Bioaccumulation of Some Heavy Metals (Pb, Cu, Cd, Zn and Fe) in Crab (*Callinectes amnicola*) living in Ebrie Lagoon, Jacqueville Sector (Cote d'Ivoire, West Africa)

Kouamé Kouamé Victor [1*], Yapi Dopé Armel Cyrille [1], Kouadio Kouakou Norbert[2], Djedje Gokou Jean Marie[3] and Tidou Abiba Sanogo[1]
1. University of Jean Lorougnon Guédé, Department of Environmental Sciences, B.P 150 Daloa, CÔTE D'IVOIRE
2. University of Man, Department of Engineering, Agronomy, Forestry and Environment, BP 20, Man, CÔTE D'IVOIRE
3. University of Nangui-Abrogoua, Department of Management and Environmental Sciences, 02 BP 801, Abidjan, CÔTE D'IVOIRE
*kvictor2@yahoo.fr; kouame_victor@ujlg.edu.ci

Abstract
In this study, some heavy metals (Pb, Cu, Cd, Zn and Fe) were seasonally determined in water, sediment and crab (Callinectes amnicola) from Ebrie lagoon. The study aimed to estimate the bioaccumulation levels of heavy metals in crab. Heavy metal levels were analyzed by inductively coupled plasma spectrophotometer (ICP-MS).

The obtained results showed that the average values of Cu, Pb and Zn in water were higher during the dry season with the concentrations of 243.02 ± 27.87, 12.10 ± 0.81 and 0.36 ± 0.09 µg.L^{-1} *respectively. Relatively to the sediment, heavy metals concentration in the same season were 307.19 ± 14.17, 68.34 ± 3.71 and 0.36 ± 0.04 µg.g*$^{-1}$ *for Cu, Pb and Cd respectively. These concentrations of heavy metals were found to exceed guideline values recommended by WHO and FEPA. Consequently, consumption of crab from Ebrie lagoon could cause long-term toxic effects for human health. BCFw and BCFs were in the order Zn > Cu > Fe > Cd > Pb and Cd > Zn > Fe > Cu > Pb respectively. This study indicates that control measures and action plans must be taken for reducing Ebrie lagoon contamination by heavy metals due to human activities.*

Keywords: Bioaccumulation factors, Crab contamination, Trace metals, Pollution, Coastal water.

Introduction
Human activities have significantly raised the concentration of heavy metals in aquatic ecosystems[23]. These metals from natural and contaminated effluents enter the estuarine ecosystem through direct discharges from coastal communities, ships, rivers and atmospheric deposition and through land runoff[10]. Metals are taken up and subsequently accumulated by aquatic organisms. The pollution levels of the aquatic environment by metals can be estimated by analyzing water, sediments and aquatic organisms[11]. Aquatic species have large body to accumulate pollutants and providing appropriate material to use them as bioindicator for indirect estimation of the concentration of pollution in their living environment[16]. The coastal areas such as lagoon and bay are important grounds for vital activities of valuable aquatic organisms such as fish, crabs, oysters and mussels.

In Côte d'Ivoire, the Ebrie Lagoon is threatened by pollutants of various kinds: domestic sewage, garbage, industrial effluents and agricultural run-off[5]. Since 2012, various studies have been carried out on some species of this lagoon. Catfish (*Chrysichthys nigrodigitatus*) was used to compare the potential impact of water pollution on fish quality using histological pathology in the fish tissues due to the exposure to heavy metals[19]. Seasonal accumulation levels of cadmium, copper, lead, mercury and zinc were determined in sediments, water and black-chinned tilapia organs (*Sarotherodon melanotheron*) to assess the public health risks associated with consuming fish harvested from this area[2,24].

Callinectes amnicola is one of the important fishery commodities in Ebrie Lagoon and constitutes a product of great consumption for the population of Côte d'Ivoire[20]. The present study was conducted to examine the bioaccumulation levels of heavy metals (Pb, Cu, Cd, Zn and Fe) in crab (*Callinectes amnicola*) collected from Ebrie lagoon.

Material and Methods
Sampling site, sample collection and preparation: The study was carried out at Ebrie lagoon (Côte d'Ivoire, West Africa) during the rainy season and the dry season. This lagoon extends over 130 km along the coastline. Based on hydrological data, Ebrie lagoon was divided into six sectors[3]. The present study was carried out at IV and V sectors (05°16'N-04°15 – 4°30 W). Three sampling sites were selected according to size of fishing area of crab (*Callinectes amnicola*), Ahua and Songon for IV sector and N'Djem for V sector (figure 1). Specimens of crab were collected free from fishermen at the study sites. A total of 36 crabs for each site were collected (108 crabs for all sites) and were analyzed based on their weight and size according to carapace width.

The organisms were grouped into two composite samples (juvenile and adult) for each site. Adults flesh, juveniles' flesh and female's eggs were analyzed. The crabs were stored into glacial ones containing ice until the laboratory for analysis. Prior to the analysis, the crabs were cleaned with running tape water and thawed at room temperature. The carapace, claws, walking legs and gut region of each crab were dissected by using a pair of sterile stainless-steel scissors to prevent contamination.

Acid digestion and chemical analysis: The acid digestion method was performed to digest the samples; this involved heating 0.5 g of dried tissues of crabs in a Teflon beaker with mixed concentrated acids [Hydrogen Peroxide (H_2O_2), Nitric acid (HNO_3), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4)] according to Kamaruzzaman et al.[7] The digestion was diluted with distilled water appropriately in the range of standards prepared from stock standard solution of the metals (Merck). After dilution process samples were analyzed using Inductively Coupled Plasma Spectrophotometer (ICP-MS) and metal concentration was presented as $\mu g.g^{-1}$ of dry weight.

In addition, temperature, pH and dissolved oxygen of the water were measured at the time of sampling using a multi parameter (TURO T-611) *in situ*. Water samples for trace metal analyses were also collected from Ebrie lagoon in pretreated plastic bottles while sediment samples were collected in pretreated polythene bags and taken to the laboratory for analyses. Water samples were acidified with 65% nitric acid and filtered through Whatmann qualitative filter paper, thereafter each sample was diluted to 100 mL with distilled water and kept at 6°C.

Sediment samples were dried at 110°C for 24 h until weight stabilized, after which each sample was sieved to obtain a homogenous sample. This was then finely ground with mortar and pestle, then 1 g of sample was digested with 1:1 ratio of 55% nitric acid and 32% hydrochloric. The samples were allowed to cool only after a clear solution was obtained and filtered through Whatmann qualitative filter papers and the filtrate was diluted to 100ml with distilled water. The concentrations of trace metals in various samples were determined by ICP-MS.

Calculations and Statistical Analyses: Bioconcentration factor was calculated for crabs' body and eggs for each metal. It is giving an indication of the degree of trace metal accumulation in the crab from the water (BCFw) and sediment (BCFs). The formula used to calculate the BCF is[18]:

$$BCF = \frac{\text{Metal in crab sample } (\mu g.g^{-1})}{\text{Metal in water sample } (\mu g.L^{-1}) \text{ or in sediment } (\mu g.g^{-1})}$$

One-way ANOVA and Tukey multiple range test were performed to test the differences of the metals' levels among sites. Differences were considered statistically at $p < 0.05$. All statistical analyses were performed with STATISTICA 7.1 software.

Results and Discussion

Metals concentrations in the water at each site during wet season and dry season are shown in table 1. Significant differences were not observed between the two seasons ($P<0.05$). The concentrations of Cu, Pb and Zn were higher at Songon station during the dry season (243.02 ± 27.87, 12.10 ± 0.81 and 0.36 ± 0.09 $\mu g.L^{-1}$ respectively). The relatively high levels of Cu, Pb and Zn in water of the Ebrie lagoon are suggestive of the influence of both domestic sewage sources and industrial and agricultural effluents. This suggests the anthropogenic influence due to industrialization and urbanization within the catchments of the lagoon. Tuo et al[21] have reported that the urban area of this important lagoon is increasingly contaminated with toxic substances.

The lowest concentrations of metals were recorded for Fe (20.00 ± 2.74 $\mu g.L^{-1}$), Pb (3.01 ± 0.40 $\mu g.L^{-1}$) and Zn (0.87 ± 0.10 $\mu g.L^{-1}$) during the same season at Ahua station. During the rainy season, Cd was higher accumulated at Songon station (18.10 ± 1.95 $\mu g.L^{-1}$) while the lowest value (9.69 ± 2.11 $\mu g.L^{-1}$) was observed at Ndjem station. Temperature, pH and dissolved oxygen values of different station were also given in table 1. Temperature was between 29.84 ± 0.79 and 27.90 ± 0.55°C. Highest value was obtained at Ndjem in the dry season and the lowest at Ahua during the rainy season. Dissolved oxygen was higher during the dry season than in the rainy season.

In addition, sediments play a significant role in determining the overall environmental quality of an estuarine system[6]. Heavy metals concentrations in sediment from sites are in table 2. In general, values were highest in dry season. These concentrations decrease slightly during the rainy season likely due to the dilution effect. Relatively to the sites, high concentrations of Cu, Pb and Cd (respectively 307.19 ± 14.17, 68.34 ± 3.71 and 0.36 ± 0.04 $\mu g.g^{-1}$) were obtained at Songon station while Fe and Zn with concentrations of 61.05 ± 5.15 $\mu g.g^{-1}$ and 11.50 ± 1.42 $\mu g.g^{-1}$ respectively were high at Ndjem station.

The lowest concentrations of heavy metals in sediment were recorded at Ahua station during the rainy season. The values of Pb, Zn and Fe were 20.61 ± 3.08, 10.35 ± 0.95 and 51.88 ± 4.88 $\mu g.g^{-1}$ respectively. According to Chapman[1], sediments are also an important biological habitat, uptake of toxicants into the food web is influenced by toxicant concentrations in sediment. Thus, heavy metals accumulation in the groups of crabs was evaluated.

Results on heavy metal concentrations in the crabs are shown in figure 2. Tukey's multiple comparison test made between the heavy metals accumulation in the organs of crabs (adults flesh, juveniles flesh and eggs) from the study area shows that Fe, Cu and Pb were more accumulated in adults flesh (25.08 ± 8.05, 26.54 ± 4.73 and 0.30 ± 0.08 $\mu g.g^{-1}$, respectively) than in juveniles flesh and eggs. Although there was no significant difference between the heavy metals' accumulation in the organs of Crabs (adults flesh, juveniles flesh and eggs) in the three sites, significant differences in metal accumulation were observed between the metals. Fe was more accumulated in the adults' flesh than in the juveniles flesh and in eggs.

On the contrary, a similar accumulation of Zn and Cu in the adults, in the juveniles and in eggs was observed. The elevated concentration of Fe, Zn and Cu in crab (*Callinectes amnicola*) might be due to the major role played by these elements in maintaining the physiological functions of the organism. This observation corresponds with the previous study of Kanakaraju et al[9], Mutlu et al[12] and Oluowo and Olomukoro[14] who postulated that these metals play an important role as an essential element in all living systems from invertebrates to human, hence the organisms tend to accumulate high concentration of Fe from the surrounding environment.

According to Kamaruzzaman et al[8], this observation might also be due to the organism's capacity to regulate and accumulate elevated concentration of these metals. On the other hand, Pb accumulation showed the lowest levels in crab organs. Followed by Pb accumulation, Cd had the second lowest levels. The values were between 0.24 ± 0.06 and 0.30 ± 0.8 $\mu g.g^{-1}$ for Pb and between 0.40 ± 06 and 0.43 ± 09 $\mu g.g^{-1}$ for Cd. In addition, the detected concentrations of heavy metals in different groups of crab (*Callinectes amnicola*) were above the standard limits set by FEPA[4] and WHO[22]. Trace metals such as Cd and Pb have no known beneficial effect on organisms and their accumulation over time in the bodies of animals can cause serious illness[17].

The metals accumulation degree in the crab from the water (BCFw) and sediment (BCFs) was evaluated by Bioconcentration factor. The BCFw and BCFs results are shown in table 3. Generally, BCFw were higher than BCFs. Significant differences were observed between BCFw of the metals. Zn had the highest BCFw and Pb had lowest BCFw for all the organs. Higher values for the adults' flesh, the juveniles flesh and the eggs were 13.53 ± 1.11, 12.42 ± 1.11 and 11.01 ± 1.11 respectively. These results were similar with the previous study of Omuvwie and Atobatele[15].

The lowest values were 0.04 ± 0.01 for the eggs, 0.05 ± 0.01 for the juvenile and 0.06 ± 0.01 for the adults. The higher values of BCFs were obtained for Cd with 5.09 ± 0.91 for the juveniles' flesh, 4.81 ± 0.91 for the eggs and 4.64 ± 0.91 for the adults' flesh. Heavy metals bioconcentration factor in the crabs from the water decreased in the sequence for the adults' flesh, the juveniles' flesh and for the eggs as Zn > Cu > Fe > Cd > Pb. BCFs for all organs were in order as follows Cd > Zn > Fe > Cu > Pb.

Heavy metals accumulation levels in organisms found in the present study complement those reported by other authors on different species of freshwater organisms[13,25]. According to Nsikak et al[13], these results can indicate that *Callinectes amnicola* can serve as a sentinel organism for the biomonitoring of Zn, Cu, Fe, Cd and Pb in fresh and brackish water ecosystems.

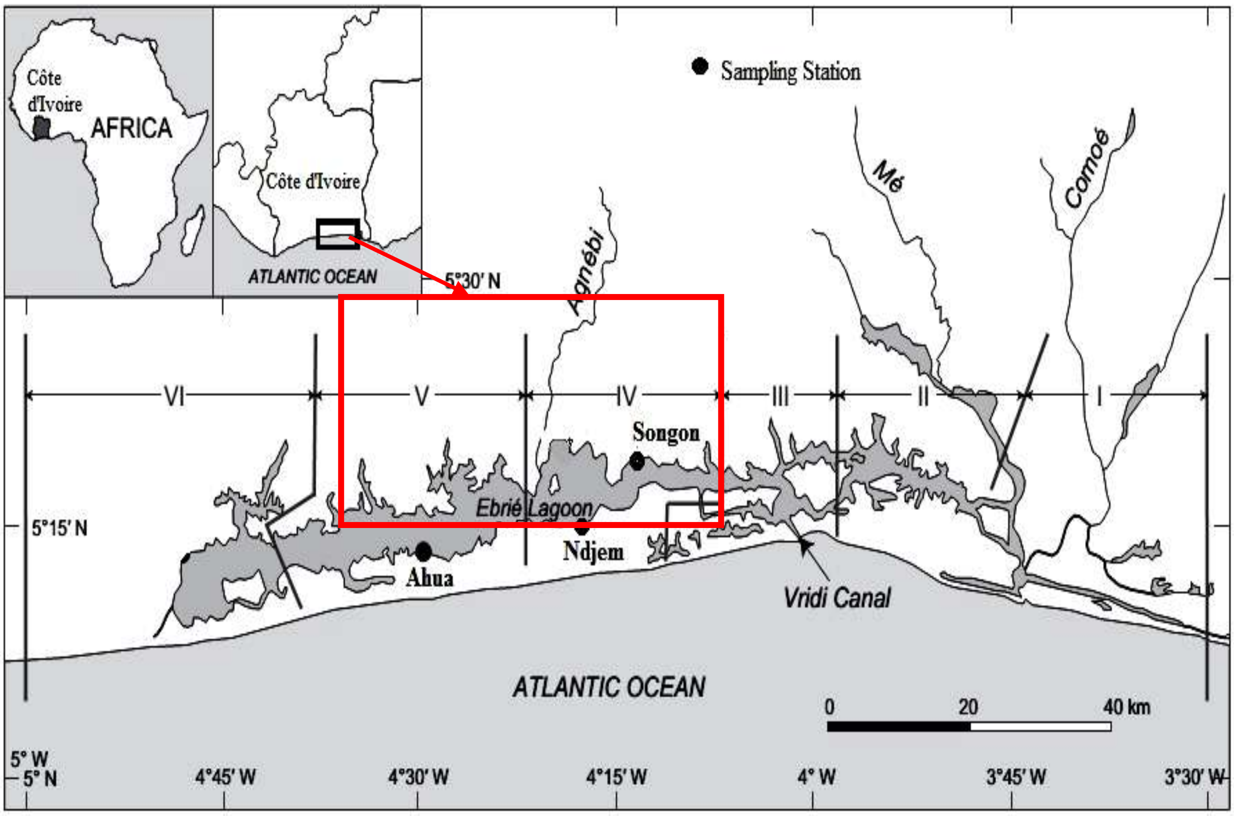

Figure 1: Location map of sampling stations.

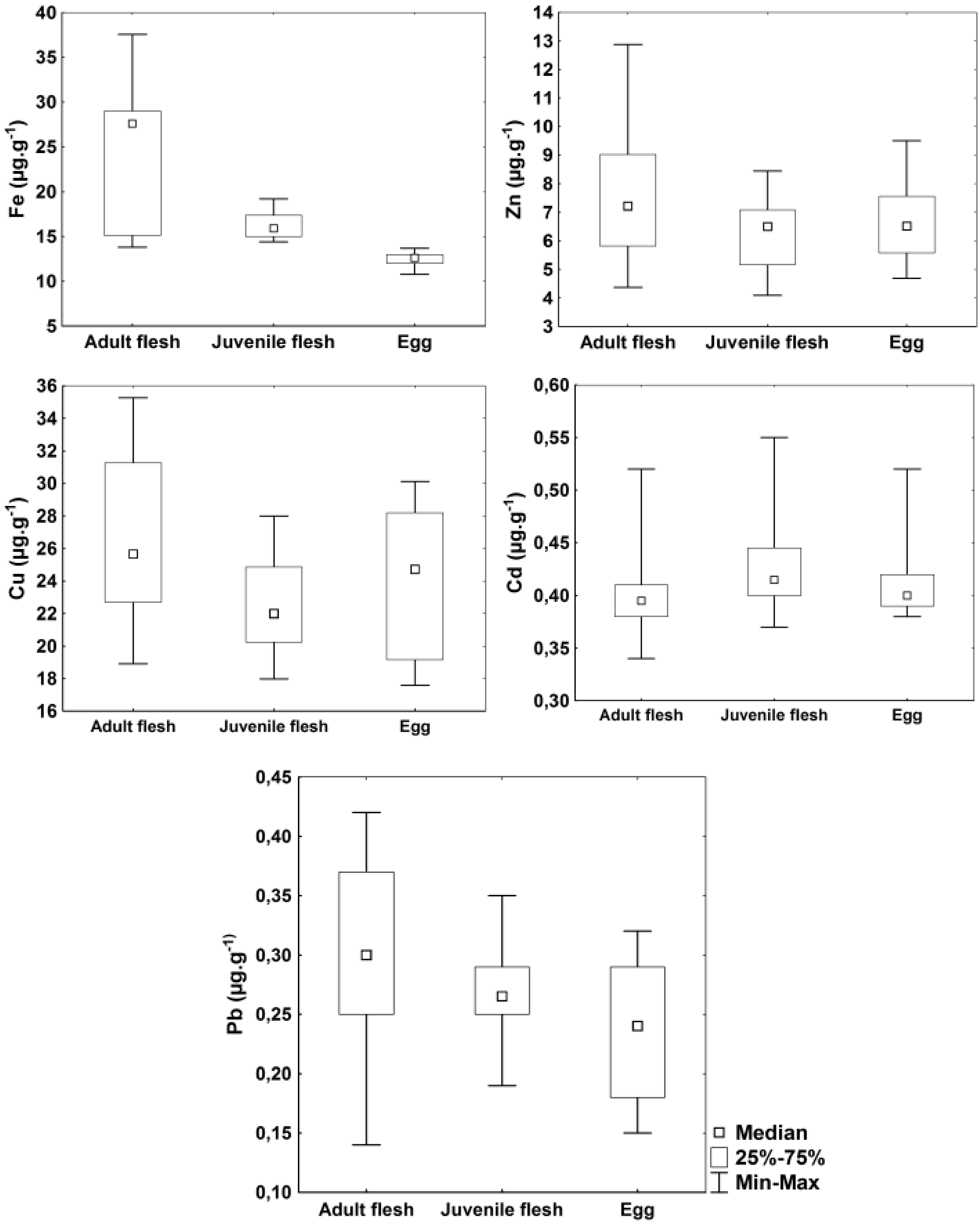

Figure 2: Comparison of heavy metals concentrations in the crabs

Table 1
Mean and standard deviation for environmental variables (Temperature, pH, dissolved oxygen) and the concentrations of heavy metals in the water during rainy and dry seasons.

Parameters	Site	Fe (µg.L^{-1})	Zn (µg.L^{-1})	Cu (µg.L^{-1})	Cd (µg.L^{-1})	Pb (µg.L^{-1})	T°C	pH	O_2 (mg.L^{-1})
Rainy season	Songon	30.02 ± 3.85	0.41 ± 0.06	126.01 ± 25.62	18.10 ± 1.95	10.45 ± 0.57	28.27 ± 0.69	6.94 ± 0.19	4.19 ± 1.13
	Ndjem	31.40 ± 4.24	0.73 ± 0.08	86.24 ± 22.21	2.93 ± 0.84	5.95 ± 0.42	28.38 ± 0.72	7.08 ± 0.19	3.88 ± 1.03
	Ahua	26.22 ± 3.01	0.73 ± 0.11	41.80 ± 23.02	9.69 ± 2.11	8.28 ± 0.63	27.90 ± 0.55	7.20 ± 0.21	7.41 ± 1.20
Dry season	Songon	29.49 ± 3.88	0.36 ± 0.09	243.02 ± 27.87	13.43 ± 1.97	12.10 ± 0.81	29.83 ± 0.88	7.32 ± 0.86	6.27 ± 1.08
	Ndjem	34.79 ± 4.15	0.75 ± 0.15	103.90 ± 28.18	15.30 ± 1.64	4.28 ± 0.43	29.84 ± 0.79	7.35 ± 0.69	6.08 ± 0.99
	Ahua	20.00 ± 2.74	0.87 ± 0.10	43.49 ± 18.92	1.79 ± 0.90	3.01 ± 0.40	29.41 ± 0.66	7.67 ± 0.54	8.24 ± 1.66

Table 2
Mean values of heavy metals concentrations in sediment from sites.

Parameters	Site	Fe (µg.g^{-1})	Zn (µg.g^{-1})	Cu (µg.g^{-1})	Cd (µg.g^{-1})	Pb (µg.g^{-1})
Rainy season	Songon	64.70 ± 5.10	7.97 ± 0.90	245.76 ± 12.21	0.36 ± 0.04	52.19 ± 3.36
	Ndjem	60.97 ± 5.09	10.43 ± 1.24	102.85 ± 10.67	0.06 ± 0.02	29.76 ± 3.08
	Ahua	59.56 ± 4.87	11.12 ± 1.30	66.10 ± 11.01	0.20 ± 0.02	36.37 ± 3.28
Dry season	Songon	59.66 ± 5.02	8.37 ± 0.68	307.19 ± 14.17	0.28 ± 0.04	68.34 ± 3.71
	Ndjem	61.05 ± 5.15	11.50 ± 1.42	149.73 ± 9.82	0.30 ± 0.02	21.39 ± 2.80
	Ahua	51.88 ± 4.88	10.35 ± 0.95	77.68 ± 10.20	0.04 ± 0.02	20.61 ± 3.08

Table 3
The bioconcentration factor in the crab from the water (BCFw) and from the sediment (BCFs) in Groups and Eggs.

Groups		Adult flesh	Juvenile flesh	Egg
BCFw	Fe	0.94 ± 0.04	0.60 ± 0.04	0.47 ± 0.04
	Zn	13.53 ± 1.11	11.01 ± 1.11	12.42 ± 1.11
	Cu	0.87 ± 0.33	0.68 ± 0.33	0.79 ± 0.33
	Cd	0.10 ± 0.02	0.11 ± 0.02	0.10 ± 0.02
	Pb	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
BCFs	Fe	0.43 ± 0.02	0.28 ± 0.02	0.21 ± 0.02
	Zn	0.78 ± 0.04	0.64 ± 0.04	0.71 ± 0.04
	Cu	0.22 ± 0.02	0.19 ± 0.02	0.20 ± 0.02
	Cd	4.64 ± 0.91	5.09 ± 0.91	4.81 ± 0.91
	Pb	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001

Conclusion

The knowledge on metal bioaccumulation in native species is very important for their management and utilization of these species for human consumption and to determine the useful bioindicator species. Trace metals (Cd, Cr, Pb, Ni and Zn) were detected in crab (*Callinectes amnicola*), sediment and water collected from Ebrie Lagoon. The concentrations of these metals were found relatively high in water and sediments mostly in dry season. These metals were also accumulated in the adults' flesh, in the juveniles flesh and in eggs of crab.

The mean concentrations of heavy metals in this study were found to exceed guideline values recommended by WHO and FEPA. BCFw and BCFs were in the order Zn > Cu > Fe > Cd > Pb and Cd > Zn > Fe > Cu > Pb respectively.

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