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Assessment of the levels of some heavy metals in mudfish (*Clarias anguillaris*) from River Okpokwu, Apa, Benue State, Nigeria

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Abstract

This study employed recommended analytical procedures to determine the levels of As, Mn, Cu, Pb and Fe in whole *Clarias anguillaris* (mudfish) obtained from river Okpokwu, Apa Local Government Area of Benue State, Nigeria. Samples of the fish, water and sediment were collected weekly at three commercial fishing locations: Auke, Odejo and Ocholonya in October, 2010 and February, 2011 and analysed for levels of the heavy metals using Unicam Solar (32) Atomic Absorption Spectrophotometer. The results obtained across the sampling points showed that the heavy metals concentrations in *Clarias anguillaris* varied between 0.05±0.01µg/g As to 0.44±0.03µg/g Fe in October, 2010 and 0.08±0.03µg/g Pb to 0.34±0.05µg/g Fe in February, 2011. In the river water and sediment, the results ranged from 0.06±0.02µg/L As to 1.24±0.05µg/L Fe and 0.36±0.04µg/g As to 3.79±0.20µg/g Fe in October, 2010 but varied between 0.04±0.01µg/L As to 0.59±0.10µg/L Fe and 0.41±0.06µg/g As to 0.97±0.03µg/g Fe in February, 2011 respectively. There were positive correlations between the metal contents of the samples. T-test analyses of the mean concentrations of the heavy metals between the sampling periods were statistically significant ($P < 0.05$). The concentrations of the heavy metals recorded in this study were below the WHO's permissible limits. Therefore, water and *Clarias anguillaris* from the study area have not become contaminated by the heavy metals studied. However, periodic review should be sustained to monitor possible accumulations of the heavy metals in future due to the proliferation of anthropogenic activities around river Okpokwu.

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Introduction

The pollution of water bodies in the urban, industrial and agricultural areas has resulted to increased concentration of heavy metals and minerals in the internal body of fish (Farkas *et al*., 2000). The occurrence of heavy metal contaminants especially in excess of natural loads has assummed an important worldwide problem due to the potential health effects if found in the food chain. Report has shown that the list of sites contaminated with trace metals grows every year, presenting a serious problem for human health and a fearful danger to the environment (Martin and Meybeck, 1979). This situation has been aggravated by the rapid growth of population, increased urbanization and expansion of industrial activities, exploration and exploitation of natural resources as well as the modern agricultural practices and lack of environmental regulations (FAO, 1992; Giguere *et al*., 2004; Gupta *et al*., 2009). As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals (Linnik and Zubenko, 2000) and present great dangers to organisms along the food chain. Research works have shown that heavy metals can be bioaccumulated and biomagnified via the food chain and finally assimilated by human consumers with the attendant adverse health effects (Agah *et al*., 2009). As a consequence, fish are often used as indicators of heavy metals contamination in the aquatic ecosystem because they occupy high trophic levels and are important food source (Agah *et al*., 2009). Lawani and Alawode (1996), reported unacceptable levels of lead and mercury in the tissues of African catfish, *Clarias gariepinus* from River Niger. Similarly, Omoregie *et al.* (2002), noted that the levels of lead, copper and zinc in the Nile tilapia, *Oreochromis niloticus* caught from River Delimi, Jos are too high for frequent human consumption.

The functioning of the natural ecosystems is increasingly being affected by human activities. Skjeikvale *et al.* (2001), reported that it is difficult to find a river or other water bodies whose natural regime has not been modified by man's activities. Despite the natural sources of heavy/trace metals in the environment, anthropogenic supply to aquatic ecosystems from industrial effluents/wastes; agricultural and domestic wastewaters laden with metal toxicants out weights the former (Benson *et al*., 2006). Among the rural populace also, there has been indiscriminate usage of dangerous agrochemicals in farm lands at the banks of rivers which provide sources of water to the host communities for domestic and agricultural purposes. The result is that toxic and hazardous materials including heavy metals are transported to several water bodies (rivers, streams, lake, and ponds) with potential effects on aquatic organisms.

Over the years, there has been a remarkable degradation in the quality of River Okpokwu and its potability due to the proliferation of chemical-based agriculture, mining and block industry activities in the major settlements along the river. Agricultural contamination of many resources through fallout, drainage and runoff erosion is highly probable in rivers within the vicinity of intensive agricultural activities. River Okpokwu provides one of the highest fishing ground of Zone C in Benue State Nigeria but there is no clear documentaion of the effects of high anthropogenic activities around it. Thus, it becomes pertinent to carry out relevant study of the fallouts of chemical-based agriculture, mining and cottage block industries along the river with a view to providing baseline data for the purpose of monitoring and managing the effects of these activities around the river.

Materials and methods

The study area

River Okpokwu is one of the relief features in Apa LGA of Benue State, Nigeria which provides one of the highest fishing ground of Zone C senatorial district. It is located on longitude 7.80^{o}E and latitude 7.58^{o}N. The River is about 4km away from Ugbokpo, Apa Local Government Headquarters. River Okpokwu meets river Ochekwu at Odejo, Agatu LGA and they flow into River Benue. Unlike other rivers within the locality, River Okpokwu never dries up completely during dry season and its volume and size increase very rapidly during rainy season. Thus, it sustains high agricultural and fishing activities. Near the source of this river, there is high gravel mining activity while along the river, there are high chemical-based farming and block industries activities. These constitute potential sources of heavy metals contamination of surface and underground waters.

Samples collections and preservation

Samples of mudfish (*Clarias anguillaris*), water and sediment were collected from three high fishing locations (Auke, Odejo and Ocholonya) in River Okpokwu during the months of October, 2010 and February, 2011. These periods were chosen to represent the peaks of rainy and dry seasons in the study area. *Clarias anguillaris* is a subsurface feeder that lives and feeds from the bottom sediment of the river. Eighteen (18) samples each of mudfish, sediment and the river water were collected at each sampling point. Water samples were collected at both extremities and median part of the river by immersion of polythene bottles to approximately 30cm below the water surface and allowed to overflow. The sediments were collected by scooping with plastic hand trowel (0.25cm deep) and immediately put in sterilized polythene bags, closed and labeled. They were taken to laboratory and dried at 40°C in an oven, prior to analysis (Maitera *et al.*, 2011). All containers used for sampling and storage were thoroughly washed with solution of detergent and distilled water. They were then soaked in 10% "Analar" nitric acid overnight followed by rinsing with distilled water to remove trace elements contamination (Wufem *et al.*, 2009). The fish were collected and transported to the laboratory in ice boxes and rinsed with deionized water prior to analyses (Ahmed *et al.*, 2010). The samples were preserved in a refrigerator at 5°C (Farkas *et al.*, 2000).

Samples preparation and analysis

Water sample

Raw water was subjected to wet digestion. 100ml unfiltered water was measured into a 250ml beaker and 20ml "Analar" nitric acid solution plus 10ml of 50% hydrochloric acid solution were added. The acidified water was then evaporated to almost dryness on a hot plate and 5ml of 50% hydrochloric acid was again added and heated for 15 minutes. The beaker was removed and cooled to room temperature before transferring the contents into a 100ml volumetric flask and made up to the mark with distilled water (Wufem *et al.*, 2009).

The digested water was filtered and analyzed for the levels of As, Mn, Cu, Pb and Fe using Unicam Solaar (32) Atomic Absorption Spectrophotometer (AAS).

Fish samples

The fishes were discaled and inviscerated and then transferred into electric oven at 40°C. They were dried in the oven at this temperature for 24hours and then pulverized in a clean dry porcelain mortar (Ahmed *et al.*, 2010). The pulverized samples were dried further for 1hour at a reduced temperature of 20°C and put into clean dried bottles (Farkas *et al.*, 2000; Adeniyi *et al.*, 2005). 3.0g of the dried fish samples were weighed into a silica crucible and ashed in a muffle furnace at a temperature of 600°C for 5hours (Farkas *et al.*, 2000; Adeniyi *et al.*, 2005). The ashes were cooled to room temperature and sieved to remove particles and then transferred into a 250ml conical flask. Thereafter, 20ml of concentrated HNO_3 was added and diluted to 50ml with deionised water and swirled gently after which the volume was made up to 100ml with deionised water and analyzed for heavy metals as with the water.

Sediment

Samples were dried, ground and sieved with 200mm mesh screen. For each sample, 5.0g of sediment were measured into 150ml flasks. 50ml of 0.1M HCl was added and the flasks were agitated for 30mins at 200 rev/min. The mixture was filtered into 50ml flask and made up to the mark with deioninsed water (Ahmed *et al.*, 2010).

Statistical analysis

Analyses of the variations of concentrations of the heavy metals between the sampling periods (October, 2010 and February, 2011) were carried out using t-test and results were considered statistically significant at $p < 0.05$.

Results and discussion

Table 1 presents the concentrations (µg/g) of the heavy metals in *Clarias anguillaris* from River Okpokwu in the months of October, 2010 and February, 2011. Across the sample locations, the levels of the heavy metals recorded in the fish in October, 2010 showed that As varied between 0.05±0.01 to 0.11±0.03 (µg/g), Mn ranged from 0.08±0.01 to 0.13±0.05 (µg/g), Cu ranged from 0.10±0.03 to 0.18±0.05 (µg/g) while Pb and Fe varied between 0.04±0.02 to 0.06±0.02 (µg/g) and 0.32±0.03 to 0.44±0.10 (µg/g) respectively. In February, 2011, the concentrations of the heavy metals varied between 0.12±0.02 to 0.15±0.04 (µg/g) As, 0.17±0.04 to 0.23±0.07 (µg/g) Mn, 0.17±0.05 to 0.29±0.06 (µg/g) Cu, 0.07±0.03 to 0.10±0.01 (µg/g) Pb and 0.11±0.02 to 0.34±0.08 (µg/g) Fe. T-test analyses of the variations in levels of paired means of these heavy metals (October, 2010 and February, 2011) were mostly statistically significant ($P < 0.05$). The fish contents of the heavy metals recorded in this study were below the documented regulatory permissibe limits of 0.03mg/g Cu, 0.10mg/g Fe, 0.02mg/g Pb and 0.01mg/g Mn respectively (WHO, 2006).

The concentrations of the heavy metals in the river water (Table 2) showed results of 0.06±0.02 to 0.09±0.04µg/L As, 0.34±0.08 to 0.41±0.04µg/L Mn, 0.52±0.07 to 0.74±0.02µg/L Cu, 0.13±0.02 to 0.31±0.02µg/L Pb, and 1.03±0.05 to 1.24±0.05µg/L Fe in October, 2010 while similar results in February, 2011 revealed 0.04±0.01 to 0.05±0.01µg/L As, 0.20±0.03 to 0.30±0.06µg/L Mn, 0.27±0.06 to 0.36±0.04µg/L Cu, 0.08±0.03 to 0.11±0.03µg/L Pb and 0.24±0.04 to 0.59±0.02µg/L Fe. The levels of these heavy metals recorded in the water were below the regulatory permissible limits of 0.05mg/L As, 1.0mg/L Cu, 0.01mg/L Pb, 0.30mg/L Fe and 0.10mg/L Mn (WHO, 2006).

Table 1. Concentrations (µg/g) of the Heavy Metals in *Clarias anguillaris* (mudfish) from River Okpokwu (October, 2010 and February, 2011).

Heavy metals	Auke		Odejo		Ocholonya		WHO RL
	Oct. 2010	Feb. 2011	Oct. 2010	Feb. 2011	Oct. 2010	Feb. 2011	(mg/g)
As	0.05^{a}±0.01	0.11^{b}±0.03	0.09^{a}±0.03	0.13^{a}±0.01	0.11^{a}±0.03	0.15^{a}±0.04	-
Mn	0.13^{a}±0.05	0.22^{b}±0.06	0.08^{a}±0.01	0.17^{b}±0.04	0.11^{a}±0.01	0.23^{b}±0.07	0.01
Cu	0.10^{a}±0.03	0.17^{b}±0.05	0.18^{a}±0.05	0.21^{a}±0.02	0.16^{a}±0.03	0.29^{b}±0.06	0.03
Pb	0.05^{a}±0.01	0.07^{a}±0.04	0.06^{a}±0.02	0.08^{a}±0.03	0.04^{a}±0.02	0.09^{a}±0.01	0.02
Fe	0.44^{a}±0.10	0.34^{b}±0.08	0.35^{a}±0.09	0.15^{b}±0.01	0.32^{a}±0.03	0.11^{b}±0.02	0.10

T-test of paired mean (October, 2010 and February, 2011) with different alphabets are statistically significant ($P<0.05$). Data presented are mean±SD of replicate analyses (n=3). SD = standard deviation. RL = Regulatory Limit

The results of concentrations of the heavy metals in sediment (Table 3) showed record ranging from 0.36±0.04 to 0.61±0.02µg/g As, 0.76±0.03 to 0.78±0.03µg/g Mn, 0.73±0.02 to 1.01±0.07µg/g Cu, 0.50±0.06 to 0.85±0.05µg/g Pb and 2.23±0.15 to 3.79±0.10µg/g Fe in October, 2010 but varied between 0.41±0.06 to 0.47±0.03µg/g As, 0.50±0.04 to 0.74±0.06µg/g Mn, 0.62±0.05 to 0.81±0.02µg/g Cu, 0.50±0.06 to 0.85±0.05µg/g Pb and 0.52±0.04 to 0.92±0.03µg/g Fe in February, 2011. The results of t-test analyses of the variations in concentrations of paired means (October, 2010 and February, 2011) of the heavy metals in both the river and sediment were also statistically significant ($P<0.05$). This may be due to the differential rates of imput and sedimentation of the heavy metals in water and sediment over the sampling periods. The sediment levels of the heavy metals obtained in this study were below the reported WHO's regulatory limits of As (0.01mk/kg) Mn (0.5mg/kg) Fe (0.30mg/kg) (Butu and Iguisi, 2013) and the Lowest Effect Levels (LELs) of Pb (31mg/kg) and Cu (16mg/kg) in sediment quality guidelines established by the Ontario Ministry of Environment and Energy (Uzairu *et al.*, 2009).

Table 2. Concentrations (µg/g) of the Heavy Metals in River Okpokwu's water (October, 2010 and February, 2011).

Heavy metals	Auke		Odejo		Ocholonya		WHO RL
	Oct. 2010	Feb. 2011	Oct. 2010	Feb. 2011	Oct. 2010	Feb. 2011	(mg/L)
As	$0.06^{a}\pm0.02$	$0.04^{b}\pm0.01$	$0.07^{a}\pm0.03$	$0.05^{a}\pm0.01$	$0.09^{a}\pm0.04$	$0.04^{b}\pm0.04$	0.05
Mn	$0.34^{a}\pm0.08$	$0.20^{b}\pm0.03$	$0.41^{a}\pm0.04$	$0.30^{b}\pm0.06$	$0.36^{a}\pm0.05$	$0.23^{b}\pm0.03$	0.01
Cu	$0.54^{a}\pm0.05$	$0.35^{b}\pm0.02$	$0.52^{a}\pm0.07$	$0.36^{b}\pm0.04$	$0.74^{a}\pm0.02$	$0.27^{b}\pm0.06$	1.00
Pb	$0.31^{a}\pm0.02$	$0.11^{b}\pm0.03$	$0.13^{a}\pm0.02$	$0.08^{b}\pm0.03$	$0.19^{a}\pm0.03$	$0.12^{b}\pm0.01$	0.30
Fe	$1.06^{a}\pm0.05$	$0.24^{b}\pm0.04$	$1.03^{a}\pm0.05$	$0.58^{b}\pm0.03$	$1.24^{a}\pm0.05$	$0.59^{b}\pm0.02$	0.10

T-test of paired mean (October, 2010 and February, 2011) with different alphabets are statistically significant ($P<0.05$). Data presented are mean±SD of replicate analyses (n=3). SD = standard deviation. RL = Regulatory Limit

Figure 1 presents the overall mean concentrations (µg/g) of the trace metals in the samples of *Clarias anguillaris*, water and sediment from river Okpokwu during the study period. Samples collected in October, 2010 recorded significantly higher levels of the trace metals than the same samples collected from the same locations (Auke, Odejo and Ocholonya) in February, 2011. Usually, October marked the peak of raining season in the study area during which the river overflows its bank and receives maximum effluents from drainages through farms, quarry, waste dumps and cottage industries situated in communities along the river. High levels of agrochemical wastes: chemical fertilizers, herbicides and pesticides from farm lands as well as fuels/oils and other domestic waste materials being washed in surface runoff into the river contstitute important sources of heavy metals comtamination of surface water bodies. Karanja *et al.* (2010), reported that water and soil pollutions from heavy metals in urban areas particularly those in the developing regions are as a result of poor disposal of industrial and urban wastes. Similar effects are also expected in a highly populated rural communities, particularly in the areas of this study where there are high incidences of intensive agriculture involving indiscrimate usage of agrochemicals, indiscriminate disposal of domestic and industrial effluents among other anthropogenic activities within the vicinity of water bodies. In Southwest England, the waters and sediments of several rivers and estuaries have been found to contain high amount of heavy metals, largely due to mining activities in the region (Aston and Thornton, 1977). Thus, the quarry activity at the source of river Okpokwu may constitute another significant point of entry of the heavy metals in the water.

The correlation analyses of the mean concentrations of the heavy metals in *Clarias anguillaris*, water and sediment samples (Table 4) showed strong positive correlation. This suggests that trace metal contaminations of fish and sediment reflects the receiving load of metal contaminants by the river. Apart from anthropogenic contaminations, trace metals may be recycled via chemical and biological processes, within the sedimentary compartment and back to the water column (Tessier and Campbell, 1987). Harikumar and Jisha (2010), reported that trace metal contaminations of sediment could affect water quality and the bio-assimilation and bio-accumulation of metals in aquatic organisms, resulting in potential long-term implications on human health and ecosystem.

Table 3. Concentrations (µg/g) of the Heavy Metals in River Okpokwu's Sediment (October, 2010 and February, 2011).

Heavy metals	Auke		Odejo		Ocholonya		WHO RL (mg/kg)
	Oct. 2010	Feb. 2011	Oct. 2010	Feb. 2011	Oct. 2010	Feb. 2011	
As	$0.61^a±0.02$	$0.44^b±0.05$	$0.55^a±0.02$	$0.41^a±0.06$	$0.36^a±0.04$	$0.47^b±0.03$	0.01
Mn	$0.76^a±0.03$	$0.50^b±0.04$	$0.77^a±0.04$	$0.51^b±0.03$	$0.78^a±0.03$	$0.74^a±0.06$	0.5
Cu	$1.01^a±0.07$	$0.81^b±0.02$	$0.73^a±0.02$	$0.62^a±0.05$	$0.97^a±0.05$	$0.70^b±0.05$	16**
Pb	$0.85^a±0.05$	$0.57^a±0.03$	$0.50^a±0.06$	$0.43^a±0.06$	$0.55^a±0.02$	$0.51^a±0.06$	31**
Fe	$2.23^a±0.15$	$0.92^b±0.03$	$3.79^a±0.10$	$0.52^b±0.04$	$3.58^a±0.03$	$0.80^b±0.03$	0.30

T-test of paired mean (October, 2010 and February, 2011) with different alphabets are statistically significant (P<0.05). Data presented are mean±SD of replicate analyses (n=3). SD = standard deviation. RL = Regulatory Limit, ** = LELs (Lowest Effect Levels in provincial)

Table 4. Correlation coefficients between the mean concentrations of the heavy metals in the samples.

		As	Mn	Cu	Pb	Fe
Sediment and Clarias anguillaris	As	1.0000				
	Mn	0.9999	1.0000			
	Cu	0.9999	1.0000	1.0000		
	Pb	0.9939	0.9932	0.9934	1.0000	
	Fe	0.9963	0.9958	0.9958	0.9997	1.0000
		As	Mn	Cu	Pb	Fe
Sediment and Water	As	1.0000				
	Mn	0.9390	1.0000			
	Cu	0.8880	0.9920	1.0000		
	Pb	0.9863	0.9829	0.9518	1.0000	
	Fe	0.9579	0.9982	0.9826	0.9922	1.0000
		As	Mn	Cu	Pb	Fe
Water and Clarias anguillaris	As	1.0000				
	Mn	0.6700	1.0000			
	Cu	0.5792	0.9932	1.0000		
	Pb	0.6074	0.9967	0.9994	1.0000	
	Fe	0.5468	0.9879	0.9992	0.9972	1.0000

Figures 2 presents the bio-accumulation factors of the trace metals in *Clarias anguillaris* from the river and sediment. The results showed elevated bio-accumulations of trace metals in February, 2011 than October, 2010. Among the inorganic contaminants, heavy metals consideration are important due to their non-degradable nature leading to bioaccumulation through tropic level which may have deleterious biological effects (Kar *et al.*, 2007). In similar studies, Atta *et al.*, (1999) and Adeniyi *et al.*, (2005) reported that sediment constituted the major repository of heavy metals in aquatic systems and a large reservoir of heavy metals in the sediments can act as significant source to the overlying water column after their input to the ecosystem has ceased (Officer *et al.*, 1989). Thus, sediments may play a considerable role in the remobilization of trace metals from bottom sediments into the water body under suitable conditions and consequently, fish ingestions and bio-accumulations. The release of heavy metals from sediments may not only result from re-suspension of particulate matters, but also through the activities of micro-organisms within the sediments and at sediment-water interface, resulting in bio-transformation to more volatile or soluble forms (Gilmour *et al.*, 1985). *Clarias anguillaris* showed significantly high potential for bio-accumulating arsenic from the river water in this study; although, it has been observed that fish and shellfish mainly accumulate the less toxic organic arsenic from their environment (WHO, 2011). However, acute high level exposure to arsenic has been linked to vomiting, diarrhoea and liver damage while chronic exposure was linked to skin disease, hypertension, some form of diabetes and cancer (Centeno *et al.*, 2005).

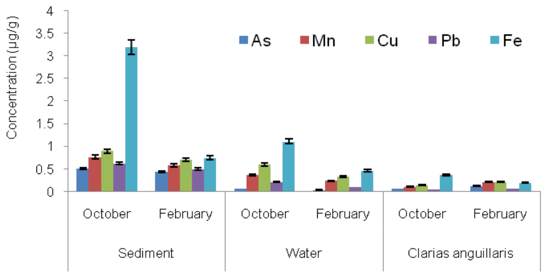

Fig. 1. Overall mean Concentrations (ug/g) of the heavy metals in the samples.

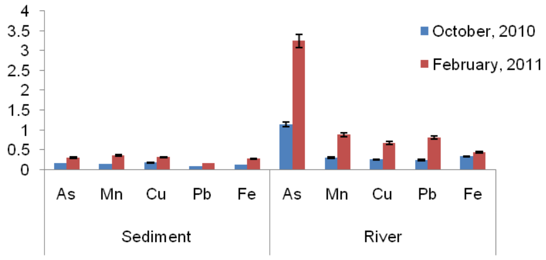

Fig. 2. Bio-accumulations of the trace metals in *Clarias anguillaris* from sediment and water of river Okpokwu.

Conclusion

The results of this study revealed variable concentrations of As, Mn, Pb, Cu and Fe in *Clarias anguillaris* (mudfish), water and sediment samples obtained from river Okpokwu in October, 2010 and February, 2011. The water recorded higher levels of the trace metals in October which may be due to the high surface runoff of effluents from agricultural lands, quary industries and domestic waste into the river. The fish contents of the trace metals were higher in February, probably due to the effect of bioaccumulation over the periods of its habitation and feeding in the water and sediment environment. However, the levels of the trace metals recorded in this study were below the WHO's permissible limits. Accordingly, the water and *Clarias anguillaris* obtained from river Okpokwu have not become contaminated by the trace metals studied. However, periodic review should be sustained to monitor possible accumulations of the trace metals in future due to the proliferation of anthropogenic activities in the study area.

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