



Effects of Heavy Metals on Benthic Invertebrate Abundance in Owena River

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Abstract

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Heavy metals are pollutants of ecological concern in the aquatic ecosystem. Macrofauna plays significant roles as bio-indicators in the aquatic ecosystems as well as in food sources for fish. This study examined the presence of heavy metal contaminants in Owena river and if the presence affects the abundance and diversity of benthic invertebrates from the river. The results showed two classes of phylum Mollusca, class Gastropoda (three) and Bivalvia (five) which are dominating class of macrozoobenthos. The Shannon diversity index of Owena River (H) was 1.38 while the Evenness (E) showed 0.66. The concentrations of heavy metals investigated in the river were within World Health Organization's (WHO) limits. However, in the sediments the concentrations of Fe (19.37 ± 0.355 - 26.00 ± 0.006 mg/l), Cd (0.13 ± 0.000 - 0.20 ± 0.00 mg/l), Zn (1.13 ± 0.006 - 1.35 ± 0.006 mg/l and Cr (0.26 ± 0.017 - 0.35 ± 0.003 mg/l) were seen to be higher than the permissible limits of WHO. The order of pollution in the surface water was as follows Fe > As > Cd > Zn > Pb > Cr while that of the sediments was Fe > Zn > Cr > Cd > As > Pb. Positive correlation was found between *Margaritifera margaritifera* and Pb, while *Lymnaea stagnalis* showed significantly positive correlation with Zn, Cd and As. The occurrence and absence of some benthic macroinvertebrates in Owena River suggests the effect of pollution from anthropogenic activities. Therefore, a good management program is necessary so as to conserve the significant biota present in the river and prevent pollution for domestic purposes.

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INTRODUCTION

One of the most important factors of the natural ecosystems is rivers. Rivers are frequently seen as the eco-friendly canal of waste and energy flow between the terrestrial and aquatic ecosystems (Islam et al., 2015). Rivers serve as homes to various organisms including benthic macroinvertebrates and play different ecological roles in nutrient recycling and the food web. In addition, rivers provide some unique functions in supporting domestic activities, irrigation, food production, and transportation

(George et al. 2010). It has been argued that human activities pollute the aquatic environment more and such cause increase in microplastics and heavy metals which may in turn affect water, sediments, and aquatic faunas and floras (Ali et al. 2016). Benthic macroinvertebrates are organisms that live at the bottom of the water column and are visible to the naked eye. Examples are crustaceans, molluscs, polychaetes, and arthropods. They live in all types of freshwater substrates from soft mud to rocky bottoms. However, they have well-known sensitivity and quick responses to environmental degradation.

They are therefore used as biological indicators (Varsh, 2013).

Increase in the concentration of heavy metals pollute water and sediments depend on the nature of the activities in the aquatic ecosystem (Sharma and Chatterjee 2017). Heavy metals are of pressing concern in terms of their pollution strength in the aquatic ecosystems. This is because of their persistence, environmental toxicity, bioaccumulation, etc. (Zhang *et al* 2014; Redwan and Elhaddad, 2017; Jordanova *et al*; 2018). As an important component in riverine ecosystems, sediment serves as both a sink and a source of heavy metals (Pejman *et al*, 2015; Huang *et al*, 2019). Most heavy metals are deposited into the sediment after entering the freshwater environment, and are much more concentrated in the sediment than in the water systems (Shyleshchandran *et al.*, 2018; Liu *et al.*, 2018). Akindele and Olutona (2014) observed that sediments that usually moved by fluid flow, for example, regularly settle on the waterbodies when the water swiftness is too low to prevent them from interrupting the environment. Thus, heavy metals may be linked to particulate matters and also transformed into benthic sediments. Costa *et al.*, (2018) argued that long-term accumulation of heavy metals in the river could lead to much higher concentrations in sediments than in water samples. Exposure to heavy metal in the river has been shown to have serious effects in sediments and can undergo extremely adjustable physicochemical reactions, for example, adsorption and desorption, oxidation and reduction, and precipitation and dissolution (Şengör *et al.*, 2007).

Previous studies have recognised the extreme toxic and non-biodegradable nature of heavy metals in the global aquatic environment (Fu *et al.*, 2014; Zheng *et al.*, 2015). However, in Nigeria, several studies have been conducted on heavy metal assessment in water, sediments and aquatic organisms (Olayinka-Olagunju *et al.*, 2021; Akindele *et al.*, 2020). Only few studies were found on the evaluation of water quality using benthic macrofauna in Ondo State, Nigeria. In view of this, the aim of the present study is to assess heavy metal in Owena River using diversity of benthic macrofauna as indicator.

MATERIALS AND METHODS

Study Area

The study area is located at Bolorunduro Owena, Ondo state 7°11' 37.02" N, 5° 1'21.53" East. The area is a region with a maximum of 420 meters' elevation.

The total surface area is 16km, the area is highly vegetated and drained by several small rivers which tributaries drain into the Owena River (Figure 1).



Figure 1: Map of Owena River showing sampling area. (Source: Google map)

Sample Collection

Collection of benthic macroinvertebrate organisms

Benthic macroinvertebrates were collected from the bottom sediment with the use of sediment grab in triplicates from the upper (0-5cm), middle (5-10cm) and lower stream (10-20cm) according to the methods described by Wang *et al.*, (2019). These sediments were kept in Ziplock bags using the procedures outline by Olawusi-Peters (2017). The sediments were rinsed and filtered *in situ* through a 1mm x 1mm mesh size metal sieve. The filtrates left in the sieve were poured in a tray and macrobenthos were hand-picked, stored and preserved with 70% ethanol (Liu *et al.*, 2018; Wang *et al.*, 2019) and transported to the Animal and Environmental Biology laboratory for further identification of species.

Heavy metal sample collection

Surface water samples were collected very early in the morning from three different locations in the river [upper stream (UP), mid-stream (MD) and lower stream (LS)]. Samples were collected in triplicate monthly from February to April, 2021 (Olayinka-Olagunju *et al.*, 2021; Ali *et al.*, 2016). A total of nine water samples were collected from the site and

transported to the Animal and Environmental Biology laboratory for analysis. In addition, sediment grab was used to collect sediments samples from three different points in triplicate. A total of nine sediment samples were collected and kept in a Ziplock bag using the procedures outlined by Olayinka-Olagunju et al., (2021) and Huang et al., (2019). Samples were transported to the laboratory for analysis.

Laboratory analysis

Macroinvertebrates

A standard identification chart (Identification guide to freshwater macroinvertebrates) was used to classify the sample into various taxa and species. Diversity index was estimated using Shannon Wiener Diversity Index (Shannon and Wiener, 1963).

$$(H) = -\sum[(pi) * \ln (Pi)]$$

Heavy metals

The sediment samples were air dried in the laboratory, ground and sieved using 2-mm mesh sieve. The water and homogenized sediment samples were digested with 5.0 mL of HNO₃/HCl (3:2) and heated on a hot plate at 130°C (Akindele et al., 2020; Olayinka-Olagunju et al., 2021). Heavy metals were analyzed using atomic absorption spectrophotometer ((N1100A model).

The standard methods outlined by Yi et al., (2011) and AOAC (2005) were used during the heavy metal analysis. The following metals zinc (Zn), lead (Pb), chromium (Cr), arsenic (As), iron (Fe) and cadmium (Cd) were examined in surface water and sediment samples. The instrument was calibrated as follows (0, 2, 4, 6, 8 and 10 mg/L). The results were read from the graph and concentrations were reported in mg/L.

Statistical Analysis

SPSS 24.0 statistical package was used to analysed the mean concentrations of the metals investigated from the UP, MS and LS. One-way analysis of variance (ANOVA) was used to compare the mean scores and significant differences (p < 0.05). In addition, correlation analysis

RESULTS AND DISCUSSIONS

Table 1 represents the composition and Relative Abundance (RA) of macrozoobenthos in Owena river. A total of 139 individuals of phylum Mollusc belonging to class Gastropoda and Bivalvia. were recorded. *Lymnaea stagnalis* had the highest number

of species with RA of 44.60% (Figure 2) followed by *Magaritifera margaritifera* with RA of 32.37%. *Unio crassus* and *Anodonta anotina* had the lowest number of individuals recorded.

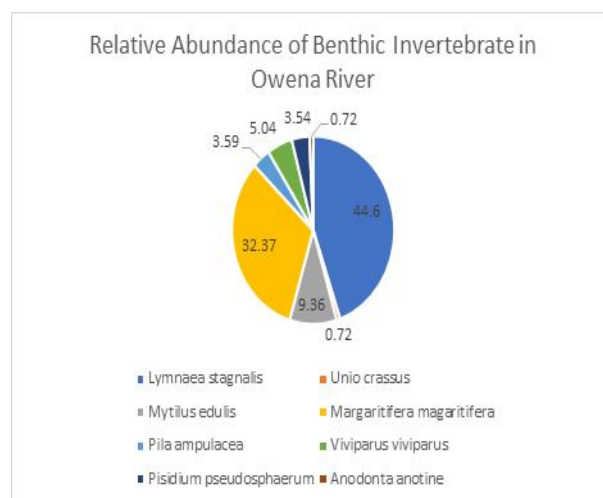


Figure 2: Pie chart showing the Relative Abundance (%) of species in Owena River

Heavy metal concentration in surface water

Table 2 presents the mean concentrations results of the heavy metals investigated in the surface water. Mean concentrations of Fe ranged from 1.100±0.000-1.500±0.000 mg/l. The result suggests that Fe was lower than the regulatory limit of 5.00mg/l (WHO, 2006). There was a significant difference between the Fe mean concentration when compared for the three months. Lead (Pb) was detected in the months of April and the concentration was not higher than the permissible limits of 0.10 mg/l. More so, no significance different was seen in Pb when all concentrations were compared.

Mean concentration of Zn did not exceed the permissible limit of 5.00 mg/l. Zinc further showed significance difference (p< 0.05) between the months. Chromium was only detected in the month February (LS) surface water. The table further showed that Cd ranged from not detected to 0.037±0.000 mg/l. From the data in Table 2, Cd was not detected in all water sample collected but where detected, concentrations were high. For example, in the month of February, Cd was high and within the concentration limits in the month of March while others were above the permissible limit. Significant difference was observed when the mean concentrations were compared. The mean scores for As concentrations (0.01 mg/l-0.050±0.006 mg/l) were found to be above the permissible WHO (2008)

limit of 0.01 mg. No significant difference was observed between all As concentrations.

Heavy metal concentration in Sediments

The mean values of heavy metals observed in the sediments of Owena River and the WHO (2006) permissible limits are showed in Table 3. The results in Table 3 illustrates that Fe ranged from 19.37 ± 0.355 - 26.00 ± 0.006 mg/l which implies that the observed concentrations were higher than the limits of 5 mg/l stipulated by WHO (2008). In addition, Fe was statistically significant ($p < 0.05$) when the concentrations were compared. The mean concentrations of Pb ranged from 0.13 ± 0.000 - 0.19 ± 0.006 mg/l. The results showed that the results were below WHO (2006) limits of 5 mg/l and the results and statistical significance was observed. More so, Zn was found to be higher than the regulatory limits of ≤ 1 mg/l, ranging from 1.13 ± 0.006 - 2.14 ± 0.000 mg/l and also there was a significant difference in the results ($p < 0.005$). The table further showed that Cr ranged from 0.19 ± 0.005 - 0.34 ± 0.006 mg/l implying the results were higher than the permissible limit of 0.01 mg/l. Cadmium and arsenic ranged from 0.13 ± 0.000 - 0.216 ± 0.008 mg/l and 0.026 ± 0.003 - 0.066 ± 0.003 mg/l respectively. The results showed that Cr concentration were higher than the permissible limits while that of As was within the permissible limits (WHO, 2011). The elements were significantly different when the concentrations were compared.

Correlation Analysis

Table 4 presents the results of Pearson correlation coefficient (r) examined amongst the heavy metal concentrations and *Margaritifera margaritifera* (Mm) values from the overall mean of the various study locations. Significant positive correlation was determined between Mm of Pb ($r = 0.919$, $P < 0.01$).

The data presented in Table 5 suggests the results of Pearson correlation coefficient (r) between *Lymnaea stagnalis* (Ls) and heavy metal concentrations values from the overall mean of the study location. Significant positive correlation was determined between Ls of Zn ($r = 0.967$, $p < 0.01$), Ls of Cd ($r = 0.977$, $p < 0.01$) and Ls of As ($r = 0.799$, $p < 0.05$).

Composition and Relative Abundance of macrozoobenthos

Macrozoobenthos is an aquatic organism that lives at the bottom of the waters. Rosa et al. (2014) stated that a macrozoobenthos organism is easily available, has diverse types and sensitive to different types of

pollutants. Macrozoobenthos is also a good indicator to predict an aquatic habitat condition (Kumar and Vyas, 2014). They are the main organisms in sediments, and their community structure have a coupling relationship with the heavy metal pollutants in their surrounding environment (Xinxin et al., 2019). Sediments contaminated with heavy metals may inhibit or promote the growth of macroinvertebrate, in turn, macroinvertebrate community also reflects the qualities of sediment (Yi et al., 2011). Based on this study conducted in Owena River, three species of Gastropoda and five species of Bivalvia which belong to the dominating class of macrozoobenthos were recorded. The Shannon diversity index of Owena River (H) gives 1.38 and the Evenness (E) gives 0.66. This result is consistent with the study conducted by Hidayah (2003), which stated that the type of macrozoobenthos of Classes Gastropoda and Bivalvia were most commonly found in estuary of Donan River. The research conducted at the mouth of Cimandiri River also obtained the results that class Gastropod dominated the area with the highest species composition. The present study revealed that *Lymnaea stagnalis* with the highest individual recorded showed a positive correlation with Zn ($r = 0.967$, $P < 0.01$), Cd ($r = 0.977$, $p < 0.01$) and As ($r = 0.799$, $p < 0.05$). *Margaritifera margaritifera* is also significantly correlated with concentration of Pb ($r = 0.919$, $P < 0.01$). It implies that these species have high tolerance for the heavy metal concentrations examined. In the study conducted by Xinxin (2019), Cr and Pb showed significant positive correlation with the individuals of gastropods. This showed that Gastropod and Bivalvia have high pollution tolerance. This result is in agreement with the findings of Khalil (2016) who stated that Gastropods and Bivalvia are pollution tolerant species. It can also be said that their hard-outer covering may serve as protectant against absorbing pollutant. Sahin (2012) states that Gastropod has a fairly high adaptability and considerably high tolerance to the water condition and different seasons. However, Sharma & Chatterjee (2017) suggested that Gastropoda was found in a few numbers compared to those of the other macrozoobenthos in Sungai Kunda (India). According to Olawusi-Peters (2017) who worked on composition and abundance of macro benthic invertebrates in Owena Reservoir revealed that the macro-benthic invertebrate of Owena reservoir is characterized by low diversity; two phyla (Mollusca and Arthropoda) and five species. The river is further characterized Mollusca as the most diverse and abundant macro- benthic invertebrates. This could be as a result of the quality of the environment and the nature of the sediment. According to Victor and Onomivbori (1996), the quality of an aquatic ecosystem depends on environmental factors, which in turn can influence the structuring of aquatic communities.

Surface water and sediments pollution concentration

Four out of the six heavy metals (Fe, Zn, Cr and Pb) examined in Owena surface water were within the permissible limits of WHO (2006). This result implies that the river was free from high concentrations of these metals. This may be due to the period of sample collection, peak of the dry season and beginning of the wet season resulting to constant flowing of the river (Akindele *et al.*, 2020; Olayinka-Olagunju *et al.*, 2021). However, Cd and As were seen to be higher in concentration than the regulatory limits recommended (WHO, 2006).

The presence of high concentration of Cd, Cr, Fe and As in both surface water and sediments from this present study might be linked to serious anthropogenic activities such as the release of agricultural activities, flooding, domestic waste and industrial activities (Hazarika *et al.*, 2017). The concentrations of heavy metals in sediments from this study were observed to be more polluted than the surface water. This may be because when the river receives contaminants, they settle at the bottom of the sediments causing sediments pollution (Singh *et al.*, 2004).

Cadmium can occur through natural and anthropogenic activities. Naturally, Cd can be found in zinc, lead, copper ores and from volcanic eruption, other activities include coal and other fossil fuels burning. Deposits from these activities may therefore affect ground and surface waters (WQA, 2022). Studies on aquatic sediments suggest that Cd is a good indicator for water and sediment pollution due to its accumulative nature (Rzetala, 2016). High concentrations of Cd in water may be very toxic and can be injurious to organs and tissues (e.g., kidney, liver, bone and blood damage) in aquatic organisms and human (Arao *et al.*, 2009). Furthermore, Cd may have a cytotoxic effect on blood mononuclear cell which are independent of the induction of apoptosis (programmed cell death).

Arsenic is abundant in nature and is usually found in drinking water. Previous studies observed that when the arsenic concentrations in water exceeds the maximum contaminant level, it can result to serious public health issues. Furthermore, study by OEHHA (2004), suggests that consumption of arsenic can lead to lung and urinary bladder cancer risk. On the other

hand, exposure to high concentration of As may also cause vascular or skin effects.

The concentrations of Fe in water were seen to be within WHO limits however, in the sediments they were higher. Iron according to some previous studies, is an important metal needed by fish for blood production (Ali and Khan 2019). Iron is usually found in soils and rocks that contain minerals. Although, the presence of Fe in water does not pose any health risk, however, high concentrations of iron may increase the growth of some harmful organisms and particularly harmful bacteria. In addition, excess concentrations of Fe may affect the benthic aquatic organisms by resulting to oxidative stress in the fish (Igharo *et al.* 2014). High concentrations of iron in the study area may be because Nigeria's parent soils (Irmak *et al.* 2008; Olayinka-Olagunju *et al.* 2019; Olayinka-Olagunju *et al.* 2021) contain high concentration of iron. Findings from this study agrees with the study of Olatunji-Ojo *et al.* (2019) who described high concentrations of Fe in some rivers in Ilaje LGA, Nigeria as well as Olayinka-Olagunju *et al.* 2021 that observed high iron concentrations in Ogbese river, Ondo State.

Zinc was another element that exceeded the WHO permissible limits in the sediments. The metal Zn occurs naturally in the air, soil and water. However, Zn enters the environment through some industrial activities such as mining, coal burning, waste combustion and steel processing (Lenntech, 2022). Zinc is a trace element, essential to plants, humans and animals but may be dangerous at high concentration. More so, plants cannot cope with zinc at high concentrations. Vardi and Chenji (2020) are of the opinion that zinc does not biomagnify in aquatic organisms because it is majorly from water and sediments but not in what they consume. Ana *et al.* (2011) suggest that the bioavailability of dissolved Zn depends on environmental parameters like pH, hardness, and dissolved oxygen. Zinc concentration obtained from this present study is similar to Olayinka-Olagunju *et al.*, 2021 who reported high concentration of Zn in Ogbese river in Ondo State Nigeria.

The concentration of Cr was higher in sediments than in surface water from this study. Chromium, is used as metal alloys and pigments for paints, cement, paper, rubber, and other materials. Although the values of Cr in water and air is usually, low but it may be excess in the environment as a result of

mining from chromite ore (Lenntech, 2022). Bioaccumulation of Cr in aquatic life is hazardous and may contribute to the danger of consuming aquatic organisms. Consumption of aquatic organisms with high concentrations of Cr by human may result to the disruption of the circulatory and respiratory system, nerve tissues, kidney and liver damage are some of the health effects of Cr. More so, exposure of the skin to Cr even at a low concentration may result to skin irritation and cause ulceration. This result is similar to the results of Olayinka-Olagunju et al., (2021).

sediments is as follows; Fe>Zn> Cr > Cd > As>Pb. This imply that Fe was the dominant element in the sediments and surface water while the least in water was Cr and that of sediments was Pb. *Margaritifera margaritifera* (Mm) showed positive correlation with Pb, while *Lymnaea stagnalis* showed significantly positive correlation with Zn, Cd and As. These results implied that *M. margaritifera* showed a similar relationship with only Pb while *L. stagnalis* showed significant relationship with Zn, Cd and As. The finding is similar to the results obtained from the studies of Wang et al., (2019).

The order of pollution in the surface water is as follows; Fe > As > Cd > Zn>Pb > Cr while that of the

Table 1: Composition and Relative Abundance (%) of Benthic invertebrate in Owena River

PHYLUM	CLASS	SPECIES	FEB	MARCH	APRIL	TOTAL	RA (%)
<i>Mollusca</i>	Gastropoda	<i>Lymnaea stagnalis</i>	34	13	15	62	44.60
	Bivalvia	<i>Unio crassus</i>	1	0	0	1	0.72
	Bivalvia	<i>Mytilus edulis</i>	13	0	0	13	9.35
	Bivalvia	<i>Margaritifera margaritifera</i>	13	11	21	45	32.37
	Gastropoda	<i>Pila ampulacea</i>	1	1	3	5	3.59
	Gastropoda	<i>Viviparus viviparus</i>	0	6	1	7	5.04
	Bivalvia	<i>Pisidium pseudosphaerum</i>	0	5	0	5	3.59
	Bivalvia	<i>Anodonta anatine</i>	0	0	1	1	0.72
	Total			62	36	41	139

Table 2: Heavy metal concentration in Owena River

Heavy metals	Iron (Fe)	Lead (Pb)	Zinc (Zn)	Chromium (Cr)	Cadmium (Cd)	Arsenic (As)
Months						
February US	1.100±0.006	ND	0.017±0.003	ND	0.020±0.000	0.027±0.003
February MS	1.120±0.000	ND	0.010±0.000	ND	0.013±0.003	0.050±0.006
February LS	1.490±0.040	ND	0.053±0.009	0.010±0.000	0.037±0.003	0.060±0.000
March US	1.100±0.000	ND	0.030±0.000	ND	ND	0.010±0.001
March MS	1.497±0.136	ND	0.030±0.000	ND	ND	0.020±0.000
March LS	1.500±0.000	ND	0.010±0.006	ND	0.020±0.000	0.040±0.000
April US	1.143±0.009	0.020±0.000	0.080±0.060	ND	0.010±0.000	0.020±0.000
April MS	1.207±0.003	0.030±0.006	0.041±0.000	ND	0.010±0.000	0.050±0.005
April LS	1.410±0.017	0.060±0.000	0.043±0.009	ND	0.030±0.000	0.020±0.000
WHO limits	5.00 0mg/l	0.1mg/l	5mg/l	0.05 mg/l	0.01mg/l	0.01mg/l

Note: ND - Not Detected; US - Upper Stream; MS – Middle Stream; LS – Lower Stream

Table 3: Heavy metal concentration in sediments of Owena River

Heavy metals	Iron (Fe)	Lead (Pb)	Zinc (Zn)	Chromium (Cr)	Cadmium (Cd)	Arsenic (As)
Months						
February US	20.21±0.017	0.15±0.006	1.16±0.000	0.31±0.000	0.15±0.009	0.05±0.000
February MS	19.37±0.355	0.13±0.000	1.13±0.006	0.31±0.003	0.13±0.000	0.04±0.000
February LS	21.06±0.000	0.19±0.006	1.41±0.009	0.34±0.006	0.16±0.006	0.08±0.003
March US	21.06±0.006	0.14±0.006	1.35±0.006	0.27±0.003	0.19±0.006	0.05±0.000
March MS	20.30±0.012	0.14±0.000	1.40±0.012	0.26±0.017	0.14±0.000	0.03±0.000
March LS	23.17±1.00	0.18±0.003	1.44±0.012	0.35±0.003	0.21±0.009	0.06±0.003
April US	23.09±0.033	0.17±0.006	1.21±0.006	0.26±0.017	0.14±0.003	0.60±0.060
April MS	21.59±0.009	0.14±0.006	1.21±0.026	0.28±0.000	0.16±0.012	0.06±0.000
April LS	26.00±0.006	0.18±0.003	1.23±0.009	0.31±0.006	0.20±0.00	0.08±0.006
WHO	5.00 0mg/l	5.00mg/l	≤1mg/l	0.1 mg/l	0.1mg/l	0.5-1.5mg/l

Note: ND - Not Detected; US - Upper Stream; MS – Middle Stream; LS – Lower Stream

Table 4: Correlation Coefficient values for *M. margaritifera* of heavy metal concentrations

Metals		Iron	Lead	Zinc	chromium	cadmium	Arsenic	<i>M. margaritifera</i>
Iron	Pearson Correlation	1	-.448	-.505	. ^a	-.491	-.503	-.482
	Sig. (2-tailed)		.227	.165	.	.179	.167	.189
Lead	Pearson Correlation		1	-.438	. ^a	-.439	-.130	.919**
	Sig. (2-tailed)			.238	.	.237	.739	.000
Zinc	Pearson Correlation			1	. ^a	.992**	.656	-.268
	Sig. (2-tailed)				.	.000	.055	.485
Chromium	Pearson Correlation				. ^a	. ^a	. ^a	. ^a
	Sig. (2-tailed)					.	.	.
Cadmium	Pearson Correlation					1	.722*	-.254
	Sig. (2-tailed)						.028	.509
Arsenic	Pearson Correlation						1	.143
	Sig. (2-tailed)							.713
<i>Margaritifera margaritifera</i>	Pearson Correlation							1
	Sig. (2-tailed)							

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

CONCLUSION

The study revealed that six heavy metals zinc (Zn), lead (Pb), chromium (Cr), arsenic (As), iron (Fe) and cadmium (Cd) were present in the water and sediment of Owena River. They can either promote or inhibit the growth of macrobenthos which have direct contact with the sediment and water. Therefore, regular assessment of heavy metals in aquatic environment should be carried out in order to monitor the level of changes in its water quality and biodiversity.

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