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Full Length Research Paper

Fish (*Arius heudelotii* Valenciennes, 1840) as bio-indicator of heavy metals in Douala Estuary of Cameroon

Fonge, B. A.[1*], Tening, A. S.[2], Egbe, A. E.[1], Awo, E. M.[1], Focho, D. A.[3], Oben, P. M.[1], Asongwe, G. A.[3] and Zoneziwoh, R. M.[4]

[1]Department of Plant and Animal Sciences, Faculty of Science, University of Buea, P. O. Box 63, Buea, Cameroon.
[2]Department of Chemistry, Faculty of Science, University of Buea, P. O. Box 63, Buea, Cameroon.
[3]Department of Plant Biology, Faculty of Science, University of Dschang, Dschang, Cameroon.
[4]Department of Geology and Environmental Science, Faculty of Science, University of Buea, P. O. Box 63, Buea, Cameroon.

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This study assessed and monitored accumulation levels of heavy metals (Fe, Pb, Cu, Zn and Mn) in a prominent benthic mangrove fish species (*Arius heudelotii* Valenciennes, 1840) from the Douala Estuary, Cameroon. Fish species selection was based on signs of deterioration in taste and texture. Sampled estuarine water and tissue parts (liver, gut, flesh, gills and bones) of the captured fish were analyzed using atomic absorption spectrophotometer. The results show that concentration of the heavy metals except Mn in water was below the permissible limits imposed by WHO and USEPA for aquatic life. The metal levels in fish collected were significantly higher ($p<0.05$) than the levels in water, indicating bioaccumulation. Metal levels in the fish organs varied considerably with the highest mean concentrations of Pb and Zn found in the flesh and gills. The high concentration of heavy metals in these parts makes the fish unsafe for consumption as the majority of consumers depend on these parts for their livelihood. This calls for environmental surveillance and monitoring of these water bodies and their surrounding environment.

Key words: *Arius heudelotii,* bioaccumulation, heavy metals, estuary, environmental surveillance.

INTRODUCTION

The role of heavy metals in aquatic environments is increasingly becoming an issue of global concern at private and governmental levels with heavy metals bio-magnifying and increasing their toxicity. High concentrations of heavy metals in surface water could lead to health hazards in man, either through drinking of the water and/or consumption of fish and other aquatic life forms (Duruibe, 2007). Many aquatic organisms have the ability to accumulate and bio-magnify contaminants like heavy metals, polycyclic aromatic hydrocarbons (PAHs) which are mutagenic and carcinogenic (Davies et al., 2006). The ingestion of these contaminants may affect not only the productivity and reproductive capa-bilities of these organisms, but ultimately affect the health of man that depends on these organisms as a major source of protein.

Urban contamination remains a problem in developing countries and many industries have contributed in a number of ways in introducing these toxic materials to the environments (United State Department of Agriculture - USDA, 2001). Seventy percent (70%) of the industries

*Corresponding author. E-mail: ambofonge@yahoo.com. Tel: (237) 77793752.

(soap, brewery, metallurgy, food processing, salt, petroleum and pharmaceutical) in Cameroon are located within the Douala Estuary (Asangwe, 2006). Most of these industries are found in the city of Douala (the economic headquarters of Cameroon). Discharges from these industries are all emptied in the coastal environment. This has led to pollution problems in the lagoon complex and adjacent wetlands that are potential sinks (Asangwe, 2002; Angwe et al., 2005).

It has been shown that soils under mangrove forests sequestered considerable amounts of heavy metals with most of them retained in the surface soils (Amusan and Adeniyi, 2005). The authors also observed that the anthropogenic heavy metals were mostly adsorbed probably due to the negatively charged sites of organics and clay. Organic amendments have a profound effect in the reduction in the delivery of suspended solids to watercourses. These amendments may release heavy metals that flow through runoff avenues either as particulate or dissolved forms. (De Siervi et al., 2005). Mangrove soils are reported to possess a remarkable capacity to retain heavy metals (Dwivedi and Padmakumar, 1983; Chen and Lin, 1988; Tam and Wong, 1993) but can also be sources of contamination. There is the possibility that these metals may only be accumulated temporarily and will be remobilized back to the water column, becoming a secondary source of pollution. The adsorption and desorption of heavy metals in wetland soils depend on one or a combination of soil properties such as pH, cation exchange capacity (CEC), organic matter, clay content, and alternating aerobic and anoxic conditions (Kerner and Wallmann, 1992; Orson et al., 1992; Lacerda et al., 1993; Tam and Wong, 1996). Heavy metals in sediments and aquatic biota can be toxic even in trace amounts to both the fish themselves and humans who depend on them for their livelihood. Moreover, the concentration of heavy metals in fish is related to several factors, such as the food habits and foraging behaviour of the organism, trophic status, source of a particular metal (Chen and Folt, 2000) and this varies from organ to organ. It is a product of equilibrium in the organism's environment and its rate of ingestion and excretion.

Arius heudelotii is one of the prominent fish species consumed and in abundant in the Douala Estuary, Cameroon (FAO, 2002). According to Heath (1991), fishes can regulate metal concentration to a certain limit above which bioaccumulation occurs. In Douala just as in other parts of Cameroon, *A. heudelotii* commonly called 'Yanda' have shown signs of deterioration in taste and texture from market survey, but there exist limited information on the causative factors. The present study aimed to investigate water samples and the levels of heavy metal accumulation in *A. heudelotii* organs from the Wouri Estuary, River Dibamba and River Wouri.

MATERIALS AND METHODS

Study area

This study was carried out in the Douala Lagoon situated in the Gulf of Guinea. It is part of the coastal plain of Cameroon, which stretches from Rio-Del-Rey through Limbe, Tiko, Douala, and Kribi down to Equatorial Guinea. It covers an area of 85 km^2 and is situated between latitude 3° 5' and 4° 1'N of the Equator and Longitude 9° 4' and 9° 5' E of the Greenwich Meridian (Figure 1). The area has a tropical climate with two major seasons: the wet (April to October) and dry (November to March) seasons. Rainfall is bimodal, peaking at July and August. Minimal rainfall is in January and February. Mean relative humidity in the area is about 80%. The Wouri and Dibamba rivers, with their tributaries, constitute the drainage system in the entire Douala municipality. They flow all year round, with the Wouri flowing within the Akwa, Bonaberi and Bassa regions, while the Dibamba flows in the eastern outskirt of the town. Intermittent streams that are usually loaded with waste from the industries empty into these rivers.

Sampling strategy and treatment

Sampling sites were established using a Global Positioning System (GPS). The sites were chosen based on ecological settings and human activities in the area. Water samples were collected in the wet season in 2008 in 500 ml (0.5 L) plastic bottles at a depth of 20 cm below the surface by dipping the containers into the water. Before the samples were collected, the bottles were rinsed several times with the sample to be collected, and two drops of concentrated sulphuric acid were added to the samples. The samples were then transported immediately in an ice-containing cooler to the Chemistry Laboratory of the University of Buea and later transferred to the Analytical and Environmental Chemistry Laboratory of the University of Dschang for chemical analyses. Before analysis, the water samples were filtered using Whatman no. 40 filter papers. They were mixed vigorously and aspired into an atomic absorption spectrophotometer for heavy metal determination.

Fish samples were randomly captured in triplicates every forth night for eight weeks from the Douala Estuary. They were placed in plastic bags and stored at -4°C after cleaning with distilled water to remove adhering dirt. All frozen samples were allowed to thaw at room temperature. The fish samples after defrosting were dissected with the help of a stainless steel knife to obtain liver, gut, bones and flesh (muscle). The different organs were chopped into fine pieces and oven-dried at 105 ± 20°C to constant weight. One gram of each sample was digested using 1:5:1 mixture of 70% perchloric acid concentrated nitric acid and concentrated sulphuric acid at 80 ± 5°C in a fume chamber, until a colourless liquid was obtained (Pauwels et al., 1992). Each digested sample was made up to 20 ml with de-ionised water and analyzed for heavy metals in an atomic absorption spectrophotometer.

Statistical analysis

Data on the concentration of the heavy metals were presented as means ± SEM. Means were subjected to One-Way Analysis of Variance (ANOVA) and separated using the Tukey's test at 5% probability level. Pearson correlation coefficient was used to compare whether there were any significance differences in the metal levels in the three sites (River Wouri, River Dibamba and

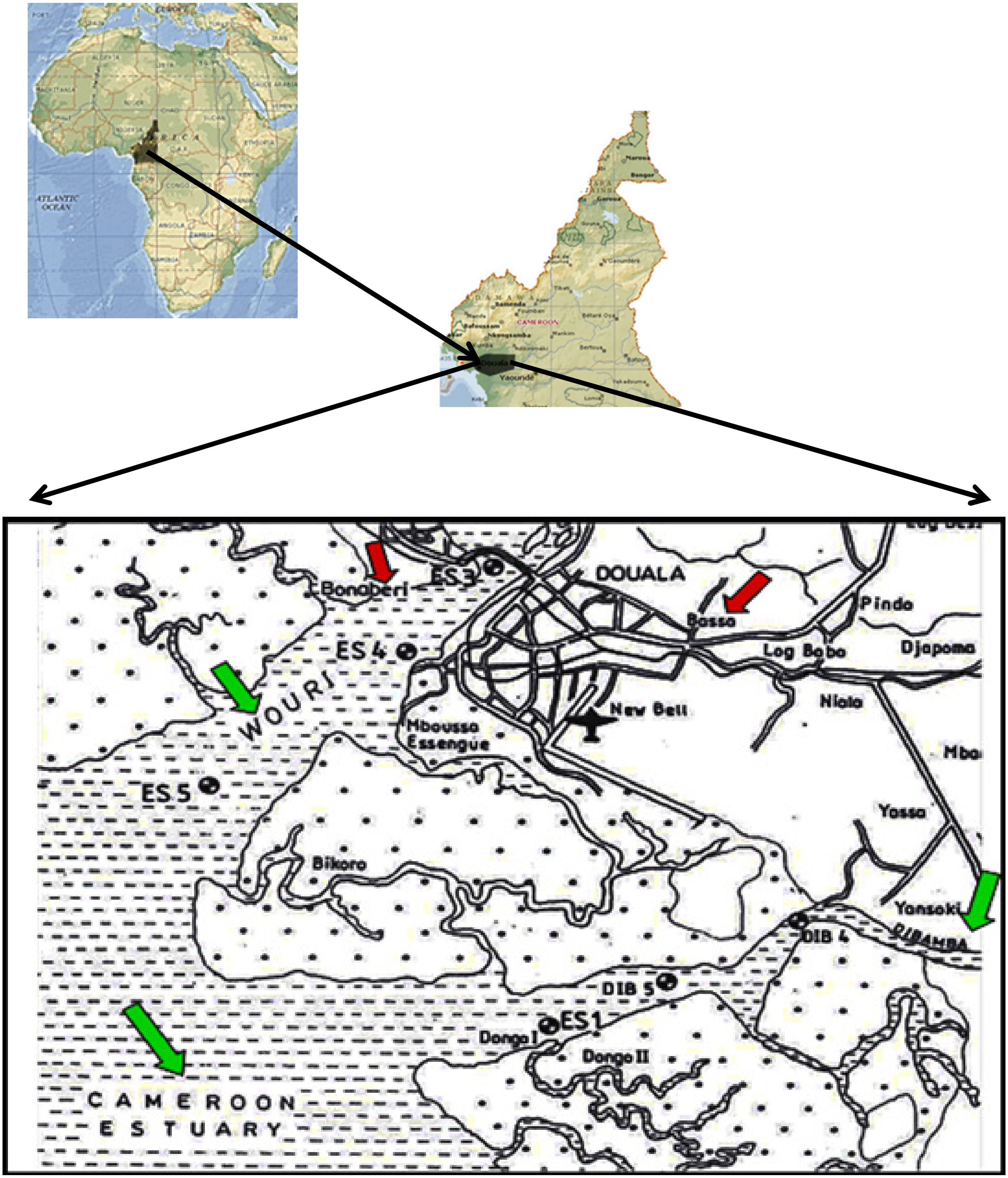

Figure 1. Study area in River Wouri, River Dibamba and Wouri Estuary showing different sampling sites.

Table 1. Mean concentrations of heavy metals in water in Rivers Wouri and Dibamba and the Wouri Estuary during the wet season.

Site	Mg/L				
	Fe	Mn	Pb	Zn	Cu
River Wouri	0.06	0.04	0.03	0.01	0.01
River Dibamba	0.09	0.04	0.02	0.03	0.03
Wouri Estuary	0.05	0.54	0.01	0.03	0.03
WHO (1985) limit	1.00	0.01	0.01	5.00	1.00
USEPA (1986) limit	1.00	0.05	0.05	1.00	1.00

Table 2. Pearson's Correlation Coefficients for the three sites.

Site	River Wouri	River Dibamba	Wouri Estuary
River Wouri	-	-	-
River Dibamba	0.81^{ns}	-	-
Wouri Estuary	0.69^{ns}	0.75^{ns}	-

ns, non significant.

Table 3. Heavy metals in fish organs.

Organ	Fe	Pb	Cu	Zn	Cr
	mg/kg				
Bones	0.057 ± 0.036^{b}	0.378 ± 0.000^{c}	0.346 ± 0.001^{c}	0.359 ± 0.001^{d}	N.D*
Flesh	0.072 ± 0.000^{b}	0.395 ± 0.000^{a}	0.35 ± 0.001^{b}	0.379 ± 0.000^{b}	N.D
Gills	0.025 ± 0.003^{c}	0.407 ± 0.000^{a}	0.361 ± 0.000^{a}	0.385 ± 0.002^{a}	N.D
Gut	0.124 ± 0.012^{a}	0.383 ± 0.000^{b}	0.353 ± 0.001^{b}	0.369 ± 0.001^{c}	N.D
Liver	$0.130 \pm .032^{a}$	0.381 ± 0.000^{b}	0.346 ± 0.002^{c}	0.363 ± 0.001^{d}	N.D
WHO (2005)	0.3	0.2	10	5	1.3

Data followed by the same letter in a column are not significantly different at P≤0.05. *N.D= Not detected.

Wouri Estuary).

RESULTS

Heavy metal levels in water

The concentrations of Fe, Mn, Pb, Zn and Cu in the water are presented in Table 1. The concentrations of Fe, Pb, Zn and Cu fell within the limits of heavy metals in drinking water set by World Health Organization (WHO) (1985) and the U.S. Environmental Protection Agency (USEPA) (1986). For Mn, its concentration (0.54 mg/L) in Wouri Estuary was above the limits set by WHO and USEPA. The highest Fe concentration was found in river Dibamba (0.09 mg/L) and the least in the Wouri estuary (0.05 mg/L). The concentration of Pb was highest in river Wouri (0.03mg/L) and least in the Wouri estuary (0.01 mg/L). Zinc and Cu were high in river Dibamba and Wouri Estuary, both having a value of 0.03 mg/L in the two water bodies. Both metals were low in River Wouri, having a value of 0.01 mg/L. Pearson correlation co-efficient showed that there was no significant difference in the levels of heavy metals between the three sites (Table 2).

Heavy metal concentration in fish organs

Table 3 shows the concentrations of Fe, Pb, Cu and Zn in the fish. The accumulation of Fe in the organs ranged from 0.025 to 0.13 mg/kg with the highest in the liver and the least in the gills. Lead concentration in the organs also had a range of 0.381 to 0.407 mg/kg, with the least in the liver and the highest in the gills. These values were above the WHO standard of 0.3 mg/kg (WHO, 1985). The concentration of Pb was also significant at $p<0.05$. There were significant differences ($p<0.05$) in Cu concentrations in the organs. Copper concentrations had a range of 0.346 to 0.361 mg /kg with the least in the liver and bones and the highest in the gills. The concentration of Zn in the organs also showed significant differences ($p<0.05$) with a range of 0.359 to 0.385 mg/kg. The highest concentration was noted in the gills and the least in the bones. The results also indicate that Cr was not detected in the organs of the fish.

The accumulation of heavy metals in fish organs was as follows:

Fe: Liver ≈ Gut > Flesh ≈ Bones > Gills
Pb: Gills ≈ Flesh > Gut ≈ Liver>Bones
Cu: Gills > Gut ≈ Flesh > Liver ≈ Bones
Zn: Gills > Flesh > Gut >Liver ≈Bones.

Figure 2 shows the variation of the heavy metal concentrations in the fish organs with time. The concentration of Zn in the organs was highest in weeks five and eight in the liver and bones, respectively. The lowest value was observed in week eight in the flesh, gills and flesh. The liver had the highest concentration of Fe in week eight and the lowest value in week three. In addition, while the highest value of Pb was recorded in week one in the liver, the lowest value was observed in weeks five and eight in the gut and bones, respectively. The highest and lowest values of Cu were found in the liver in weeks five and eight having the highest and week one the lowest.

DISCUSSION

According to Yilmaz et al. (2006) and Agarwal et al. (2007), fishes are considered as biomonitors of aquatic ecosystems for estimation of heavy metal pollution and risk potential for human consumption. The result showed that the levels of the heavy metals were within the limits set by WHO (1985) and USEPA (1986) for drinking water. The concentration of Mn was above the 0.01 and 0.05 mg/L set by WHO (1985) and USEPA (1986), respectively for drinking water. Water from this area is therefore undesirable for drinking as this can result in many heart diseases from Mn toxicity. This implies that the water within the Douala Estuary thus poses some health risk with respect to heavy metals. The high concentration of Pb in River Wouri could be associated to the constant discharge of effluent from garages and petrol stations in the Douala metropolitan city. The high concentration of Fe in River Dibamba is not uncommon as the majority of the metallurgy industries such as Fe recycling industry are located around this area. There could also be a possibility that the sediments accumulate some of these metals in the adsorbed state which are in equilibrium with those in water (Harbinson, 1986; Kerner and Wallmann, 1992; Tam and Wong, 1993; De Siervi et al., 2005). Davies (2006) pointed out that the most toxic metals accumulate in mangrove sediments. Thus, the high density of mangrove wetlands along Douala Estuary may contribute to the high concentration of some of these metals in it. According to Angwe et al. (2005), the Douala Estuary has two main industrial zones (the Bonaberi and Bassa); these zones have most of the industries situated along the Wouri, Dibamba estuary line and all their effluents are discharged into the two rivers. The Bassa industrial zone all empties its waste into river Dibamba and its tributaries.

Though the mean concentrations of Fe, Cu and Zn found in the fish were below the maximum permissible values, continual increase in urbanization may result to deleterious levels given that heavy metals biomagnify in a mangrove ecosystem (Davies, 2006). Porter et al. (1975) and Forstner and Wittmann (1981) also reported that the enhanced levels of metals in fish tissues arise through bio-magnification at each trophic level and carnivorous bottom feeders concentrate higher metal levels. Moreover, lead was found to be above the WHO (2005) threshold value in all the organs of the fish. These implies that *A. heudelotii* from the Douala estuary may not be fit for human consumption as Pb which has high affinity for thiol groups, turn proteins and peptides susceptible to structural modifications in sub-cellular compartments and tissues as in skeletal muscle (Paris-Palacios et al., 2000). Hechtenberg and Beyersmann (1991) also demonstrated the inhibitory effects of lead on Ca^{2+}-ATPase activity from sarcoplasmic reticulum in rabbits. It has been shown that lower levels of lead exposure appear to cause a small decrease in the intellectual development of young children (Alloway and Ayres, 1993). Children are more vulnerable because their nervous system is still developing. Lead and lead compounds have been classified as carcinogenic to humans based on evidence from animal experiments (IARC: International Agency for Research on Cancer, 1996).

The highest concentrations of Pb (0.407 mg/kg), Cu (0.361 mg/kg) and Zn (0.385 mg/kg) were found in the gills of *A. heudelotii.* This may be due to the feeding habit of the fish, *A. heudelotii*, are detritus species feeding mostly in the mangrove habitat. They also take in heavy metals through the gills, and with the fine nature of the gills it turns to accumulate the metals. Metal concentration in the gills could also be due to complexing of the elements with mucus remaining between the gill lamella, which is hard to remove completely from the gills before preparation of the tissues for analysis (Demirak et al., 2005). The gills had the lowest concentration of Fe. This may be due to high Fe metabolism in the gill which is involved in the transport of oxygen to the other tissues in

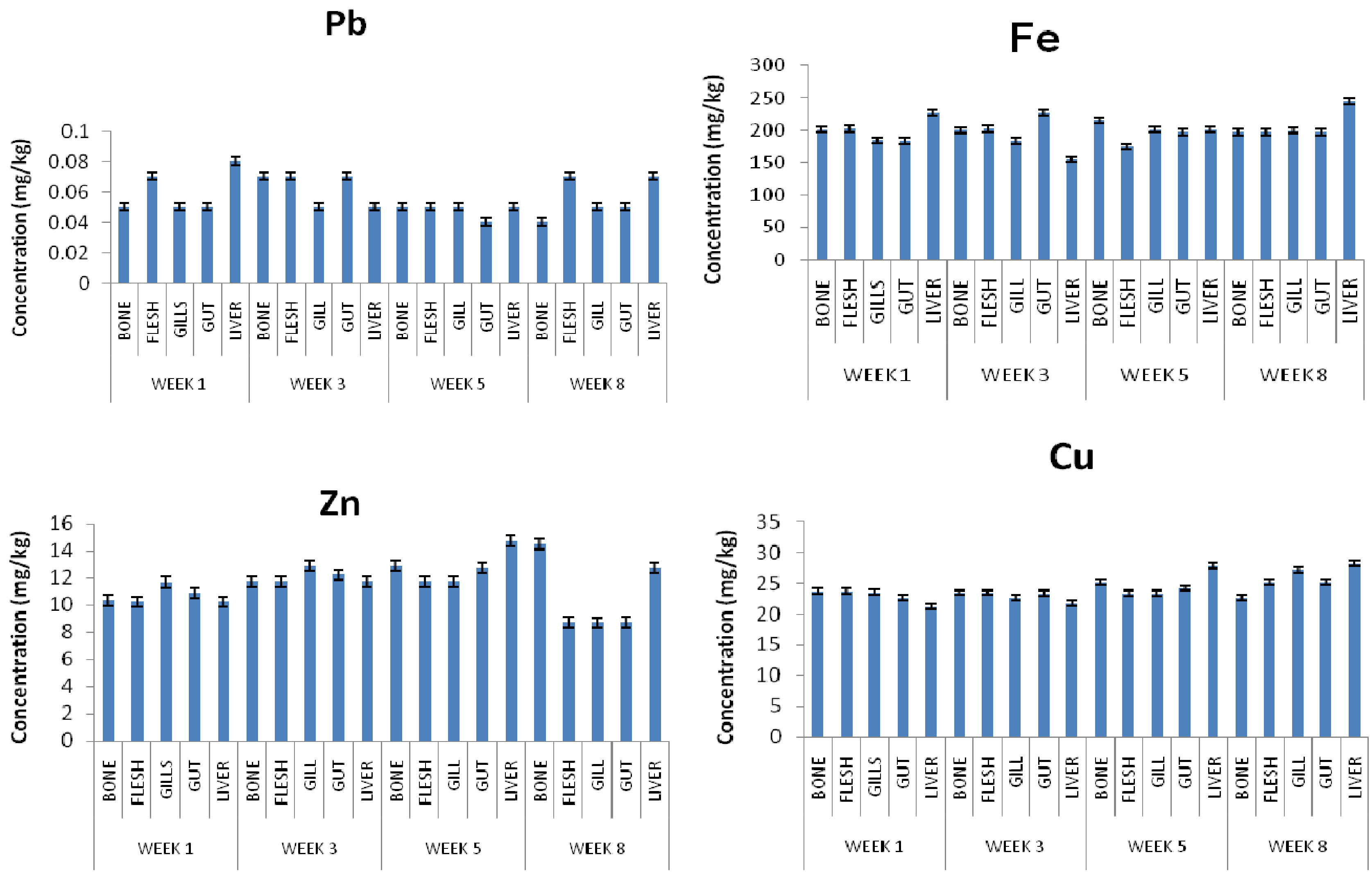

Figure 2. Concentrations of heavy metals in fish organs with time.

the fish. Heavy metals accumulate mainly in metabolic organs such as liver that stores metals by producing metallothioneins, which appears as a metal detoxification mechanism within the body (Roesijah and Robinson, 1994; Peakall and Burger, 2003). Metallothioneins are a family of low molecular weight cysteine-rich protein that have been reported in vertebrate and invertebrates. Their synthesis can be induced by a wide variety of metal ions including cadmium, Cu and Zn. Metallothioneins have been proposed as biomarkers to indicate the presence of high levels of metals in the environment, (Peakall and Burger, 2003; Pratap and Wendelaar Bonga, 2007). The accumulation of metals in the liver of food fish does not directly affect human health because this is not an edible part. Nevertheless, the predatory animals such as birds which consume the whole fish are at risk of excess metal contaminants. This observation is in line with the findings of Nussey et al. (2000), Ogusie (2003) and Obasohan and Eguavoen (2008) who reported that the liver accumulates more heavy metals than the flesh.

Furthermore, the concentrations of the heavy metals in the water were below those in the fish tissues which may be due to bio-accumulation. Similar findings by Fonkou et al. (2005), Senarathne and Pathiratne (2007), Mohammed et al. (2009) and Abdel-Baki et al. (2011) also showed that the metal levels in fish were higher than the levels in both water and sediment, thus indicating bio-accumulation. However, there was no particular trend in the concentrations of heavy metals in all the fish organs with time. This could be attributed to the fact that sampling was carried out in an uncontrolled environment and hence the heavy metal load is bound to be irregular as reflected in the different organs.

In conclusion, this study shows that water from the Wouri Estuary has a very high Mn load making, it dangerous for potable water as Mn toxicity is related to many heart diseases. It was also shown that *A. heudelotii* can accumulate Pb in its gills, flesh and liver up to levels above the WHO threshold value. This implies that this fish species from the Douala Estuary is not fit for human consumption. Regular monitoring of heavy metal levels in the water and fish within this vicinity is therefore necessary.

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