	April 09	1.27	13.22	0.14	5.40
	May 09	0.96	4.63	0.09	5.83
	June 09	0.77	4.08	0.09	5.48
	July 09	0.49	5.28	0.08	5.15
	Aug. 09	0.65	5.95	0.12	4.95

4.11.2 Index of Geoaccumulation

The geoaccumulation index (Igeo), originally defined by Müller (1979) was applied to get a quantitative measure of the metal pollution in aquatic sediments from the Ada and Aveglo sampling stations in Table 4.6.

The formula used for the calculation of Igeo is: Igeo= $\text{Log}^2 \left[\frac{Cn}{1.5Bn}\right]$(Muller, 1979).
Cn is the measured content of element "n", and Bn the element" s content in "average shale" (background concentration) (Turekian and Wedepohl, 1961). The content accepted as background is multiplied each time by the constant 1.5 in order to take into account natural fluctuations of a given substance in the environment as well as very small anthropogenic influences.

Table 4.5 *Background concentrations of the studied metals*

	Mn	Zn	Fe	Hg
Shale Standard (Background concentration)	850*	95*	46700*	0.04†

***** Turekian and Wedepohl, 1961
† GESAMP, 1988

The calculated Igeo values were compared to description of sediment quality Igeo classification table (Table 3.5) (Müller, 1979) to ascertain the pollution intensities of the two sampling sites.

Based on the geoaccumulation calculations, it was realized that the sediments from the two sampling stations are unpolluted (class 0) as far as the heavy metals, Manganese, Zinc, Iron and Mercury are concerned. Igeo values were well below zero (0) for all the studied metals with Manganese values ranging between -1.69 and -3.64. Igeo values for Zinc fell between -4.57 and -7.54 for the sediments from the two sampling stations for the 18-month sampling period indicating that the sediments from the two stations were practically very unpolluted as far as Zinc was concerned. A comparison of the Igeo values of Iron and Mercury with the Sediment Classification Table revealed that the sediments

were unpolluted with the two metals. Igeo values for Iron fell between -4.32 and -6.66 whiles that of Mercury ranged between -1.32 and -2.98 (Table 4.6).

Table 4.

6 *Results for Sediment Geoaccumulation Index of the studied metals for Ada and Aveglo*

PERIOD	SAMPLING SITE	Mn	Zn	Fe	Hg
March 2008	Ada	-2.84	-5.57	-4.68	-2.95
	Aveglo	-2.94	-5.57	-5.51	-1.51
April 2008	Ada	-2.74	-5.57	-5.51	-2.95
	Aveglo	-2.74	-6.16	-5.88	-1.32
May 2008	Ada	-2.64	-5.57	-4.80	-2.95
	Aveglo	-3.64	-6.16	-5.88	-2.60
June 2008	Ada	-2.74	-5.57	-4.80	-2.91
	Aveglo	-3.64	-6.16	-5.97	-1.38
July 2008	Ada	-3.06	-7.16	-5.38	-2.94
	Aveglo	-3.18	-5.57	-5.38	-2.91
Aug. 2008	Ada	-3.06	-5.64	-6.66	-2.10
	Aveglo	-2.12	-4.84	-4.32	-2.58
Sept. 2008	Ada	-2.12	*	-6.16	-3.12
	Aveglo	-2.40	-4.57	-4.44	-2.33
Oct. 2008	Ada	-3.06	-6.16	-6.16	-2.76
	Aveglo	-2.94	-7.16	-5.32	-2.70
Nov. 2008	Ada	-3.47	-7.16	-5.38	-2.98
	Aveglo	-2.74	-7.16	-5.27	-2.76
Dec. 2008	Ada	-2.64	-6.16	-5.01	-2.58
	Aveglo	-2.74	-6.16	-5.97	-2.67
Jan. 2009	Ada	-3.18	-5.64	-5.80	-2.67
	Aveglo	-3.32	-6.16	-5.44	-2.50
Feb. 2009	Ada	-3.47	-6.16	-5.72	-2.85

Table 4.

	Aveglo	-2.64	-5.57	-5.88	-2.63
March 2009	Ada	-1.69	-5.64	-5.11	-2.85
	Aveglo	-3.18	-5.57	-5.51	-2.66

6 Cont" d *Results for Sediment Geoaccumulation Index of the studied metals for Ada and Aveglo*

PERIOD	**SAMPLING SITE**	**Mn**	**Zn**	**Fe**	**Hg**
April 2009	Ada	-3.06	-5.64	-5.44	-2.92
	Aveglo	-3.18	-5.57	-5.57	-2.89
May 2009	Ada	-2.64	-6.16	-5.51	-2.75
	Aveglo	-3-32	-4.16	-5.51	-2.85
June 2009	Ada	-2.84	-4.16	-5.44	-2.89
	Aveglo	-3.06	-4.16	-5.38	-2.84
July 2009	Ada	-2.84	-5.57	-5.21	-2.71
	Aveglo	-2.40	-4.34	-4.60	-2.71
Aug. 2009	Ada	-2.84	-4.57	-5.51	-2.75
	Aveglo	-2.74	-4.57	-5.44	-2.66

4.11.3 Contamination Factors

The contamination factors (CF) of the metal pollutants in the Volta Estuary were also evaluated to contextualise the degree of anthropogenic contribution to the total heavy metal pollution.

Table 4.

The low CF values realized for both sampling stations (Table 4.8) indicate very little anthropogenic effects as far as the studied metals are concerned. The concentration of heavy metals in the sediments were well below the natural background concentrations

7 *Contamination factors (CFs) of the studied heavy metals for the two sampling stations*

PERIOD	SAMPLING SITE	Mn	Zn	Fe	Hg
March 2008	Ada	0.21	0.03	0.06	0.20
	Aveglo	0.19	0.03	0.03	0.53
April 2008	Ada	0.23	0.03	0.05	0.21
	Aveglo	0.22	0.02	0.03	0.60
May 2008	Ada	0.24	0.03	0.05	0.20
	Aveglo	0.12	0.02	0.02	0.25
June 2008	Ada	0.22	0.03	0.05	0.20
	Aveglo	0.12	0.03	0.02	0.58
July 2008	Ada	0.18	0.01	0.04	0.20
	Aveglo	0.16	0.03	0.04	0.20
Aug. 2008	Ada	0.18	0.03	0.01	0.35

Table 4.

	Aveglo	0.34	0.05	0.07	0.25
Sept. 2008	Ada	0.35	N/A	0.02	0.17
	Aveglo	0.29	0.06	0.07	0.30
Oct. 2008	Ada	0.18	0.02	0.02	0.22
	Aveglo	0.19	0.01	0.04	0.24
Nov. 2008	Ada	0.13	0.01	0.04	0.19
	Aveglo	0.23	0.01	0.04	0.22
Dec. 2008	Ada	0.23	0.02	0.05	0.25
	Aveglo	0.22	0.02	0.02	0.24
Jan. 2009	Ada	0.16	0.03	0.03	0.24
	Aveglo	0.15	0.02	0.03	0.27
Feb. 2009	Ada	0.14	0.02	0.03	0.21
	Aveglo	0.23	0.03	0.03	0.24
March 2009	Ada	0.46	0.03	0.04	0.21
	Aveglo	0.16	0.03	0.03	0.16

8 Cont" d *Contamination factors (CFs) of the studied heavy metals for the two sampling stations*

PERIOD	**SAMPLING SITE**	**Mn**	**Zn**	**Fe**	**Hg**
April 2009	Ada	0.17	0.03	0.03	0.20
	Aveglo	0.16	0.03	0.03	0.21
May 2009	Ada	0.23	0.02	0.03	0.21
	Aveglo	0.15	0.08	0.03	0.20
June 2009	Ada	0.20	0.08	0.03	0.20

Table 4.

	Aveglo	0.18	0.08	0.04	0.21
July 2009	Ada	0.21	0.09	0.04	0.22
	Aveglo	0.29	0.07	0.06	0.23
Aug. 2009	Ada	0.21	0.07	0.04	0.22
	Aveglo	0.22	0.06	0.03	0.24

CHAPTER 5

DISCUSSION

5.1 Trends in the Physicochemical Water Parameters

The study observed that a sandy substratum characterized both clam fishing sites. This may well relate to the fact that clams preferred the sediment characteristics, granule size and organic matter content and nutrients in the estuary. Salinity, pH, conductivity and TDS remained fairly constant for both locations. This may well represent tolerable water quality limits for the clams at the estuary. Dissolved Oxygen levels at Ada and Aveglo were reasonably high and fairly constant throughout but appeared to decline for both locations in July August and September 2008. These periods coincided with the peak of the rains, a period during which the estuary possibly received polluted run-off from various metal fabrication factories, waste disposal sites and farming locations along the basin. This could well impact negatively on the dissolved oxygen levels.

The sudden drop in salinity from 0.03 to 0.02 at both stations after April 2009 could be attributed to the dredging of the Volta estuary, initiated and recently carried out in April 2009 by the Volta River Authority (VRA) aimed at breaking down "islands" built by heaps of sand at the estuary. The dredging process has allowed intrusion of more of the River water into the sea shortening the retention period of the sea water during high tides and may have caused the re-suspension of anoxic sediments leading to their oxidation, which results in the formation of sulphuric acid causing a lowering of the pH and the release of heavy metals. According to Peltola and Astrom (2002), dredging results in the oxidation of the reduced sulphur in the sediments resulting in a lowering of pH, which in turn leached metals.

5.2 Heavy Metal Levels in the Clams and Sediments

The role of organic matter and sediment grain size in relation to the accumulation of heavy metals to the sediments has been emphasized (Davies *et al.*, 1991, Sakai *et al.*, 1986, Thorne and Nickless 1981). Increases in heavy metal concentrations are associated with finer grain sediments sizes and organic matter and this can be seen at the two sites where the metal concentrations were well below the standard concentrations (GESAMP, 1982), probably because of the coarse nature of the sediments from the two stations (Appendix 3). However, sediments are the major depository of metals, in some

cases, holding more than 99 percent of total amount of a metal present in the aquatic system (Odiete, 1999)

An assessment of the metal concentrations in the clams revealed that the concentrations of all the studied metals were well below the WHO guideline standards (WHO, 2000). This phenomenon suggests that metal levels in the surrounding biota are very low and are not interfering with the normal metabolic processes of *G. paradoxa.* Similar observations and assertions were made by Anderlini (1992) and Vazquez *et al*., (1993) in their studies of the effects of heavy metals in selected bivalve species.

Fe concentrations in the sediments were relatively high, but this could be due to natural processes instead of anthropogenic activities as Fe occurs abundantly in the natural environment and may come from background levels in the sediments.

According to Sholokovitz (1978), Wilson *et al.* (1986) and Din (1992), metal levels that originate from natural processes such as erosion and flocculation of metals may cause elevated levels in sediments and biota unrelated to anthropogenic sources.

Agricultural activities around the Volta Estuary, especially at Ada Foah is usually limited mainly to small scale holdings and subsistence agriculture, and poverty often causes the farmers to open more land for cultivation. The environmental problems that arise from rural agriculture include absence of fallow periods, decline in soil fertility, deforestation and wetland drainage. These problems usually result in increased exportation of silt, organic material and nutrients from the surrounding agricultural lands into the Estuary. Of the heavy metals investigated in this study, Mn and Zn are present in most of the agrochemicals used in Ghana and more specifically around the estuary. The use of agrochemicals in Ada Foah and the other surrounding agricultural communities is not widespread, but the major users of these agrochemicals are smallholders who have had little, if any training or skills in application, use, storage or disposal. The amount of chemicals entering the estuarine environment although presently insignificant might have adverse environmental effects in future due to bioaccumulation.

The actual sources of the metals in the Estuary are difficult to identify. One possibility is that the high concentrations of Fe and Zn could be associated with the erosion and weathering of soils and parental rocks in the surrounding catchment as well as mobilisation of metals from the sediments.

5.3 Temporal Variations in Heavy Metal Levels in Sediments and Clam Whole Soft Tissues

One of the specific objectives of this research was to examine the temporal trends of heavy metal levels in the tissues *of G. paradoxa* and sediment samples of the Volta Estuary over the 18-month sampling period for peculiar temporal trends and variations and whether or not the monthly concentrations varied significant. Although variations in the concentrations of the four studied metals were significant, clear-cut trends were observed. Peak concentrations for most of the metals however, fell between the months of June and November.

A number of authors have suggested that the analysis of heavy metals in bioindicators such as bivalves should be based on more than one sampling date or period to account for the variability found in the results. This study was carried out over 18 months spanning over the major and minor rainy seasons of 2008 and 2009 as well as the dry season (the Harmattan) from December 2008 to March 2009 thus giving ample time to study the temporal variations in metal concentrations.

The metal tissue level and level of bioaccumulation in the *G. paradoxa* are synergistically influenced by a number of abiotic and biotic factors. Among biotic factors, a major role is played by the shellfish age, sex, size, genetic type and physiological condition, whereas major abiotic factors include the habitat, water circulation, chemical form of the metal present in water, between-metal competition, temperature, pH, dissolved oxygen, light, salinity, season, and degree of particular biotope contamination (Phillips, 1976; Martincic *et al.*, 1980; Marcus and Thompson, 1986; Giordano *et al.*, 1991; Gold- Bouchot *et al.*, 1995).

In natural conditions, seasonal variations predominantly appear as a result of combined effects of the above-mentioned factors. Variations are often ascribed to changes in the freshwater inflow, changes in the metabolism rate of an organism and changes in soft tissue weight (Phillips, 1976; Cooper *et al.*, 1982; Marcus and Thompson, 1986; Martincic, 1987; Mitra *et al.*, 1994; Hunter *et al.*, 1995; Morel and Koffi Koffi, 1995; O" Connor, 1998).
Borchardt *et al.* (1988) suggested that seasonal variations of metal levels in bivalves such as *M. edulis* follow a sinusoidal curve. Their logic is as follows: the growth period in late spring and early summer causes rapid increases in biomass, which results in lower metal levels when they are expressed in

relation to this increased biomass. If these data are then used as a baseline, increases in metal levels observed in summer can appear to be enhanced. Expressed graphically, such levels appear as a sinusoidal curve. If such curves exist, they may be metal specific, as different metals are known to bioaccumulate at different rates (Phillips 1980; Phillips and Rainbow 1994). The rate of accumulation and the ability of the bivalves to detoxify particular metals also differ greatly (Rainbow *et al.*, 1990) and the estuarine environment especially, is not static. The levels of metals present are dependent on the anthropogenic input into the estuary. This would disrupt a sinusoidal curve pattern.

The results from the present study suggest that seasonal variation of the studies heavy metal levels in *G. paradoxa* from the Volta Estuary was irregular and did not follow sinusoidal curves suggested by Borchardt *et al.*, 1988. Although most of the studied metals exhibited peak levels just prior to or during the clam spawning season which spans from June to November, a definite sinusoidal curve was not observed.

5.4 Factors Affecting the Temporal Variations in Heavy Metal Levels in Bottom Sediments and Clam Whole Soft Tissues

Trace metal concentrations in clams depend on numerous environmental and biological factors (Cossa, 1989; Kramer, 1994; Kljaković-Gašpić, 2007). Many authors have related these seasonal variations to a great extent to seasonal changes in flesh weight during development of gonadic tissues (Cossa and Rondeau, 1985; Joiris *et al.*, 1998; Otchere *et al.*, 2000, 2003).

The pattern of variation of heavy metal accumulation in whole soft tissues of the clams from the Ada and Aveglo sampling stations appears to be influenced largely by the reproductive cycle of the organism, similar to trends observed by Etim in 1990 and Etim *et al.,* (1991) after studies on *Galatea paradoxa* in the Cross River, Nigeria. The studies revealed that clam spawning starts in June (when mean dry tissue weight maximum occurs), and is completed between October and November (when mean dry tissue weight minimum occurs). This observation corroborates the current study in which the spawning season of the Volta clam, *G. paradoxa*, coincides with the onset of the second rainy season in Ghana and is completed by the start of the dry season.

It is believed that during the spawning period, proteins and carbohydrates contents, which have a high affinity for heavy metals, are accumulated for gonad tissue production, energetic storage and consumption (Latouche and Mix, 1982; Páez-Osuna *et. al.,* 1995; Lima, 1997). Galstoff, 1961; Etim *et al.,* 1991; observed that the ripe oyster gonad may comprise 31 to 41% of the total body weight. On the basis of this, Cunningham and Tripp (1975) argued that if metals were accumulated in the gonad tissues, an appreciable loss might occur during spawning. The accumulation of proteins and carbohydrates prior to spawning explains why most of the peak metal concentrations coincided with the onset of the spawning period of the *G. paradoxa.* This phenomenon probably explains why the peak concentrations of most of the metals coincided with the spawning season of the clam with sharp drops in concentration in the months following the spawning season.

The seasonal pattern of variation for heavy metals, especially the essential heavy metals in the tissues of *G. paradoxa* is similar to results observed by Etim *et al.,* (1991) which showed peak values in July especially for Zinc, an essential heavy metal.

Variability of heavy metal levels in the clams can also be caused by changes in the physiological conditions of the clams (Phelps *et al.,* 1985) and environmental parameters including temperature, salinity, oxygen concentrations (Phelps *et. al*., 1985; Phillips, 1976; Luoma and Bryan, 1982), some of which were variable during the sampling period.

Another factor that can explain the observed heavy metal trends at the two sampling stations is the clam fishing season. Very intense clam fishing is done between the onset of rainy seasons in March each year (the start of the open season) and December (the start of the close season and the Harmattan. During this period, heavy metals could be introduced into the Estuary as a result of the intense fishing activities from sources such as fuels leakages and fumes from outboard motors of the fishing boats and from the motorized air compressors used by the divers in the their clam fishing activities (Plate 3). Metals could also be introduced from sources such as the paint coatings of the fishing boats. This trend is corroborated by Chouba *et al.,* (2007) found higher levels of heavy metals in the mullet, *Mugil cephalus* during high rainfall periods and the times for most intense fishing activities In Tunisia. The elevated concentrations of heavy metals in this fishing period could also be attributed to surface water run-off into the Volta estuary during periods of rainfall.

The study did not observe any known point source of pollution, similar to the phenomenon observed by Otchere (2003) in his study on heavy metal concentrations in the tissues of some bivalves from three lagoons in Ghana. The results of this research, in conformity to Otchere (2003), provide evidence that even clams from areas with no known point sources of contamination may have measurable body burdens of heavy metals. This may probably be due to the processes of natural weathering and supply from locations further upstream.

The relatively high concentration of essential heavy metals in the clam and sediment samples, particularly Manganese and Iron might be attributed to local hydrological conditions, weathering and the intensive leaching of mineralized rocks in surrounding area during rainstorms.

Another principal factor that might explain the relatively high wet season metal concentrations is the use of galvanized iron sheets as the principal roofing material in the settlements surrounding the Volta Estuary. This could also account for the relatively high levels of Fe in the clams and sediments. According to Otchere (2003), higher wet season levels of Fe and Zn might as well be due to import from surrounding settlements as most roofing in Ghana are made of galvanized iron sheets, most of which are presently rusty. Many metals are also found in agricultural products such as fertilisers. Those present in fertilisers include Mn and Zn. Eventually, these metals may accumulate in agricultural soils and become exposed to water bodies and the organisms present in them through run-offs during the rainy season (Otchere 2003).

Other environmental processes such as freshwater runoff, particulate matter re-suspension and primary production can affect the bioavailability of trace metals in the Volta Estuary. Many of these processes are highly variable on monthly and even daily scales and could possibly account for the variations in monthly metal concentrations in the clams and in the sediment in particular.

5.5 Spatial Variations in Heavy Metal Concentration in the Sediment and Clam Tissues

Comparing the different clam size classes (small vs. small, medium vs. medium and large vs. large) from the two sampling stations using the Kruskall-Wallis Test of Significance, no significant differences ($p>0.05$) were observed for Mn, Zn, Fe and Hg concentrations in the tissues of the compared class sizes.

This phenomenon could be due to similarities in bioavailability of the heavy metals to the clams at both sampling locations (Ferreira *et al.*, 2004), similarities in suspended particulate matter, food sources, and homogeneity in environmental and physicochemical water parameters at the two sampling stations.

With the exception of Mercury all the heavy metals examined in this study are essential metals and have intracellular regulatory mechanisms to keep their concentrations in equilibrium in the organisms (Ferreira *et al.*, 2004). This explains the absence of any significant spatial variations in metal concentration between the two sampling sites.

5.6 Heavy Metal Uptake and Accumulation in Clams

It is generally agreed that heavy metal uptake occurs mainly from water, food and sediment. However, effectiveness of metal uptake from these sources may differ in relation to ecological needs and metabolism of animals and concentrations of the heavy metals in water, food and sediment as well as some other factors such as salinity, temperature and interacting agents (Roesijiadi and Robinson, 1994).

Analyses of the clam and sediment samples revealed no distinct relationship between heavy metal levels in clam tissues and sediments in which they thrive. An observation of the concentrations of all the clam size classes and sediments from the two stations revealed no significant relationship patterns, indicating no distinct trend in metal uptake by the clams as far as the sediment in/on which they are found is concerned. Heavy metal accumulation in clams may not be directly or solely derived from sediments (Huanxin *et al.*, 1999). Other sources of heavy metals in bivalve tissues are derived from living or dead suspended particles and from dissolved metals in the water (Huanxin *et al.*, 1999).
Because the release of heavy metal from sediments is controlled by the behaviour of heavy metals themselves, and the physical and chemical condition of the environment, there are no clear

relationships between the concentration of heavy metals in oyster tissue, and those in sediment (Huanxin *et al.*, 1999).

The relatively consistent monthly concentrations of Mn, Fe and Zn in whole soft tissues of *G. paradoxa* may well represent efficient metabolism and detoxifying processes that include transportation, transformation, sequestration and/or excretion of excess metals (Connell *et al.*, 1999). The results further suggest that the levels of contamination of these metals do not exceed the clam" s capacity of regulation (Amiard *et al.*, 1985; Durou *et. al.*, 2005).

The relatively higher concentrations of Zn in the clam tissues compared to the concentrations in the sediments suggests a high rate of accumulation by the clams, a physiological mechanism induced by exposure or even a high relevance of the water as an additional source of contamination (Cardoso *et al.*, 2008).

It is in this light that according to Canterford *et al.*, (1978) it is useful to express results in terms of biota-sediment accumulation factors (BSAFs) when comparing the order of uptake of metals. BSAFs evaluate the efficiency of metal uptake by the clams and to describe the accumulation of studied metals.

For example, although monthly concentrations of Fe in the sediments from both stations were generally higher than Mn, Fe BSAFs were generally lower (Tables 4.4). This phenomenon occurs because Fe is deposited much more quickly but is strongly bound to the sediments under the estuarine conditions (Huanxin *et al.*, 1999). Fe is, thus, not readily available to the clams as far as the sediments are concerned as a heavy metal source. Mn on the other hand can be said to be released much more easily from sediments than Fe and thus more available to the clams accounting for the higher BSAFs for Mn. Hg has much higher monthly BSAF values because it is a non-essential trace metal, which is not metabolised in the tissues of the clam and thus accumulates in the clams.

Peak BSAF values for most of the heavy metals were recorded just prior to or at the beginning of the spawning season lending credence to accumulation of heavy metals prior to spawning.
The release of heavy metals from sediments is controlled by the complex dynamics of the heavy metals and the physical and chemical conditions of the environment. Hence, there was no clearly defined relationship between the heavy metal concentrations in the clam tissues and in the sediments. Other factors of the environment are certainly implicated in this observation.

Biological variables such as size, sex or changes in tissue composition and reproductive cycle as well as the season of sampling and the hydrodynamics of the estuary have to be considered. Seasonal variations have been reported to be higher in winter/dry than in summer/wet. These seasonal variations have been related to a great extent to seasonal changes in flesh weight during development of gonadic tissues (Cossa and Rondeau, 1985; Joiris *et al.*, 1998; Otchere *et al.*, 2000, 2003).

5.7 Implications for Human Consumption of Whole Soft Tissue of Clams from the Volta Estuary

Analysis of risks levels associated with the consumption of clams and comparison to WHO Safety Reference Standards for Bivalves revealed that the concentration of the heavy metals found in the clam tissues were within permissible limits. It should, however, be noted that consumption data used in the assessment are based on national average shellfish consumption rates as there is no documented data on the shellfish consumption levels of the riparian communities where the study was conducted. However, based on the calculated RQs and the national rate of shellfish consumption, it will take a daily consumption level of approximately 12–227 g of *G. paradoxa* flesh to cause manganese toxicity, 86–528 g for iron toxicity, 306–1159 g for zinc toxicity and 594–1532 g for mercury toxicity. These values are very much likely to be above the daily shellfish consumption values for the communities around the Volta estuary and thus the heavy metals studied are unlikely to cause harm to human consumers in these areas.

However, it should be pointed out that exposure estimates for heavy metal intake from shellfish consumption are based on the national average shellfish consumption data which may not be appropriate for estimating exposures of particular subpopulations or individuals residing in specific regions and towns of the country, such as coastal settlements and locations of active shellfish production, where more shellfish is consumed.

5.8 Variations in Heavy Metal Concentrations in the Tissue of G. Paradoxa in Relation to Body Size

In line with one of the hypotheses of this research that there are significant differences in metal concentrations in the tissues of *G. paradoxa* in relation to size, it was realized that the Volta clam, *Galatea paradoxa,* accumulates Mn, Zn, Fe and Hg in their tissues, with no clearly-defined trend as far

as size was concerned, although some positive metal-size relationships were observed in certain months.

Positive relationships between metal concentrations in whole body tissues and body size have been reported occasionally from a variety of bivalves and gastropods (Boyden 1974, 1977, Cossa 1989, Odzak *et al.,* 1994). It is likely that when tissues grow more quickly than the metal can be absorbed, there will be a reduction in metal concentrations in soft tissue. Since in nearly all species, smaller (younger) individuals grow faster than the older ones, dilution of metal concentrations by tissue growth should have a greater effect in smaller individuals than in larger ones, causing a positive slope in the metal concentration-body size relationship (Strong and Luoma, 1981). Although no clear trend was defined as far as metal concentrations and clam sizes were concerned, the observations by Strong and Luoma, (1981) could explain why metal concentrations in the clams from Ada and Aveglo exhibited a positive trend (an increasing trend with increasing size) in certain sampling months. On the other hand, positive relationships observed in some mollusc species have been explained in terms of extremely slow rates of elimination of a metal from the body of an organism with non-regulatory uptake (Langston & Zhou 1987a, 1987b). This suggests that the net accumulation of the metals may occur throughout the life of the organisms and higher concentrations in the larger (older) individuals may reflect previous longer-term exposures (Boyden 1977).

Bioaccumulation of Mn and Zn appear to be linked to gonadal recrudescence and are accumulated prior to spawning and in the rainy seasons. There appears to be some regulatory mechanism for relatively large clams to regulate to some extent the concentrations of Mn, Zn and Fe but they do not appear to be able to regulate concentrations of mercury because it is not one of the essential elements for gonadal recrudescence. Some environmental factors possibly contribute to the concentrations of the metals. There were, however, significant temporal variations in the concentrations of heavy metals in soft tissues of clams.

Variations in the mean heavy metal concentrations in the different clam size classes for both sampling stations were not significant ($p>0.05$) except for Hg concentrations in the Ada clams (Fig 4.19). This could be due to similarities in bioavailability of the heavy metals to the clams (Ferreira *et al.*, 2004) and homogeneity in environmental and hydrographic parameters at the two locations (Figs 4.1 and 4.2). The high peak Mn concentrations of 867 and 629 μg/g at Ada (Table 7) for the smallsized clams in the months of July and August 2008 as opposed to the relatively lower concentrations in the medium

and large clams suggests that the medium and large size classes are sexually-mature and have an efficient metabolism and detoxifying processes (Connell *et al.*, 1999) to keep the concentrations of Mn, Fe and Zn (essential heavy metals) relatively lower. This possibly explains why there was a significant variation ($p<0.05$) in the concentrations of mercury, a non- essential heavy metal, in the tissues of the Ada clams. Essential heavy metals have intracellular regulatory mechanisms to keep their concentrations in equilibrium in the organisms (Luoma and Rainbow, 2008). The study did not observe any discrete point source of pollution in the confines of the estuary. Variability of heavy metal concentrations can also be caused by changes in the physiological conditions of the clams (Phelps *et al.*, 1985; Ferreira *et al.*, 2004) and environmental parameters including temperature, pH, salinity, oxygen concentrations (Phillips, 1976; Luoma and Bryan, 1982). Except Hg, all the metals examined in this study are essential for clams and have intracellular regulatory mechanisms (Luoma and Rainbow, 2008) and equilibrium maintenance in the organisms.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The Volta clam, *Galatea paradoxa*, accumulates Mn, Zn, Fe and Hg in their tissues, regardless of sizes and bioaccumulation of Mn and Zn appear to be linked to gonadal recrudescence and are accumulated prior to spawning and in the rainy seasons. There appears to be some regulatory mechanism for

relatively large clams to regulate to some extent the concentrations of Mn, Zn and Fe but they do not appear to be able to regulate concentrations of mercury because it is not one of the essential elements for gonadal recrudescence. There were, however, significant temporal variations in the concentrations of heavy metals in soft tissues of clams. The concentrations of Mn, Fe, Zn and Hg in the Volta clams are within acceptable limits and therefore, are safe for human consumption according to WHO Safety Reference Standard for Bivalves and Molluscs (2000).

Analyses of the clam and sediment samples revealed no distinct relationship between heavy metal levels in clam tissues and sediments in which they thrive indicating that heavy metal accumulation in clams may not be directly or solely derived from sediments but from other sources such as living or dead suspended particles and from dissolved metals in the water.

Concentrations of the studied metals varied significantly between the clams and sediments for both stations though both samples showed different affinities for the studied metals. The results further suggest that the levels of contamination of these metals in the estuary do not exceed the clams" capacity of regulation.

One of the objectives of this study was to evaluate the risk implications for human consumption of the whole soft tissue of *G. paradoxa* in the light of various health standards. Analysis of risks levels associated with the consumption of clams and comparison to WHO Safety Reference Standards for Bivalves revealed that the concentrations of the heavy metals found in the clam tissues were within permissible limits using various indicators such as Tolerable Daily Intake (TDI) ESAADI, RSC, Risk Quotients and LOCs Risk. Against this background, the clams can be said to contain acceptable levels of manganese, zinc, iron and mercury for human consumption.

6.2 Recommendations

The assessment of the concentrations of the Mn, Zn, Fe and Hg in the whole soft tissues of the clams and sediments of has provided very valuable and comprehensive baseline information and data on the pollution status of the Volta Estuary. This data can serve as a guideline for future researchers and environmental managers to identify future anthropogenic impacts at the study locations with respect to the studied metals, and better assess the need for remediation by monitoring for changes from the

existing levels. The data and findings of this research can also be useful for the management and sustainable development of the studied localities as far as heavy metal pollution is concerned.

To preserve the unpolluted state of the Volta Estuary, it remains important that allochtonous inputs from the catchment area are devoid of heavy metals and regulatory mechanism should be enforced to ensure that current trends are not exacerbated.

Despite the fact that the clams are wholesome for human consumption as far as the studied metals are concerned, it should be pointed out that exposure estimates for heavy metal intake from shellfish consumption are based on the national average shellfish consumption data. This may not be appropriate for estimating the risk associated with shellfish consumption of particular subpopulations or individuals residing in specific regions and towns of the country, such as coastal settlements and locations of active shellfish production, where more shellfish is consumed.

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APPENDIX 1: *Monthly Physical Measurements of the clam size classes* **March**

2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)

SMALL	31.31	25.26	12.1	1.6662	0.4320
	31.07	26.99	12.4	2.1097	0.4534
	35.55	29.65	17.6	3.2311	0.6322
	37.33	26.55	22.0	2.2764	0.4232
	35.24	28.77	19.0	2.3726	0.5423
	33.60	27.90	17.8	1.7821	0.4064
	31.71	26.75	15.7	1.8290	0.4089
	33.96	30.14	17.1	2.3057	0.5112
	28.62	24.74	12.0	1.3471	0.2941
	34.26	29.22	18.3	2.0864	0.4388
MEDIUM	43.10	35.80	37.3	3.5047	1.0147
	46.60	38.36	41.4	5.0087	1.1481
	44.39	34.77	28.7	3.2366	1.0103
	45.57	38.08	31.6	3.7396	0.7749
	45.81	36.58	32.7	5.1701	1.0860
	45.91	37.42	33.5	5.0203	1.0338
	43.47	35.62	24.3	4.7121	1.0691
	46.82	40.36	42.3	5.4139	1.1883
	42.77	34.51	28.2	3.8874	0.8124
	44.97	40.03	33.9	5.2665	1.1638
LARGE	64.95	53.41	76.6	12.5755	2.6234
	59.45	45.57	59.2	10.1191	2.2853
	56.34	45.20	63.2	7.8870	1.6048
	62.73	49.10	74.5	9.8477	2.0435
	67.29	54.57	84.3	13.9559	3.1842
	60.72	47.91	64.5	9.3419	1.9173
	64.68	50.82	68.7	9.4779	1.9191
	56.38	44.48	56.3	8.4737	1.5466
	58.95	44.85	51.6	10.3565	2.4695
	61.04	44.90	47.0	11.4509	2.5802

March 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)

SMALL	38.65	31.54	23.6	3.5620	0.8290
	36.13	32.54	21.4	2.6678	0.6003
	38.49	35.50	26.8	2.8073	0.5671
	31.29	26.32	12.1	1.4258	0.2513
	36.91	31.95	24.7	2.2185	0.4560
	33.17	29.34	18.0	1.6572	0.3100
	33.78	28.79	18.4	2.0197	0.4143
	30.75	26.78	14.7	1.8075	0.3723
	33.93	31.24	21.4	1.8976	0.3973
	32.84	29.17	18.6	2.1174	0.4631
MEDIUM	47.44	38.53	44.6	4.4508	1.0622
	47.94	36.04	26.5	3.6444	0.4775
	42.02	36.78	32.5	3.4774	0.7048
	45.63	37.02	31.4	4.3066	0.7592
	46.93	35.73	33.9	4.6265	1.0674
	41.42	35.07	30.9	4.1675	0.8822
	46.93	37.73	36.2	5.3785	1.2064
	42.47	37.34	36.3	3.6940	0.8261
	48.87	38.84	45.5	4.5834	0.8408
	44.22	37.92	35.5	4.5213	0.9464
LARGE	55.33	43.53	62.6	8.3697	1.6690
	57.75	49.27	77.4	10.4978	2.3508
	70.31	56.62	97.1	14.7981	3.2222
	57.49	42.42	71.0	9.3215	2.0456
	60.05	44.33	44.8	7.9671	1.2289
	58.59	48.28	71.2	8.8072	2.0658
	58.11	49.97	73.3	9.7220	2.0674
	56.97	43.41	54.6	7.6318	1.7199
	57.37	47.35	79.1	7.6188	1.6803
	58.00	42.78	69.0	9.4700	2.2356

April 2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	29.96	24.74	12.9	1.2658	0.3022
	28.17	24.46	9.5	1.3244	0.3997
	27.62	22.49	10.5	1.0009	0.3976
	27.69	23.39	10.6	1.2345	0.3732
	26.28	23.54	9.2	0.8976	0.3665
	36.29	30.80	20.3	3.2356	0.5897
	32.98	28.89	16.7	1.8096	0.4899
	35.15	27.62	16.6	2.7113	0.5976
	32.64	27.80	16.2	1.7067	0.4553
	38.33	31.71	22.0	3.2134	0.7712
MEDIUM	41.66	31.52	19.6	2.8100	0.6783
	42.65	32.89	15.2	2.0356	0.6356
	45.74	35.86	10.1	1.7686	0.4632
	44.93	31.98	19.0	2.8392	0.7834
	42.51	34.44	14.8	1.9793	0.5487
	45.51	38.45	23.2	3.6344	0.9945
	47.99	37.63	36.7	5.2190	1.3122
	50.69	42.74	30.6	4.7006	1.3008
	44.61	33.89	28.6	3.1112	1.2215
	47.75	37.73	29.1	4.1347	1.3034
LARGE	56.33	45.54	25.8	7.0012	1.7784
	57.75	45.23	28.1	6.8321	1.7503
	55.43	42.00	23.8	7.4665	1.8213
	69.34	51.27	50.0	15.8123	4.6566
	68.63	56.83	44.4	11.2009	4.0001
	66.92	55.94	51.5	16.6376	5.3342
	75.08	61.86	49.2	10.5127	3.1054
	56.63	43.21	33.1	6.0996	1.5640
	54.53	46.66	30.2	6.1125	1.5509
	58.32	45.55	30.1	7.8904	1.9660

April 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	26.36	24.74	10.9	1.3342	0.3543
	25.17	22.56	8.5	1.1375	0.3045
	29.09	22.49	11.1	1.5067	0.4197
	27.69	23.39	10.6	1.2122	0.3134
	24.98	19.32	8.5	0.8996	0.2231
	30.72	23.33	15.3	1.7003	0.4266
	32.98	28.89	15.4	1.8485	0.4553
	27.45	22.20	11.7	1.5612	0.3390
	32.84	27.80	16.2	1.6076	0.5566
	37.23	29.07	18.5	3.0987	1.0042
MEDIUM	42.52	31.36	18.9	2.4132	0.9956
	45.33	36.21	20.3	2.5067	1.0012
	46.90	35.86	11.0	2.1116	0.8902
	44.93	31.98	19.0	2.8975	1.1002
	49.43	37.72	25.2	2.7023	1.0664
	50.33	41.77	35.5	6.5395	1.7765
	47.99	37.63	36.7	5.2786	1.6588
	50.69	42.74	30.6	4.7099	1.6004
	51.06	42.65	32.4	5.9435	1.7154
	49.02	38.89	29.1	5.3574	1.6896
LARGE	58.00	46.55	34.6	7.7453	1.8807
	56.93	45.23	32.1	7.4889	1.7681
	55.07	42.63	28.9	8.2365	2.0014
	69.34	51.27	49.7	12.3473	4.8709
	68.63	56.83	56.4	12.4843	4.9908
	66.92	55.94	35.9	16.6364	5.6643
	75.66	63.04	55.2	24.4294	6.7533
	57.12	44.18	35.7	8.9394	2.3365

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
	60.20	49.32	45.5	9.4348	3.6751
	66.55	55.32	40.2	10.9276	4.4422
May 2008, Ada					
SMALL	30.92	25.28	13.3	1.8023	0.5007
	31.05	25.12	10.9	2.1056	0.6783
	33.21	27.14	15.8	2.1047	0.7005
	32.54	26.18	14.5	2.2087	0.8843
	30.44	25.48	13.2	1.7035	0.7684
	26.87	19.06	12.3	1.1062	0.3302
	28.40	22.14	8.0	0.9078	0.3211
	32.22	24.26	15.8	1.2030	0.3444
	31.00	26.29	12.9	1.2054	0.3005
	31.87	27.22	13.4	1.4083	0.3967
MEDIUM	44.15	38.71	24.3	2.3036	1.1120
	47.18	38.37	31.1	2.5074	1.3242
	41.80	33.17	25.1	1.9011	1.3354
	40.06	31.54	19.3	1.7024	1.0222
	43.77	38.73	33.6	2.6035	1.4475
	47.84	35.11	13.9	1.6064	0.7723
	45.57	36.77	23.5	2.2009	1.2112
	45.63	37.98	24.4	2.2020	1.1876
	41.88	32.43	15.8	1.7011	0.8843
	46.45	37.08	22.2	2.3041	1.0023
LARGE	57.60	49.59	44.6	9.3841	3.8756
	60.22	46.72	50.4	7.8105	3.3324
	59.33	43.78	40.5	9.1955	3.4453
	62.10	53.13	58.3	8.8463	3.4098

60.01	48.55	42.7	9.2153	4.0012
56.32	48.21	38.7	6.2718	3.1254
56.17	44.76	49.9	6.3132	3.5543
61.37	49.88	51.4	7.7646	3.6543
66.90	49.35	53.4	8.3300	3.7320
56.33	44.87	47.7	7.4312	3.2087

May 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	33.74	29.41	15.2	2.1033	0.6034
	32.09	27.31	14.1	1.9563	0.4432
	29.99	26.04	14.0	1.9654	0.4501
	39.43	31.76	19.4	2.2120	0.6123
	34.45	24.32	14.9	1.9294	0.3995
	34.46	26.72	15.4	2.1264	0.4002
	26.55	21.37	10.2	1.2861	0.1994
	33.00	26.98	13.2	1.5124	0.2130
	35.48	28.69	20.5	2.4095	0.6651
	33.20	25.65	13.2	1.9689	0.4902
	31.46	26.35	15.0	1.6793	0.3781
	36.33	27.56	17.3	1.8090	0.4301
MEDIUM	41.09	30.44	19.4	2.0102	0.6032
	41.17	35.16	29.6	2.3001	0.6113
	44.22	38.14	33.0	2.5673	0.6334
	42.60	37.10	27.8	2.3982	0.5998
	42.30	34.47	27.6	2.2129	0.5864
	45.76	38.39	35.1	2.6094	0.7723
	43.67	36.34	26.7	1.9097	0.4100
	47.07	40.75	36.7	2.6023	0.6410
	46.36	41.50	39.0	2.8112	0.6788

		Width (mm)	Weight (g)		(g)	Dry Tissue Weight (g)
	48.78	39.88	40.7	2.9991		0.6598
LARGE	57.72	49.59	48.9	9.3841		3.5564
	59.04	46.72	54.4	7.8105		3.0656
	61.76	43.78	41.5	9.1955		3.4453
	60.67	52.22	59.7	8.8463		3.2012
	65.98	51.91	87.4	11.4012		3.9906
	63.58	51.91	74.4	10.7023		3.9975
	57.91	47.21	65.6	8.1012		3.2123
	59.63	49.31	67.2	8.1443		3.2987
	56.90	46.72	53.4	7.7754		2.9985
	65.59	52.27	75.8	10.4810		3.8793

June 2008, Ada

Size Class	Length (mm)		Wet Tissue Weight		
SMALL	31.05	26.40	14.1	1.8123	0.3865
	30.53	26.22	13.3	1.9462	0.4325
	33.45	27.73	16.9	2.2623	0.4562
	30.58	24.76	11.8	1.5129	0.3099
	34.46	26.72	15.4	2.1783	0.4203
	25.45	21.75	8.5	0.9439	0.1546
	31.09	25.20	11.8	1.4932	0.3214
	35.48	28.69	20.5	2.4983	0.5212
	34.02	25.64	13.2	1.6215	0.3111
	31.25	26.68	14.2	1.7008	0.3657
MEDIUM	41.98	35.02	21.3	2.6342	0.5664
	42.28	33.78	27.0	2.2956	0.4437
	44.96	34.82	30.5	2.2223	0.4389

	42.52	34.28	27.5	3.6113	0.5462
	42.45	35.38	26.2	3.6853	0.5134
	37.86	31.21	19.2	3.4054	0.5010
	41.16	31.51	21.5	2.1110	0.4122
	36.46	29.20	17.3	3.4545	0.5220
	41.87	33.40	19.7	2.9953	0.4760
	51.21	41.27	45.8	9.0012	2.0012
LARGE	67.33	52.44	74.2	20.1932	4.3320
	62.76	52.27	73.0	12.5983	2.7762
	66.74	55.23	74.0	9.6778	2.2100
	78.11	61.53	100.3	21.4002	4.5566
	56.17	44.76	49.9	6.32474	1.3320
	55.06	47.16	49.2	7.8536	1.6780
	58.12	47.33	50.3	6.8295	1.6122
	55.24	41.68	47.3	6.5439	1.6054
	58.26	49.77	53.0	8.3438	1.8860
	66.92	51.63	67.2	10.6105	2.3340

June 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	30.83	25.55	10.6	2.0453	0.5310
	28.48	23.91	9.5	1.3908	0.3123
	35.45	28.10	12.4	2.2123	0.5538
	28.70	25.16	10.5	1.5098	0.2990
	26.94	22.48	8.1	1.0453	0.1956
	27.07	23.98	10.4	1.2125	0.1987
	26.98	22.14	7.6	1.0009	0.1673
	33.81	24.79	7.6	2.4567	0.5590

		Width (mm)	Weight (g)		(g)	Dry Tissue Weight (g)
	31.94	26.48	14.1	1.7123		0.3855
	26.70	22.84	7.4	1.0986		0.1889
MEDIUM	41.51	33.88	24.5	3.2123		0.7880
	44.33	32.78	22.4	3.0098		0.6988
	42.64	37.61	31.8	3.2345		0.8055
	41.94	35.22	26.8	3.3094		0.8136
	46.99	41.27	39.2	5.8098		1.3509
	52.22	42.50	44.7	9.6112		2.0204
	48.63	39.55	41.6	4.0009		0.9956
	51.96	41.02	47.4	5.9567		1.4093
	42.64	37.61	31.8	3.2234		0.8880
	46.32	37.88	33.6	5.7123		1.2998
LARGE		48.41	60.3	6.6120		1.5870
	55.14					
	56.34	48.00	56.3	7.3110		1.8781
	62.37	51.44	58.2	10.2223		2.3345
	56.19	47.17	59.6	7.3345		1.4155
	57.81	43.06	47.1	7.6101		1.5609
	57.72	48.55	49.4	8.6009		1.6656
	56.11	42.23	46.4	6.4123		1.5055
	58.00	49.44	55.9	9.5009		2.1119
	59.99	49.38	62.9	14.3342		3.1243
	76.15	56.09	85.9	17.3440		3.8880

July 08, Ada

Size Class	Length (mm)	Wet Tissue Weight		
SMALL	25.90	21.18	5.3	0.9646	0.1123
	36.58	31.20	17.2	2.7526	0.5476
	40.31	34.76	25.6	2.4002	0.4356
	37.77	32.69	19.8	3.2571	0.8809
	37.64	29.85	16.6	1.5359	0.3341
	32.87	26.21	15.5	1.5000	0.3112
	37.86	32.44	20.8	2.2928	0.5081
	31.98	22.43	13.3	1.3262	0.3190
	39.13	31.82	19.7	1.3000	0.2908
	33.47	31.17	16.3	1.4489	0.3341
MEDIUM	44.12	36.31	27.6	3.4621	0.7654
	41.39	36.31	25.0	2.8189	0.5440
	40.40	33.04	23.8	2.3822	0.5223
	46.48	36.14	29.3	4.1952	0.9764
	42.50	34.13	23.1	3.4506	0.7854
	43.42	34.57	20.3	2.9980	0.6112
	47.64	39.05	37.7	4.1548	1.0098
	43.62	38.46	35.9	3.9162	0.8834
	48.39	37.07	25.6	3.9414	0.9776
	41.52	35.50	24.4	2.9546	0.6120
LARGE	57.72	49.59	48.9	9.3841	2.2431
	59.04	46.72	54.4	7.8105	1.9008
	61.76	43.78	41.5	9.1955	2.0190
	60.67	52.22	59.7	8.8463	2.1081
	59.41	49.25	62.6	9.2153	2.2021
	53.74	46.76	49.9	6.2718	1.6658
	56.17	44.76	49.9	6.3132	1.5099
	55.06	47.16	49.2	7.7646	1.8890
	55.08	43.05	41.4	7.4827	1.8600
	58.52	45.16	43.0	5.9228	1.5577

Width (mm)	Weight (g)	(g)	Dry Tissue Weight (g)

	Width (mm)	Weight (g)		(g)	Dry Tissue Weight (g)
July 2008, Aveglo					
Size Class	**Length (mm)**		**Wet Tissue Weight**		
SMALL	30.85	25.55	10.6	2.0451	0.4450
	28.48	23.91	9.5	1.3209	0.3023
	35.45	28.10	12.4	2.2573	0.5234
	28.70	25.16	10.5	1.5906	0.3451
	26.94	22.48	8.1	1.0233	0.2143
	27.07	23.98	10.4	1.2684	0.2243
	26.98	22.14	7.6	1.0895	0.1190
	33.81	24.79	7.6	2.4900	0.5510
	31.94	26.48	14.1	1.5375	0.3331
	26.70	22.84	7.4	1.0970	0.1224
MEDIUM	41.54	35.25	25.6	3.4428	0.8122
	41.99	33.65	27.8	4.8906	1.3565
	45.38	32.44	26.9	3.9336	1.2110
	42.01	34.90	24.8	3.6650	1.1321
	43.91	34.52	23.5	3.5112	1.1008
	42.57	33.88	23.5	3.1008	0.9878
	48.57	45.66	38.5	4.2503	1.2243
	42.64	37.61	31.8	3.2121	0.9112
	41.22	35.75	29.0	3.3823	1.0080
	46.99	41.27	39.2	5.8009	1.5546
LARGE	56.22	44.50	54.7	9.6658	2.3344
	55.96	43.02	47.4	5.9909	1.6570
	55.14	48.41	60.3	6.6574	1.6908
	57.80	47.26	66.9	6.9790	1.6990
	56.04	44.64	49.0	5.4976	1.5098
	55.54	42.32	54.3	6.8584	1.5990
	59.02	46.55	50.7	5.3345	1.5068
	69.19	55.62	76.5	14.9455	3.4456
	57.04	46.22	54.0	6.0009	1.6700
	55.74	43.19	49.6	5.3384	1.5011

August 2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	32.87	26.81	12.4	1.6345	0.3451
	34.04	26.61	9.4	2.0920	0.5001
	34.98	28.13	15.6	1.7940	0.3776
	31.45	28.53	14.8	1.4293	0.3190
	34.67	29.95	19.0	2.1894	0.5000
	33.88	27.46	14.6	2.0098	0.4765
	25.43	21.70	6.3	0.7230	0.1123
	31.40	25.90	11.0	1.5689	0.3546
	30.62	24.92	10.0	1.3330	0.2756
	30.85	26.51	11.1	2.0009	0.4889
MEDIUM	46.32	37.88	33.6	5.7348	1.4309
	45.87	37.88	37.0	3.7209	0.7781
	48.57	39.55	37.7	6.7283	1.5889
	43.24	38.38	28.5	4.3693	0.9976
	40.13	36.62	22.9	3.0225	0.7453
	37.90	31.07	21.9	3.6750	0.7745
	47.36	38.00	34.7	4.5357	1.2231
	45.41	37.30	33.1	4.4987	1.2050
	47.64	40.31	36.1	4.5123	1.2433
	44.88	40.16	41.0	4.2009	1.0030
LARGE	57.93	42.20	41.2	5.1100	1.2645
	57.73	46.20	44.4	6.5098	1.4112
	59.00	49.39	53.7	6.9467	1.5609
	65.41	51.91	62.1	10.4093	2.6334
	56.31	44.61	58.2	8.9965	1.7790
	68.81	52.44	75.3	21.1112	4.5590

		Width (mm)			Dry Tissue Weight (g)
	62.76	52.27	73.0	12.5090	2.9800
	65.43	53.30	74.0	8.4345	1.6579
	77.50	57.97	105.4	20.8657	5.5034
	69.68	61.33	98.0	25.2983	6.1120

August 2008, Aveglo

Size Class	Length (mm)		Weight (g)	Wet Tissue Weight (g)	
SMALL	36.38	30.16	20.1	2.8480	0.5312
	36.36	29.26	17.6	2.4891	0.4465
	37.79	31.14	20.7	2.7738	0.5244
	34.59	27.30	16.3	2.3354	0.4330
	37.89	31.54	21.5	3.1880	0.7798
	30.52	27.14	14.7	1.6500	0.3651
	28.86	26.20	11.2	1.9531	0.4031
	31.53	26.62	15.0	1.7930	0.3899
	35.89	29.33	18.4	2.1748	0.5020
	32.01	27.58	15.1	2.1024	0.4098
MEDIUM	42.84	35.00	27.3	2.4790	0.4556
	41.72	31.03	20.5	2.7470	0.5089
	40.55	30.24	23.3	3.0842	0.7700
	43.03	34.88	26.4	4.0229	0.9011
	41.11	32.16	25.3	3.2847	0.8076
	46.59	34.33	20.9	5.1091	1.2001
	42.03	32.67	24.0	3.8531	0.8712
	41.29	32.85	27.3	3.5351	0.7771
	42.40	34.70	29.8	3.9494	0.8788
	49.91	38.52	41.5	4.2733	1.1876

		Width (mm)	Weight (g)		(g)	Dry Tissue Weight (g)
LARGE	62.07	50.21	62.5	10.4721		2.3556
	56.19	47.17	59.6	7.3191		1.5009
	56.78	41.74	47.1	13.1381		2.9800
	75.81	63.57	115.3	29.1501		6.7768
	81.58	62.60	113.5	32.6806		7.0982
	75.06	55.51	85.9	16.7270		3.6570
	68.30	58.88	92.4	24.5278		6.5101
	55.83	43.55	37.9	5.5157		1.3409
	58.69	49.38	62.9	14.8098		3.1190
	71.43	56.89	95.8	20.2123		6.1120

Dry Tissue Weight (g)

September 2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	29.75	25.12	10.9	1.5546	0.4532
	24.78	22.76	8.0	1.2123	0.2650
	28.41	22.89	8.3	0.8098	0.1201
	26.02	21.92	8.7	0.8361	0.1335
	27.16	23.47	8.1	0.9920	0.2134
	30.47	26.90	14.6	1.9234	0.5089
	32.81	28.55	13.0	2.4112	0.5200
	29.88	27.08	13.6	1.2876	0.2210
	30.30	26.46	10.6	2.3085	0.5201
	29.21	26.63	12.2	1.6243	0.3650
MEDIUM	41.82	33.84	23.1	4.4097	0.9340
	43.32	32.77	20.9	5.0354	1.1145
	45.79	36.80	26.8	5.6856	1.3458
	38.99	32.64	23.3	3.9964	0.9879
	45.44	35.98	32.9	4.7254	1.2245
	45.91	35.39	29.0	4.2963	0.9466
	48.76	39.36	42.1	6.3906	1.5098
	43.65	34.44	23.3	3.9983	1.0010
	38.44	31.82	18.0	3.3123	0.8120
	39.35	32.37	23.9	3.5009	0.9102

LARGE	55.72	43.72	42.0	8.6565	1.9949
	56.11	43.68	46.4	6.3098	1.5034
	57.58	45.21	55.9	9.6112	2.0556
	59.99	49.38	62.9	14.8090	2.7809
	71.00	56.89	95.8	20.2245	4.2440
	56.50	44.71	40.8	9.5789	2.0011
	58.37	46.32	47.0	11.1098	2.7002
	56.07	43.92	40.2	6.9232	1.6609
	58.89	45.09	46.5	9.4890	2.0440
	55.39	42.85	37.6	5.2234	1.2201

September 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	
SMALL	26.25	23.02	10.9	1.5344	0.3566
	25.44	20.23	5.2	0.5083	0.0998
	25.01	22.85	8.3	1.1389	0.1100
	26.27	24.17	9.4	1.1382	0.1032
	26.27	22.95	8.6	1.0098	0.1102
	28.75	26.25	12.4	1.4877	0.3244
	29.18	24.09	9.4	1.0980	0.1004
	28.30	25.51	11.1	1.2087	0.2011
	29.59	26.29	13.5	1.7123	0.3883
	29.44	26.74	12.2	1.9786	0.5409
MEDIUM	41.37	31.90	23.7	4.0559	0.8790
	43.43	34.71	28.8	6.1134	1.4355
	48.47	35.90	26.9	4.9648	1.2234
	46.65	35.65	21.7	3.9346	0.8700
	41.33	31.52	25.2	3.4978	0.7768
	44.65	35.28	32.4	5.1123	1.1890
	44.74	35.86	32.0	4.5798	1.1109

					Dry Tissue Weight (g)
	44.93	33.98	27.9	5.3098	1.1288
	41.50	34.44	28.1	5.1234	1.1044
	45.51	38.45	40.9	5.9865	1.3350
LARGE	61.10	48.23	66.9	14.8221	3.3823
	64.62	51.44	70.4	15.7645	3.4366
	60.15	52.42	63.4	13.9120	3.1223
	74.09	62.02	115.0	18.4293	4.0156
	63.63	49.23	67.9	13.9098	3.1220
	65.51	50.99	72.1	13.6644	3.1890
	67.41	55.69	84.0	13.6099	3.0997
	55.87	47.37	62.9	14.0113	3.2996
	64.63	52.13	69.9	13.4738	3.1155
	63.31	51.09	69.1	13.7962	3.2000

October 2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	25.58	23.52	8.6	1.0250	0.1133
	27.80	25.05	11.0	1.3955	0.3245
	26.81	22.24	8.7	0.8146	0.1002
	22.92	22.91	8.0	0.8141	0.1201
	30.80	26.19	13.9	2.0390	0.5021
	26.66	24.74	10.6	1.1531	0.1329

	37.14	32.71	21.1	3.5345	0.8900
	32.71	29.45	16.4	1.9322	0.5351
	37.86	29.59	15.0	2.3148	0.5463
	39.88	33.50	22.4	4.4715	1.0090
MEDIUM		41.07	44.4	7.0335	1.5602
	53.64				
	50.75	42.19	47.8	5.8959	1.4522
	41.99	34.46	26.9	4.0236	1.3276
	46.35	34.72	32.3	4.7018	1.4309
	52.10	40.48	41.5	8.9316	2.0670
	50.67	43.14	48.9	7.9963	1.7607
	43.16	36.83	34.8	3.9365	0.7789
	47.10	38.59	33.7	6.0008	1.3340
	46.42	34.28	32.1	4.5502	1.0007
	53.40	37.30	35.0	5.8925	1.5010
LARGE		58.02	115.0	18.4418	4.1113
	74.79				
	62.63	49.13	67.9	13.0357	3.1122
	63.81	51.79	71.1	13.6590	3.2243
	68.51	53.69	84.0	13.6035	3.2900
	64.87	50.37	72.9	17.9763	4.0072
	69.01	58.57	96.5	17.6251	3.9807
	64.34	47.76	61.7	11.7092	2.8890
	64.81	49.62	61.5	9.5439	2.1451

					Dry Tissue Weight (g)
October 2008, Aveglo					
Size Class	**Length (mm)**	**Width (mm)**	**Weight (g)**	**Wet Tissue Weight (g)**	
SMALL	27.91	24.50	10.1	1.4199	0.3210
	30.08	25.42	11.8	1.6579	0.4476
	30.12	24.72	10.2	1.7322	0.4558
	27.48	23.85	10.0	1.2393	0.3002
	28.05	25.13	10.7	1.5251	0.3440
	25.70	22.80	8.0	1.0290	0.3530
	35.82	29.90	18.1	2.0963	0.4778
	37.76	31.08	19.6	2.6180	0.4809
	37.07	30.13	18.9	2.7513	0.4990
	33.21	26.66	8.9	3.1786	0.8806
MEDIUM	41.97	39.49	34.6	4.0616	0.9665
	47.16	38.74	33.5	6.5584	1.5560
	52.36	41.90	43.1	7.5153	1.6609
	45.22	34.34	21.5	4.2914	0.9711
	47.87	37.02	30.2	5.7550	1.4002
	46.39	37.79	33.0	6.2156	1.5340
	51.43	39.02	34.0	5.6589	1.3778
	52.14	40.83	42.4	7.7216	1.8009
	47.02	35.61	33.4	6.6087	1.5644
LARGE		48.01	70.6	13.7246	2.8887
	64.74				

	56.61	46.54	54.9	9.7090	2.3565
	54.84	47.03	51.4	11.0912	2.6388
	55.47	45.34	51.8	10.5192	2.4401
	59.55	46.74	57.9	13.4526	2.8923
	60.63	55.00	76.2	12.5477	2.7700
	62.05	52.19	73.3	15.0386	3.0875
	58.46	42.20	37.8	8.2324	1.9966
	60.26	41.70	38.5	10.0819	2.3080
	55.64	43.40	49.7	10.2998	2.3556

November 2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	34.93	28.44	17.5	2.0802	0.4427
	32.73	28.55	15.5	2.3604	0.5136
	23.63	22.06	9.2	0.7321	0.1621
	29.31	24.99	11.6	1.6235	0.3770
	32.13	28.81	16.1	1.8644	0.3707
	28.74	25.23	12.1	1.5063	0.3319
	33.25	28.22	11.8	2.0708	0.4193
	30.72	27.52	14.9	2.1465	0.5004
	29.45	23.68	10.6	1.9748	0.4289
	29.33	23.07	8.8	2.0289	0.4456
MEDIUM	43.45	36.21	30.7	3.0872	0.6552
	40.63	33.43	21.9	4.6990	0.9854
	50.45	37.00	25.5	5.7530	1.0726
	46.40	37.64	32.3	5.5749	1.2391
	43.54	36.40	29.9	3.9965	0.7073
	41.01	33.42	27.2	3.8491	0.7977
	43.43	37.63	31.5	4.1409	0.8405

					Dry Tissue Weight (g)
	41.76	35.69	30.3	3.4006	0.6901
	41.33	32.45	24.4	4.1188	0.8363
	43.12	35.68	26.2	6.2000	1.3512
LARGE	55.54	45.66	47.9	10.9533	2.2186
	64.38	50.82	75.2	15.9666	3.5593
	62.40	54.32	67.2	12.2110	2.1586
	55.95	45.67	45.2	8.4004	1.6487
	56.80	45.41	51.2	9.1061	2.0048
	60.13	46.52	65.7	10.5876	2.3978
	58.17	45.79	58.3	11.3804	2.4321
	55.62	44.01	34.9	5.8900	1.0758
	55.43	41.35	41.8	8.9670	2.0065
	56.34	45.78	54.1	10.4453	2.4565

November 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	30.48	27.29	12.8	1.6814	0.3451
	26.67	24.46	10.2	1.3217	0.2751
	36.69	35.57	18.6	2.1841	0.4432
	28.99	27.37	12.9	1.2902	0.2874
	34.82	31.18	19.2	2.2189	0.4422
	33.64	28.83	17.5	2.1353	0.4807
	33.65	29.70	18.2	2.3894	0.5676
	36.02	31.75	22.1	2.3185	0.4696
	32.74	29.16	17.6	1.7789	0.3874
	36.48	32.20	20.5	2.8238	0.6860
MEDIUM	49.52	40.42	43.0	5.4296	1.2333
	50.84	42.46	40.7	6.5857	1.4588
	46.04	34.55	26.9	4.2467	0.7268
	43.21	36.74	32.2	4.2792	1.0026
	42.83	35.62	27.6	4.8465	1.0454
	42.53	36.30	33.6	3.4946	0.7196
	49.95	41.64	47.3	6.2758	1.3977
	46.29	39.54	38.5	5.0914	1.1054
	45.30	39.18	35.3	5.2250	1.1401
	46.52	41.27	33.0	5.1218	1.1245
LARGE	55.87	47.29	55.1	10.1014	2.0486
	57.55	47.99	66.5	9.9465	2.2211
	56.71	46.91	61.3	11.5369	2.6404
	55.83	45.54	56.3	7.5136	1.5202
	56.21	46.06	58.2	8.9379	2.0010
	57.76	49.07	60.5	8.8653	2.0481
	56.78	46.33	62.1	7.3354	1.6793
	61.12	50.84	79.8	11.6553	2.4564
	56.68	45.35	55.5	9.0392	1.9285
	55.30	43.43	46.9	7.8978	1.5712

December 2008, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	27.53	36.80	12.2	1.3454	0.2189
	34.69	30.93	18.3	1.6089	0.2681
	35.15	30.75	19.4	2.1342	0.4125
	35.66	30.50	20.3	2.1506	0.4013
	30.48	28.01	14.1	1.9656	0.3364
	29.21	26.47	14.7	1.5723	0.2699
	33.23	27.33	18.4	1.2358	0.2020
	30.33	26.61	15.1	1.4521	0.2351
	31.46	26.03	14.2	1.4985	0.2696
	32.44	27.18	14.0	1.4470	0.2413
MEDIUM	44.43	35.49	36.4	2.9833	0.5020
	47.27	35.78	33.5	4.1254	0.8514
	46.01	38.66	34.3	6.2255	1.3134
	44.31	37.67	32.2	4.7685	0.8894
	44.41	36.77	36.3	3.3201	0.5534
	46.32	38.29	32.3	5.1754	1.0750
	41.89	36.43	30.6	4.2290	0.8524
	40.55	31.08	24.1	2.6733	0.4113
	47.93	39.71	39.8	6.4893	1.2390
	40.43	34.31	25.0	3.2208	0.6248
LARGE	62.84	50.03	69.8	11.7097	1.6761
	56.90	43.91	58.5	7.1000	1.1463
	60.18	48.05	71.9	8.6309	1.5188
	64.16	51.69	67.5	12.7855	2.0983
	56.07	46.86	50.9	7.8842	1.2772
	57.59	49.88	61.6	8.9077	1.5857
	72.04	57.74	106.9	14.0134	2.4572
	60.01	48.76	56.8	10.6054	2.3613
	58.63	46.60	54.6	11.4430	2.1534
	59.13	49.20	64.7	11.7732	2.4285

December 2008, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	27.31	26.73	12.3	0.9854	0.1788
	30.10	27.00	14.4	1.3765	0.2321
	31.87	29.32	17.6	1.8850	0.3632
	31.25	24.69	7.8	1.6588	0.2761
	28.11	25.16	11.8	1.0236	0.1608
	32.38	27.99	15.9	1.5423	0.2653
	34.76	31.19	21.6	1.6902	0.2579
	32.79	28.89	15.8	1.8832	0.3555
	28.47	24.77	10.7	0.8856	0.1367
	33.95	30.43	19.1	1.5633	0.2401
MEDIUM	46.75	40.60	39.0	4.4041	0.7249
	43.47	33.64	31.2	2.8562	0.5086
	43.83	37.06	32.2	3.7509	0.7656
	43.08	38.56	34.2	3.7387	0.6226
	44.59	41.12	45.0	4.2477	0.8806
	42.72	36.17	31.8	2.8865	0.5532
	42.56	38.10	30.6	3.9087	0.8266
	46.56	39.27	38.4	3.5113	0.6978
	51.42	41.93	41.9	5.1109	0.9239
	49.78	44.88	51.5	6.2398	1.4077
LARGE	58.72	48.40	63.1	10.2000	2.1498
	56.99	46.00	61.3	8.7005	1.8706
	55.21	46.86	59.1	7.6455	1.2158
	57.65	47.49	60.8	8.8311	1.6424
	57.13	43.39	49.8	7.3000	1.4520
	55.68	43.23	49.9	6.0923	1.1730
	55.14	48.28	61.4	7.1243	1.2921
	60.21	49.83	66.6	9.6631	1.9835
	56.61	46.32	54.0	7.1090	1.1425
	55.84	43.26	35.0	5.7645	0.7700

January 2009, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	36.29	28.66	15.2	1.5626	0.2702
	34.39	27.05	14.0	1.1525	0.2406
	32.19	26.35	10.7	0.9076	0.1712
	31.67	24.96	11.2	1.0123	0.2235
	37.05	29.73	16.3	1.4770	0.2591
	37.24	29.23	15.6	2.6703	0.4534
	35.23	29.00	15.8	1.3473	0.2763
	32.52	28.17	13.4	1.2850	0.2384
	39.99	35.14	26.7	2.5428	0.4697
	36.88	29.97	18.3	1.6755	0.3494
MEDIUM	46.88	37.87	33.1	1.7191	0.4883
	48.78	38.13	15.1	3.8752	0.5810
	45.78	36.73	28.9	3.3985	0.4883
	45.88	39.06	27.1	2.9745	0.6175
	43.71	35.03	25.8	2.1525	0.3813
	42.50	38.04	27.9	3.3772	0.6199
	45.63	38.33	34.6	3.1495	0.6456
	42.23	33.96	20.8	2.7837	0.4313
	44.23	35.55	28.8	2.5351	0.4969
	43.21	37.08	26.9	2.3560	0.4216
	45.63	38.33	34.5	3.1495	0.9457
LARGE	67.05	54.65	76.8	8.9507	1.7877
	72.32	54.42	88.5	6.7123	0.6824
	59.77	48.71	66.8	6.6712	1.1568
	66.41	52.64	84.4	3.0545	0.7298
	55.53	49.13	54.3	4.1442	0.8056
	63.45	53.44	78.0	9.0412	2.2623
	55.89	44.46	51.0	5.7890	1.8369
	58.34	45.14	45.8	4.9444	1.1261
	55.39	44.71	39.7	3.6661	0.8879

	65.06	52.47	76.6	4.8240	0.6104

January 2009, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	35.27	29.25	16.2	1.5809	0.3102
	34.97	27.03	16.3	1.2005	0.2501
	33.19	26.45	11.2	0.9112	0.1734
	34.87	27.92	14.9	1.7129	0.5237
	37.05	29.73	16.3	1.4770	0.2591
	37.24	29.23	15.6	2.6703	0.4534
	35.23	29.00	15.8	1.3473	0.2763
	32.52	28.17	13.4	1.2850	0.2384
	39.99	35.14	26.7	2.5428	0.4697
	31.76	27.90	17.0	1.4750	0.3109
MEDIUM	50.88	43.24	37.9	3.8191	0.5890
	48.78	38.13	15.1	3.8752	0.5810
	46.79	31.70	30.8	3.5956	0.4453
	44.99	37.06	28.3	2.8756	0.5170
	44.01	36.03	28.0	2.1225	0.4009
	45.50	39.80	29.8	3.3659	0.6198
	45.63	38.33	34.6	3.1495	0.6456
	44.23	33.80	23.8	2.9838	0.5093
	44.23	35.55	28.8	2.5351	0.4969
	53.21	40.08	39.9	4.3560	0.5290
	45.63	38.37	34.8	3.1443	0.5457
LARGE	67.55	56.61	77.1	8.9622	1.8099
	72.32	54.42	88.5	6.7123	0.6824
	59.77	48.71	66.8	6.6712	1.1568
	66.41	52.64	84.4	3.0545	0.7298
	55.53	49.13	54.3	4.1442	0.8056
	63.45	53.44	78.0	9.0412	2.2623
	55.89	44.46	51.0	5.7890	1.8369

	58.34	45.14	55.8	4.9444	1.1261
	55.39	44.71	39.7	3.6661	0.8879
	66.05	54.23	70.6	4.9242	0.6109

February 2009, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	28.45	26.67	12.8	0.9750	0.1709
	30.90	27.89	14.9	1.4568	0.3345
	32.70	28.44	18.9	1.7551	0.3078
	32.29	25.89	8.8	1.6595	0.2609
	28.08	27.23	13.8	1.2231	0.1607
	33.68	28.99	16.9	1.5608	0.2213
	36.67	29.70	24.7	1.5772	0.3001
	33.78	27.59	16.9	1.7830	0.3765
	27.34	22.09	10.2	0.8850	0.1097
	37.99	32.80	19.2	1.5098	0.4411
MEDIUM	49.47	38.50	29.5	2.6992	0.4321
	49.00	40.71	36.3	4.6911	0.7517
	46.22	37.70	31.2	4.1474	0.5243
	46.71	37.91	31.4	2.7945	0.4200
	45.71	37.80	30.2	3.4333	0.5507
	46.06	36.01	26.4	2.7826	0.3671
	37.86	32.73	23.1	3.0351	0.4681
	46.95	39.09	35.3	2.7205	0.4375
	42.07	45.04	24.9	2.8821	0.5037
	46.64	36.16	27.5	3.1387	0.4531
LARGE	63.87	50.81	80.4	12.2063	1.9051
	66.03	54.62	69.1	10.2812	2.0758
	66.68	53.93	88.9	10.9804	1.8946
	71.86	58.40	99.3	15.7791	2.7059
	66.96	52.95	66.0	9.1347	1.4816
	68.10	52.71	72.0	11.3265	1.7813
	64.81	55.33	71.5	9.9322	1.6013

	63.18	50.12	66.1	6.3092	0.9832
	68.83	53.63	79.5	9.9018	1.5786
	61.18	51.76	66.4	7.2919	0.8984

February 2009, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	37.22	30.97	18.9	2.3590	0.4516
	34.96	28.97	13.5	1.8015	0.3282
	37.80	30.60	20.4	1.9580	0.3628
	35.60	31.83	19.6	1.6147	0.2431
	35.19	30.96	18.5	1.9206	0.2668
	32.80	29.10	17.1	1.8206	0.3631
	34.21	28.69	18.5	1.9206	0.4046
	33.38	28.70	12.7	1.7104	0.2868
	35.92	31.33	18.9	2.3987	0.4528
	23.69	21.55	7.0	0.6945	0.1198
MEDIUM	49.53	40.91	41.4	4.7857	0.8063
	49.66	41.31	41.6	4.8626	0.9960
	40.93	35.90	26.4	3.1811	0.5594
	40.10	33.83	21.7	2.5426	0.4509
	42.95	34.76	29.5	2.7602	0.4714
	50.30	37.08	32.2	4.5380	0.6947
	52.86	44.78	47.5	6.1747	1.0410
	49.65	37.86	34.0	3.5186	0.4665
	47.84	40.66	40.8	4.0550	0.7338
	44.29	36.53	37.6	2.7914	0.4318
LARGE	56.26	41.81	41.8	6.4232	1.3031
	56.86	51.27	75.9	8.0416	1.2500

66.63	54.54	72.5	14.5251	3.1940
59.90	51.40	64.2	10.6152	2.1944
66.96	49.67	64.1	10.0696	1.8814
67.51	50.27	67.9	10.6400	1.8604
63.39	52.73	70.8	9.4114	1.6417
72.35	60.00	97.4	18.3670	3.6876
56.45	42.67	53.7	7.3451	1.5490
65.34	52.48	69.0	9.3564	1.6400

March 2009, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	24.45	23.01	9.6	1.1050	0.1120
	28.71	26.10	10.9	1.4950	0.1240
	26.81	22.40	9.4	0.8040	0.1202
	24.30	22.71	9.0	0.9130	0.1304
	31.45	26.23	13.7	2.0567	0.6126
	27.34	25.13	11.7	1.2531	0.1340
	38.34	34.45	23.1	3.6770	0.8810
	35.79	31.47	17.6	2.6322	0.5466
	39.88	30.69	17.5	2.4001	0.5490
	38.00	32.47	22.8	4.4455	1.1092
MEDIUM	52.14	40.78	45.7	7.1030	1.5709
	51.46	43.34	48.5	5.7857	1.5509
	40.45	33.65	29.9	4.1208	1.2270
	46.35	34.72	32.3	4.7018	1.4309
	53.15	44.42	42.7	8.8612	2.1070
	53.66	45.10	43.6	7.9908	1.6783
	48.55	35.65	37.8	3.8308	0.8779
	48.80	39.40	34.0	6.4733	1.4541
	46.42	34.28	32.1	4.5502	1.0098
	47.45	38.35	34.0	5.8099	1.5670
LARGE	70.09	53.10	102.0	18.5610	4.8454

63.34	48.73	69.8	12.9323	3.1560
64.86	52.79	69.12	13.6600	3.2458
65.09	51.23	77.1	11.4530	2.9908
65.11	52.00	76.3	16.0098	4.1244
67.99	55.55	93.5	16.1209	3.9887
65.37	49.75	60.4	10.9844	2.8701
66.61	51.09	62.2	9.7899	2.1489
64.48	54.22	76.8	9.6510	1.9109
62.23	53.67	76.1	10.6700	2.4630

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
March 2009, Aveglo					
SMALL	25.08	23.72	9.6	1.0255	0.1138
	27.80	24.29	11.3	1.3934	0.3244
	26.85	22.25	8.7	0.8146	0.1002
	22.90	22.92	8.0	0.8141	0.1201
	30.89	26.15	13.9	2.0390	0.5021
	26.69	24.79	10.6	1.1531	0.1329

	37.45	32.75	21.1	3.5345	0.8900
	32.67	29.42	16.4	1.9322	0.5351
	37.24	29.57	15.0	2.3148	0.5463
	39.06	33.55	22.4	4.4715	1.0090
MEDIUM	53.64	41.07	44.4	7.0335	1.5602
	50.75	42.19	47.8	5.8959	1.4522
	41.99	34.46	26.9	4.0236	1.3276
	46.35	34.72	32.3	4.7018	1.4309
	52.10	40.48	41.5	8.9316	2.0670
	50.67	43.14	48.9	7.9963	1.7607
	43.16	36.83	34.8	3.9365	0.7789
	47.10	38.59	33.7	6.0008	1.3340
	46.42	34.28	32.1	4.5502	1.0007
	53.40	37.30	35.0	5.8925	1.5010
LARGE	74.79	58.02	115.0	18.4418	4.1113
	62.63	49.13	67.9	13.0357	3.1122
	63.81	51.79	71.1	13.6590	3.2243
	68.51	53.69	84.0	13.6035	3.2900
	64.87	50.37	72.9	17.9763	4.0072
	59.01	38.59	66.5	8.6259	3.9459
	64.34	47.76	61.7	11.7092	2.8870
	64.81	49.62	61.5	9.5439	2.1451
	62.29	52.14	74.7	9.2597	1.8112
	60.64	51.17	74.1	10.4503	2.3133

April 2009, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	31.00	27.38	13.7	1.2727	0.2791
	34.02	29.15	20.3	1.7018	0.3651
	30.60	26.80	15.5	1.4066	0.2650

		Width (mm)	Weight (g)	Wet Tissue Weight (g)		(g)
	32.58	27.41	13.5	1.4098	0.2583	
	32.09	28.81	16.6	1.4856	0.2384	
	30.57	28.40	16.4	1.2458	0.2517	
	28.99	25.67	12.2	1.0568	0.2090	
	32.86	29.33	16.9	1.2696	0.2111	
	32.01	27.66	14.6	1.1902	0.2076	
	34.24	29.32	17.4	1.9252	0.4536	
MEDIUM	44.74	37.41	37.9	3.7466	0.7671	
	44.85	38.45	37.2	4.1379	0.9983	
	47.65	39.55	40.6	4.3546	0.9042	
	46.14	38.70	37.3	5.8979	1.4274	
	46.63	39.81	37.1	4.1591	0.8854	
	45.91	36.49	35.0	3.9177	0.8207	
	47.57	40.07	44.1	4.7006	0.9800	
	43.07	36.42	32.6	4.0550	0.6006	
	47.56	39.02	41.9	4.8360	0.9824	
	50.87	42.83	47.5	5.1323	0.9249	
LARGE	60.34	46.41	56.9	7.8027	1.3577	
	56.59	46.77	62.6	8.0701	1.7525	
	60.06	48.86	62.3	6.6016	1.0652	
	63.72	52.27	82.7	8.7737	1.7253	
	59.74	48.41	66.3	8.5692	1.7854	
	60.74	48.47	73.3	10.4171	2.3260	
	58.00	45.79	59.4	9.3415	2.2085	
	59.12	48.05	68.5	8.9784	2.0000	
	62.29	52.14	74.7	9.2597	1.8112	
	60.64	51.17	74.1	10.4503	2.3133	

April 2009, Aveglo

Size Class	Length (mm)				Dry Tissue Weight
SMALL	32.24	29.09	19.5	1.6582	0.3427
	32.27	29.79	18.4	1.9548	0.3947
	33.35	29.79	19.5	1.8491	0.3187
	31.48	29.49	17.5	2.0778	0.3910
	32.05	30.61	18.5	1.9923	0.4203
	25.40	24.65	10.5	0.9964	0.1929
	30.57	29.86	17.5	1.6580	0.3238
	33.09	30.75	20.0	1.8953	0.3213
	32.66	30.11	18.3	1.8748	0.3999
	37.94	32.51	21.5	3.0356	0.6497
MEDIUM	40.39	34.12	29.1	3.9251	0.8072
	46.99	34.45	36.2	5.7522	1.2239
	47.00	38.12	44.6	4.3555	0.7934
	45.97	38.91	39.3	6.5056	1.2094
	48.11	39.25	40.3	5.5269	1.0325
	43.38	37.43	33.5	4.4706	0.8493
	42.21	35.01	34.8	4.3691	0.8561
	44.49	40.42	33.4	5.0194	1.0946
	49.40	39.73	43.0	5.4477	1.1335
	46.06	39.21	39.0	5.0443	1.0575
LARGE	57.50	49.34	66.6	6.7778	1.1604
	64.31	51.14	70.7	12.5800	2.2692
	55.52	45.78	54.6	8.4637	1.4311
	56.55	49.41	53.2	7.9623	1.3144
	60.55	50.67	68.0	9.9874	1.8428
	58.24	45.14	55.4	8.8061	1.5327
	55.45	45.72	59.6	8.8299	1.6708
	57.00	49.41	73.8	7.3537	1.7135
	61.52	50.49	71.5	10.7044	1.6032
	57.67	48.41	69.1	7.6975	1.2884

	(mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	(g)
May 2009, Ada					
Size Class	**Length**				**Dry Tissue Weight**
SMALL	38.42	33.02	24.9	2.6415	0.5223
	36.59	34.56	26.9	2.1602	0.4092
	39.35	33.32	26.2	3.1586	0.6868
	29.71	27.15	15.2	1.9076	0.4165
	28.83	26.66	13.2	1.3428	0.2749
	28.52	25.52	9.8	1.4163	0.2448
	33.35	23.92	16.9	1.9117	0.3642
	33.48	28.32	17.8	1.5428	0.2361
	32.29	27.12	11.6	1.6004	0.2275
	35.96	29.92	17.3	2.9483	0.5940
MEDIUM	47.80	39.05	38.2	5.6897	1.0486
	44.81	36.80	26.8	5.2956	0.9788
	41.00	36.35	27.6	0.6883	0.7752
	46.94	40.66	36.7	5.2861	0.9785
	41.00	37.35	30.2	5.0392	1.1905
	41.89	34.50	28.3	3.8481	0.8431
	45.86	40.97	41.3	6.1594	1.3526
	48.94	41.99	40.6	5.9703	1.1417
	47.59	40.06	36.7	7.0163	1.4480
	44.05	36.64	31.0	4.6062	0.9394
LARGE	56.90	49.29	56.0	7.7454	1.2114
	57.84	49.80	68.9	6.1635	1.0119
	54.82	47.70	55.1	8.2654	1.4945
	70.24	56.93	87.1	20.1540	4.4967
	67.54	52.94	73.8	13.8231	2.5141
	58.40	44.80	55.2	9.4257	1.9024
	58.66	47.27	54.4	11.0587	2.1105
	58.46	49.35	67.1	12.1420	2.8838
	63.62	53.97	79.5	14.9032	3.2433
	57.78	47.91	62.8	10.1132	1.9682

	(mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	(g)

May 2009, Aveglo

Size Class	Length				Dry Tissue Weight
SMALL	29.39	27.17	16.6	1.5066	0.3456
	35.78	31.17	22.2	2.5034	0.5899
	21.41	28.44	18.3	1.5626	0.3346
	34.45	29.73	20.2	2.2426	0.5281
	36.31	32.53	24.0	2.5732	0.4436
	39.03	31.27	29.5	3.2432	0.8047
	30.65	27.92	18.7	1.7269	0.3874
	34.97	30.40	21.9	2.2651	0.5330
	32.65	28.32	19.3	2.2800	0.5379
	29.06	27.34	16.3	1.4491	0.3340
MEDIUM	44.91	37.63	38.3	5.6541	1.3394
	43.64	38.39	40.9	5.0793	1.1181
	43.25	37.30	40.9	4.3363	0.9771
	45.55	39.56	45.3	6.0417	1.4227
	43.97	37.08	38.6	5.4587	1.2697
	47.49	41.58	45.7	6.3898	1.4031
	45.03	40.29	43.9	5.1913	1.1954
	45.87	39.29	45.7	6.6280	1.5810
	39.56	35.16	29.1	3.3654	0.7788
	35.21	30.78	23.4	2.3073	0.5128
LARGE	57.14	46.48	63.9	9.9320	2.1901
	56.21	45.08	66.4	8.6802	1.9951
	55.26	45.15	61.6	9.3845	2.0461
	57.63	49.23	73.0	11.2733	2.6113
	57.46	46.96	62.6	8.7210	1.6766
	55.80	39.90	37.6	7.4329	1.4105

	(mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	(g)
	55.19	46.42	58.3	7.6504	1.8608
	59.97	46.83	73.5	11.0934	2.6355
	56.47	47.99	71.1	10.0102	2.2483
	68.19	55.96	112.6	17.8733	3.9516

June 2009, Ada

Size Class	Length				Dry Tissue Weight
SMALL	32.10	25.23	13.7	1.2727	0.2791
	35.55	29.00	22.5	1.7018	0.3756
	30.66	27.02	17.2	1.5909	0.2574
	32.58	27.41	13.5	1.4098	0.2583
	31.00	24.27	17.5	1.5085	0.2384
	30.45	26.90	16.7	1.2457	0.2549
	28.99	25.67	12.2	1.0568	0.2090
	32.86	29.33	16.9	1.2696	0.2111
	31.07	27.66	14.6	1.1902	0.2076
	34.29	29.32	17.4	1.9252	0.4536
MEDIUM	44.44	37.89	38.9	3.7466	0.7671
	46.85	38.32	38.2	4.1379	0.9983
	47.65	39.68	41.6	4.3545	0.9042
	46.14	38.25	42.3	5.8977	1.4274
	46.63	39.34	38.1	4.1512	0.8854
	45.32	36.49	36.0	3.9757	0.8207
	47.99	39.07	44.2	4.7456	0.9800
	43.56	36.42	32.4	4.0585	0.6006
	47.12	38.02	41.8	4.8394	0.9824
	50.09	43.83	47.7	5.1129	0.9249

	(mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)		(g)
LARGE	61.33	46.41	56.9	7.8123	1.3577	
	56.59	46.77	62.6	8.0700	1.7525	
	61.40	48.86	62.3	6.6034	1.0652	
	64.72	52.27	82.7	9.0137	1.7253	
	56.74	48.41	66.3	8.6490	1.7854	
	63.74	49.77	73.3	10.3443	2.3260	
	58.11	45.79	59.4	9.3465	2.2085	
	59.83	48.05	68.5	8.9754	2.0110	
	62.45	52.14	74.7	9.2597	1.8112	
	60.78	51.17	74.0	10.5553	2.3133	

June 2009, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	34.69	30.93	18.3	1.6089	0.2681
	35.15	30.75	19.4	2.1342	0.4125
	35.66	30.50	20.3	2.1506	0.4013
	30.48	28.01	14.1	1.9656	0.3364
	29.21	26.47	14.7	1.5723	0.2699
	33.23	27.33	18.4	1.2358	0.2020
	30.33	26.61	15.1	1.4521	0.2351
	31.46	26.03	14.2	1.4985	0.2696
	32.44	27.18	14.0	1.4470	0.2413
MEDIUM	44.43	35.49	36.4	2.9833	0.5020
	47.27	35.78	33.5	4.1254	0.8514
	46.01	38.66	34.3	6.2255	1.3134
	44.31	37.67	32.2	4.7685	0.8894
	44.41	36.77	36.3	3.3201	0.5534
	46.32	38.29	32.3	5.1754	1.0750
	41.89	36.43	30.6	4.2290	0.8524
	40.55	31.08	24.1	2.6733	0.4113
	47.93	39.71	39.8	6.4893	1.2390
	40.43	34.31	25.0	3.2208	0.6248
LARGE	62.84	50.03	69.8	11.7097	1.6761
	56.90	43.91	58.5	7.1000	1.1463
	60.18	48.05	71.9	8.6309	1.5188
	64.16	51.69	67.5	12.7855	2.0983
	56.07	46.86	50.9	7.8842	1.2772
	57.59	49.88	61.6	8.9077	1.5857
	72.04	57.74	106.9	14.0134	2.4572
	60.01	48.76	56.8	10.6054	2.3613
	58.63	46.60	54.6	11.4430	2.1534
	59.13	49.20	64.7	11.7732	2.4285

July 2009, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	31.41	26.26	13.1	1.9562	0.4380
	31.51	26.09	12.4	2.1492	0.4824
	36.59	39.65	18.3	3.2333	0.6824
	37.19	26.66	21.1	2.2962	0.4207
	33.23	27.45	17.0	2.2766	0.4881
	33.60	27.90	17.8	1.7821	0.4064
	31.71	26.75	15.7	1.8290	0.4089
	33.96	30.14	17.1	2.3057	0.5112
	28.62	24.74	12.0	1.3471	0.2941
	34.26	29.22	18.3	2.0864	0.4388
MEDIUM	50.00	39.80	40.4	5.3079	1.1043
	46.60	38.36	41.4	5.0087	1.1481
	50.31	36.79	34.3	5.2058	1.1903
	45.57	38.08	31.6	3.7396	0.7749
	45.81	36.58	32.7	5.1701	1.0860
	45.91	37.42	33.5	5.0203	1.0338
	43.47	35.62	24.3	4.7121	1.0691
	46.82	40.36	42.3	5.4139	1.1883
	42.77	34.51	28.2	3.8874	0.8124
	44.97	40.03	33.9	5.2665	1.1638
LARGE	64.95	53.41	76.6	12.5755	2.6234
	59.45	45.57	59.2	10.1191	2.2853
	56.50	42.02	64.4	7.9072	1.6358
	62.73	49.10	74.5	9.8477	2.0435
	67.29	54.57	84.3	13.9559	3.1842
	60.72	47.91	64.5	9.3419	1.9173

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
	64.68	50.82	68.7	9.4779	1.9191
	56.38	44.48	56.3	8.4737	1.5466
	58.95	44.85	51.6	10.3565	2.4695
	61.04	44.90	47.0	11.4509	2.5802

July 2009, Aveglo

SMALL	38.65	31.54	23.6	3.5620	0.8290
	36.13	32.54	21.4	2.6678	0.6003
	38.49	35.50	26.8	2.8073	0.5671
	31.29	26.32	12.1	1.4258	0.2513
	36.91	31.95	24.7	2.2185	0.4560
	33.17	29.34	18.0	1.6572	0.3100
	33.78	28.79	18.4	2.0197	0.4143
	30.75	26.78	14.7	1.8075	0.3723
	33.93	31.24	21.4	1.8976	0.3973
	32.84	29.17	18.6	2.1174	0.4631
MEDIUM	47.44	38.53	44.6	4.4508	1.0622
	47.94	36.04	26.5	3.6444	0.4775
	42.02	36.78	32.5	3.4774	0.7048
	45.63	37.02	31.4	4.3066	0.7592
	46.93	35.73	33.9	4.6265	1.0674
	41.42	35.07	30.9	4.1675	0.8822
	46.93	37.73	36.2	5.3785	1.2064
	42.47	37.34	36.3	3.6940	0.8261
	48.87	38.84	45.5	4.5834	0.8408
	44.22	37.92	35.5	4.5213	0.9464
LARGE	55.33	43.53	62.6	8.3697	1.6690
	57.75	49.27	77.4	10.4978	2.3508
	70.31	56.62	97.1	14.7981	3.2222

	57.49	42.42	71.0	9.3215	2.0456
	60.05	44.33	44.8	7.9671	1.2289
	58.59	48.28	71.2	8.8072	2.0658
	58.11	49.97	73.3	9.7220	2.0674
	56.97	43.41	54.6	7.6318	1.7199
	57.37	47.35	79.1	7.6188	1.6803
	58.00	42.78	69.0	9.4700	2.2356

August 2009, Ada

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	21.49	19.31	5.6	0.7450	0.1936
	25.57	23.03	9.6	1.1462	0.2793
	25.65	21.77	8.9	0.9990	0.2565
	21.07	19.91	5.7	0.5827	0.1416
	20.96	18.87	5.5	0.8075	0.1370
	21.97	20.34	6.3	1.1063	0.2228
	25.45	24.88	10.3	1.2671	0.2507
	28.09	25.16	11.0	1.6625	0.3333
	20.65	19.96	6.0	0.7226	0.1789
	21.75	20.61	6.5	0.7230	0.1968
MEDIUM	46.25	39.10	38.6	5.6363	1.2934
	47.20	38.67	35.5	6.6214	1.5690
	44.21	37.03	32.2	5.6810	1.3689
	40.39	44.02	23.0	3.8279	0.9728
	43.10	36.43	29.5	4.9006	1.2191
	41.72	35.08	25.4	3.8108	0.9646
	50.40	43.92	49.1	4.8426	1.7990
	51.02	41.69	25.4	7.7349	1.7126
	42.33	34.71	28.2	3.4574	0.8624
	42.07	37.03	33.9	4.9665	1.0438
LARGE	86.55	63.55	140.5	36.2513	8.5322
	70.54	57.36	86.6	18.2494	3.9720

	Width (mm)	Weight (g)	Wet Tissue Weight (g)	
63.69	56.07	92.3	17.5522	4.1883
77.14	65.24	143.7	26.5521	5.7111
77.49	61.17	130.4	27.0534	6.4417
74.39	59.58	111.9	25.1635	5.7715
82.84	64.50	119.9	25.1546	5.3390
64.42	56.46	80.5	17.5375	4.2132
56.37	45.35	74.1	6.9518	1.4437
55.58	43.09	69.3	5.4700	1.2356

August 2009, Aveglo

Size Class	Length (mm)	Width (mm)	Weight (g)	Wet Tissue Weight (g)	Dry Tissue Weight (g)
SMALL	34.62	27.34	21.6	3.3621	0.2247
	37.11	31.43	22.4	2.2934	0.4033
	31.44	25.23	17.8	1.5044	0.2674
	31.49	26.32	15.1	1.4200	0.2532
	37.54	31.92	28.7	2.2339	0.5060
	30.41	25.43	12.0	1.4834	0.2160
	33.78	28.79	18.4	2.0197	0.4143
	30.75	26.78	14.7	1.8075	0.3723
	33.93	31.24	21.4	1.8976	0.3973
	35.84	30.17	19.4	2.1154	0.4634
MEDIUM	48.54	36.22	43.5	4.5671	1.0622
	42.44	35.34	31.5	3.7544	0.5785
	42.02	36.78	32.5	3.4774	0.7048
	45.63	37.02	31.4	4.3066	0.7592
	46.93	35.73	33.9	4.6265	1.0674
	40.99	35.77	33.7	4.3675	0.9823
	46.93	37.73	36.2	5.3785	1.2064
	42.47	37.34	36.3	3.6940	0.8261
	49.23	39.81	47.0	5.0831	1.2406
	44.22	37.92	35.5	4.5213	0.9464
LARGE	55.33	43.53	63.6	9.6697	1.6678
	56.75	46.27	64.4	10.4978	2.3508
	70.31	56.62	97.1	14.7981	3.2222
	57.49	42.42	71.0	9.3215	2.0456
	60.05	44.33	44.8	7.9671	1.2289
	68.59	52.28	66.2	8.8072	2.0667
	58.11	49.97	73.3	9.7220	2.0674
	59.91	44.44	60.5	7.6318	1.7199
	57.37	47.35	79.1	7.6188	1.6803
	58.00	42.78	69.0	9.4700	2.2356

Appendix 2

Table 2 *Physicochemical Parameters of the Volta Estuary at Ada and Aveglo*

PERIOD	SITE	(PARAMETER) pH	Temp. (°C)	Salinity	Cond. (µs/cm)	TDS (mg/l)	DO (mg/l)
March 2008	Ada	6.99	28.16	0.03	62	31	8.76
	Aveglo	7.00	28.20	0.03	70	32	6.77
April 2008	Ada	7.05	28.01	0.03	60	30	7.21
	Aveglo	7.04	28.30	0.03	70	32	6.78
May 2008	Ada	6.63	29.22	0.03	61	31	5.84
	Aveglo	7.06	28.35	0.03	69	33	6.79
June 2008	Ada	6.99	28.49	0.03	66	33	6.33
	Aveglo	7.08	28.49	0.03	68	34	6.78
July 2008	Ada	6.55	28.08	0.03	70	35	3.16
	Aveglo	7.00	28.11	0.04	84	42	3.16
Aug. 2008	Ada	6.99	27.38	0.03	65	33	3.06
	Aveglo	6.90	27.33	0.03	66	33	2.70
Sept. 2008	Ada	6.48	27.28	0.03	63	32	2.48
	Aveglo	6.89	27.19	0.03	63	32	2.38
Oct. 2008	Ada	6.19	28.99	0.03	63	32	1.52
	Aveglo	6.23	29.11	0.03	64	32	1.58
Nov. 2008	Ada	6.89	29.29	0.03	64	32	2.19
	Aveglo	6.51	29.15	0.03	63	31	1.88
Dec. 2008	Ada	6.47	29.09	0.03	58	29	2.55
	Aveglo	6.65	29.04	0.03	58	29	2.44

Table 2 *Physicochemical Parameters of the Volta Estuary at Ada and Aveglo*

PERIOD	SITE	(PARAMETER) pH	Temp. (°C)	Salinity	Cond. (µs/cm	TDS (mg/l)	DO (mg/l)
Jan. 2009	Ada	8.50	29.46	0.03	59	29	6.11
	Aveglo	6.47	29.18	0.03	57	29	4.00
Feb. 2009	Ada	7.20	28.44	0.03	60	30	3.58
	Aveglo	6.87	28.94	0.03	60	30	4.74
March 2009	Ada	7.07	28.76	0.02	53	27	3.99
	Aveglo	6.76	28.80	0.03	57	29	3.83
April 2009	Ada	6.56	28.87	0.03	63	31	3.04
	Aveglo	6.65	29.05	0.03	61	30	3.15
May 2009	Ada	6.58	29.58	0.02	52	26	3.41
	Aveglo	6.65	29.78	0.02	55	28	3.92
June 2009	Ada	6.85	29.59	0.02	54	27	3.18
	Aveglo	7.00	29.62	0.02	54	27	3.03
July 2009	Ada	7.20	28.61	0.02	55	27	4.48
	Aveglo	7.17	28.73	0.02	55	28	4.07
Aug. 2009	Ada	7.41	26.95	0.02	52	26	4.53
	Aveglo	7.28	28.88	0.02	55	27	1.97

Appendix 3: Table 3 *Sediment Composition Analysis for the two sampling stations*

	Composition (%)			Particle Size Distribution (%)				
	SAND (0.02-2mm)	SILT (0.002-0.02mm)	CLAY (<0.002mm)	VFS (0.02-0.06mm)	FS (0.06-0.2mm)	MS (0.2-0.6mm)	CS (0.6-2.0mm)	TEXTURE
Mar 08 Ada	97.26	1.54	1.20					
Mar 08 Avg	96.26	1.42	2.32					
Apr 08 Ada	98.34	1.28	0.38					
Apr 08 Avg	97.18	1.46	1.36					
May 08 Ada	99.00	0.50	0.50					
May 08 Avg	99.78	0.08	0.14					
June 08 Ada	98.76	0.19	1.05					
June 08 Avg	99.16	0.12	0.72					
July 08 Ada	98.92	0.04	1.04					
July 08 Avg	99.1	0.50	0.4					

Aug 08 Ada	99.24	0.18	0.58
Aug 08 Avg	99.26	0.26	0.48
Sept 08 Ada	98.26	0.59	1.15
Sept 08 Avg	96.28	1.40	2.32
Oct 08 Ada	99.34	0.18	0.32
Oct 08 Avg	98.18	0.48	1.34
Nov 08 Ada	99.30	0.10	0.60
Nov 08 Avg	99.48	0.12	0.40
Dec 08 Ada	99.22	0.26	0.54
Dec 08 Avg	98.78	1.08	0.14
Jan 09 Ada	98.66	0.29	1.05
Jan 09 Avg	99.16	0.12	0.72

1.76	1.94	13.24	81.29	SAND 0.69	7.84	13.31	70.44	SAND
0.71	1.29	19.6	77.74	SAND 0.56	1.03	14.57	82.02	SAND
0.22	0.44	20.66	77.68	SAND 0.12	4.56	30.76	64.34	SAND
0.92	2.64	26.84	63.36	SAND 0.72	3.96	20.22	74.26	SAND
1.58	6.00	14.76	76.58	SAND 0.48	1.08	21.32	76.22	SAND
0.22	0.98	27.76	70.28	SAND 0.06	0.18	0.31	98.71	SAND
0.76	2.94	13.29	81.24	SAND 0.79	7.74	15.21	72.54	SAND
0.51	1.49	19.64	77.70	SAND 0.26	1.03	14.87	82.02	SAND
0.48	1.95	12.57	84.30	SAND 0.27	0.56	1.51	97.14	SAND
0.10	0.14	0.31	98.71	SAND 0.12	4.76	30.56	64.34	SAND

1.92	2.64	25.84	63.36	SAND	1.72	2.96	20.32	74.16	SAND

Table 3 *Sediment Composition Analysis for the two sampling stations*

	SAND (0.02-2mm)	SILT (0.06-0.02mm)	CLAY (0.2-<0.002mm)	VFS (0.6-0.06mm)	FS	MS 0.2mm)	CS (0.02-0.6mm)	(0.002-2.0mm)	TEXTURE
Feb 09 Ada	98.24	0.18	1.58	1.22		0.98	26.78	70.26	SAND
Feb 09 Avg	99.26	0.26	0.48	0.06		0.18	0.31	98.71	SAND
Mar 09 Ada	97.28	1.57	1.15	1.76		3.94	12.29	80.24	SAND
Mar 09 Avg	96.38	1.30	2.32	0.76		7.77	17.21	70.54	SAND
Apr 09 Ada	98.34	0.11	0.39	1.61		1.38	18.65	77.70	SAND
Apr 09 Avg	96.14	2.46	1.4	0.56		1.03	14.47	82.12	SAND
May 09 Ada	98.3	1.1	0.6	0.48		1.95	12.57	84.3	SAND
May 09 Avg	99.48	0.12	0.4	0.27		0.56	1.51	97.14	SAND
June 09 Ada	99.24	0.20	0.56	0.22		1.98	26.76	70.28	SAND
June 09 Avg	97.26	2.24	0.5	0.66		0.18	0.31	98.11	SAND
July 09 Ada	97.48	1.37	1.15	0.76		3.74	13.29	80.44	SAND
July 09 Avg	97.38	1.33	1.29	1.76		6.67	17.31	70.54	SAND
Aug 09 Ada	98.92	0.04	1.04	1.58		6.00	14.76	76.58	SAND
Aug 09 Avg	99.10	0.50	0.40	0.48		1.08	21.32	76.22	SAND

Legend:

AVG*: Aveglo*
CS: *Coarse sand*
MS*: Medium sand*

FS*: Fine sand*
VFS*: Very fine sand*

APPENDIX 4: *Results of the one way ANOVA and the Bonferroni's Multiple Comparison Test for significance in metal concentrations in the three clam size classes*

Manganese Ada

One-way analysis of variance					
P value	0.2036				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.642				
R squared	0.06051				
ANOVA Table	SS	df	MS		
Treatment (between columns)	55770	2	27890		
Residual (within columns)	865900	51	16980		
Total	921700	53			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	59.28	1.365	No	ns	-48.24 to 166.8
Small vs Large	74.50	1.715	No	ns	-33.02 to 182.0
Medium vs Large	15.22	0.3505	No	ns	-92.30 to 122.7

Zinc Ada

One-way analysis of variance	
P value	0.3145
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	1.183

R squared	0.04435

ANOVA Table	SS	df	MS
Treatment (between columns)	234.1	2	117.1
Residual (within columns)	5045	51	98.92
Total	5279	53	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	4.944	1.491	No	ns	-3.262 to 13.15
Small vs Large	3.556	1.072	No	ns	-4.651 to 11.76
Medium vs Large	-1.389	0.4189	No	ns	-9.596 to 6.818

Iron Ada

One-way analysis of variance

P value	0.3909
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.9568
R squared	0.03616

ANOVA Table	SS	df	MS
Treatment (between columns)	11460	2	5731
Residual (within columns)	305500	51	5990
Total	317000	53	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	25.00	0.9690	No	ns	-38.86 to 88.86
Small vs Large	34.56	1.339	No	ns	-29.31 to 98.42
Medium vs Large	9.556	0.3704	No	ns	-54.31 to 73.42

Mercury Ada

One-way analysis of variance

P value	0.0074
P value summary	**
Are means signif. different? (P < 0.05)	Yes

Number of groups	3
F	6.523
R squared	0.4202

ANOVA Table	SS	df	MS
Treatment (between columns)	0.0004527	2	0.0002263
Residual (within columns)	0.0006246	18	0.00003470
Total	0.001077	20	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	-0.004429	1.407	No	ns	-0.01274 to 0.003881
Small vs Large	-0.01129	3.584	Yes	**	-0.01960 to -0.002976
Medium vs Large	-0.006857	2.178	No	ns	-0.01517 to 0.001452

Manganese Aveglo

One-way analysis of variance

P value	0.8178
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.2020
R squared	0.007858

ANOVA Table	SS	df	MS
Treatment (between columns)	591.3	2	295.6
Residual (within columns)	74650	51	1464
Total	75240	53	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	7.889	0.6186	No	ns	-23.68 to 39.46
Small vs Large	5.556	0.4356	No	ns	-26.01 to 37.12
Medium vs Large	-2.333	0.1830	No	ns	-33.90 to 29.24

Zinc Aveglo

One-way analysis of variance

P value	0.9551
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.04603
R squared	0.001802

ANOVA Table	SS	df	MS
Treatment (between columns)	7.259	2	3.630
Residual (within columns)	4021	51	78.85
Total	4029	53	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	-0.5556	0.1877	No	ns	-7.883 to 6.772
Small vs Large	0.3333	0.1126	No	ns	-6.994 to 7.661
Medium vs Large	0.8889	0.3003	No	ns	-6.438 to 8.216

Iron Aveglo

One-way analysis of variance

P value	0.6775
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.3923
R squared	0.01515

ANOVA Table	SS	df	MS
Treatment (between columns)	4900	2	2450
Residual (within columns)	318500	51	6246
Total	323400	53	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	-3.333	0.1265	No	ns	-68.55 to 61.88
Small vs Large	18.33	0.6959	No	ns	-46.88 to 83.55
Medium vs Large	21.67	0.8225	No	ns	-43.55 to 86.88

Mercury Aveglo

One-way analysis of variance

P value	0.7753
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.2581
R squared	0.02788

ANOVA Table	SS	df	MS
Treatment (between columns)	0.00004352	2	0.00002176
Residual (within columns)	0.001517	18	0.00008430
Total	0.001561	20	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Small vs Medium	-0.002429	0.4948	No	ns	-0.01538 to 0.01052
Small vs Large	-0.003429	0.6986	No	ns	-0.01638 to 0.009524
Medium vs Large	-0.001000	0.2038	No	ns	-0.01395 to 0.01195