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Research Paper

Evaluation and Correlation Analysis of Heavy Metals Concentration in Gills of Benthic Organisms of The Great Kwa River Calabar, Nigeria

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ABSTRACT
The evaluation and correlation analysis of heavy metals concentration in gills of benthic organisms in Great Kwa was carried out bi-monthly (Twice monthly) from February to August 2018. The river is located in Calabar, Cross River State Nigeria around longitude 8° 20'E and Latitude 4° 45'N. Its position makes it prone to pollution as heavy rains washes human and industrial waste into the water body which thus, informed the choice of this work. Stations were demarked and samples (both water, sediment and benthic organisms) were collected at each stations throughout the period of study. APHA, 1998 and AOAC, 1995 protocol were used to analyze water, sediment and benthic organism while PAST software version 4.1 was used to obtain diversity index values for both phytoplankton, zooplankton and benthic organisms analysis. Three (3) indices were applied to estimate the diversity of the phytoplankton, zooplankton and benthic organisms' species. The result ofcorrelation coefficient between physicochemical parameters, phytoplankton and zooplankton showed negative correlation. A total of 163 benthic macroinvertebrates, comprising of 15 species spread across 2 taxonomic divisions (Decapoda and Gorbbiforme) were obtained. Decapoda made up 90% of the benthic population with Bittiumreticulatum, Terebiagranifera and Cardisamaarmatum (67, 25 and 15 counts respectively). Analysis on bioaccumulation of heavy metal showed gills of mudskipper (Periophthalmuskoelreutere) in sample point 1 among the benthic organism sampled to have high lead concentration. All benthic organisms encountered were somewhat pollution tolerant and pollution tolerant species. This suggests that the river could be under pollution stress as moderate benthic diversity indices were revealed. It is therefore recommended that laws are enforced to control the disposal of effluent and other waste products.

KEYWORDS*: heavy metals, Benthic organisms, Correlation, Great Kwa*

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I. INTRODUCTION

Water plays an important role in the maintenance of life. It is an essential resource for a healthy population both for crops and livestock as it is used for different purposes to include drinking, powering hydroelectric plants, transportation, infrastructural purpose, tourism, recreation and other human and economic activities [1]. Water pollution directly and indirectly results from human activity which leads to deleterious effects [2]. Water quality has become a matter of concern to the global communities as growth in population, advances in industrialization, agriculture and changes in the climate alternate the hydrological cycle has led to increased environmental risk to humans and other life forms when used. These has aroused a worldwide concern placing water control as a top priority in policy agenda [3]. Reference [4] stated that the suitability and quality of water for use is determined by its taste, odour, color and concentration of both organic and inorganic chemicals disposed inside water bodies. Water quality evaluation is therefore important as it imposes the need to transfer data and information to the public on how to take advantage and conserve these natural resources with the objective of obtaining sustainability [5]. The use of macroinvertebrate diversity has proved to be cost effective in the ecological studies of water quality [6] because it gives the actual state of the water compared to the measurement of the physical and chemical properties.

The differences in sensitivity of the microbes, bacteria, plant, animals could serve a great deal for timely assessment and prediction of environmental pollution [7]. Pollutants have the capacity to bio accumulate and bio magnify and this impacts great stress to aquatic lives [8]. Heavy metals are metals having densities that are five times higher than water [9]. Heavy metals are metallic element with relatively high density. These metals could result from natural or anthropogenic source [10]. Drinking water, food and air are major routes of heavy metal uptake, though required by the body in trace quantity for body metabolism at high concentration can lead to poisoning. Reference [11] reported that heavy metals like Chromium (Cr), Cadmium (Cd), Silver (Ag), Lead (Pb), and Zinc (Zn) are associated with being poisonous at high concentration. A study by [12] has shown that fishes, when exposed to sub lethal toxicants, exhibit different behavioral changes to the toxin.

Bio-indicators are those organisms which give an insight on the conditions of the environment where they are found [13], as their presence or absence tell about the cumulative effects of pollutants in the ecosystem and give a view on how long such pollutant may have persisted which may not be detected when physical or chemical parameter are used [14]. A good bio-indicator is therefore a representative of the study area, have narrow specific tolerance [15], and thus have a simple correlation between the contaminant concentration in their tissue and the contaminant concentration found in the environment [16]. Benthic organisms are organisms that attach themselves or live on the sea flow, their diversity is largely controlled by temperature, current and energy from wave. They form an important and significant part of the aquatic food chain. They serve as food source for sea birds, bottom feeding fish and marine mammals [17].

II. MATERIALS AND METHODS

2.1 Study site

Samples were collected fromThe Great Kwa River which source from the Oban Hills in the Cross River National park and flows southward to the Cross River Estuary [18], with broad mud flat at its lower reaches which drains the east coast of the Calabar municipality and consist of semi diurnal tides [19]. It has an estimated length of 56km and 2.8 km at the point where it empties into the Cross River Estuary. Figure 1 below shows the map of the study area.

2.2 Sample collection

Samples were collected from two stations of the Great kwa river; Esuk-Atu (sample point 1) with coordinate of latitude 4.94997N and longitude 8.35834E and Obufa- Esuk (sample point 2) with latitude 4.94192N and longitude 8.35499E between the hours of 8:00am and 12:00 noon on the days of sampling.This was carried out bi-monthly (Twice monthly) from February to August 2018.

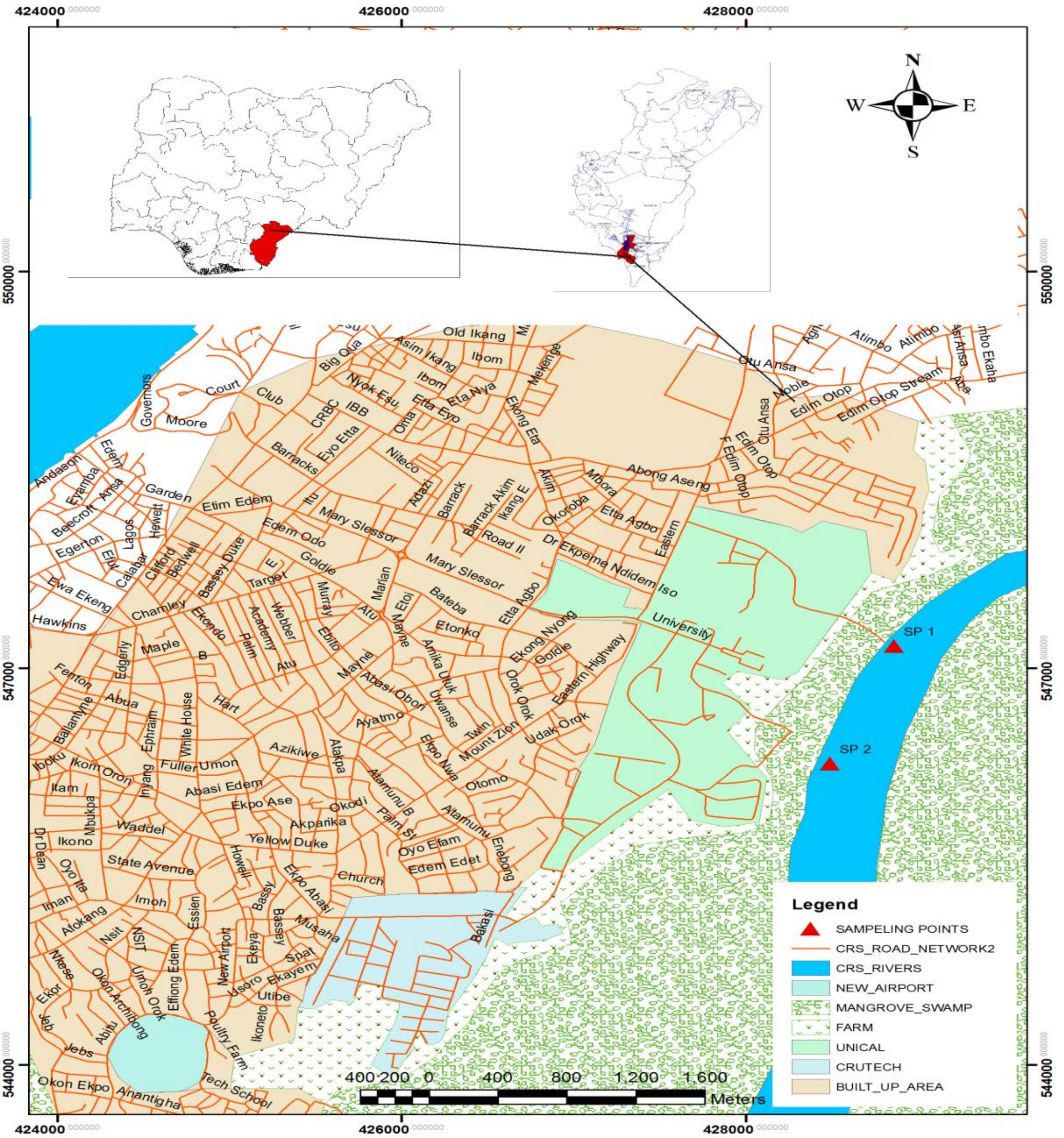

FIG. 1: Map of study area

Source: Nigerian Geological Survey Agency Calabar, Cross River.

2.3 Plankton analysis

Standard plankton net of 55 microns were used to collect samples by both vertical and horizontal towing. The filtrate was concentrated to 100ml using 4% formalin added to it to preserve the organism with few drops of lugol's iodine solution. All samples were properly stored in a cooler and conveyed to the Laboratory for identification and analyses. A homogenate of the sample fixed with 4% formalin and lughol's solution was put in a 1ml counting chamber (Ajah Model 001) and allowed to settle after covering it with a glass slide. Examination was at x100 magnification using the x10 lens. They were identified to species level using the following taxonomic keys [20] and [21]

2.4 Biochemical analysis

A van veen grab was used to collect benthic samples from each sampling stations of the study area. Using the grab three hauls were made and sediment collected. They were sieved using a sieve of 0.85mm mesh size. Thereafter they were emptied into a well labelled container, preserved with 4% formalin and then transported to the laboratory for identification. Forceps was used to sort them out into different groups and identified using reference [22] identification guide under a stereoscope. Macro invertebrates samples collected were sent to BGI Resource Laboratory Port Harcourt. A gram each of gill was collected from each sample, it was dried for 8hours using reference [23] protocol. Afterwards 0.3g of each dried sample was placed into a labelled test-tube and 5ml of a mixture of chloroform, methanol and water in the ratio of 2:1:0.5 were added. It was allowed to stand overnight. De-ionized water was used to wash before the addition of 5ml of nitric acid to each tube before sample digestion. 20ml of de-ionized water was also used to give a final concentration of 20%. Digested sample was thereafter analysed for levels of Cd, Zn, Pb, Cr, As, using absorption spectroscopy (Model-analyst 200 Perkin Elmer).

2.5 Statistical analysis

PAST software version 4.1 was used to obtain diversity index values for both phytoplankton, zooplankton and benthic organisms analysis. Three (3) indices were applied to estimate the diversity of the phytoplankton, zooplankton and benthic organism species in the samples analyzed. The indices employed were Margalef index (d) [24] and Shannon-weiner index (HI) [25].

III. RESULTS

3.1 Heavy metal concentration in gills of invertebrates obtained across spatial gradients

Analysis on the concentration of heavy metals in the gills of invertebrates (mudskipper, crab, periwinkle and shrimp) obtained across temporal gradients was conducted. The heavy metals analyzed were zinc, lead, chromium, cadmium and arsenic. Table 1 presents the results of heavy metals accumulation in benthic organism obtained in Sample point I.

TABLE 1
Mean concentration of heavy metals in gills of benthic organism obtained from sample point 1

	Zinc	Lead	Chromium	Cadmium	Arsenic
Mudskipper	0.100 ± 0.010^{a}	0.057 ± 0.006^{a}	0.327 ± 0.038^{b}	0.750 ± 0.056^{c}	ND
Crab	0.076 ± 0.002^{b}	0.026 ± 0.003^{b}	1.060 ± 0.020^{a}	4.100 ± 0.945^{a}	ND
Periwinkle	0.064 ± 0.004^{b}	0.023 ± 0.006^{b}	0.257 ± 0.015^{c}	2.487 ± 0.455^{b}	ND
Shrimp	0.030 ± 0.002^{c}	0.008 ± 0.001^{c}	1.020 ± 0.020^{a}	0.750 ± 0.154^{c}	ND

*Means with the same superscript are not significant different at $P>0.05$ and those with different superscripts are significantly different at $P<0.05$. * ND=Not detected

As could be seen in Table 1, statistical significant difference was observed in the levels of all the heavy metals analyzed in the gills of the benthic organism species obtained from Sample point I. Furthermore, the crab and periwinkle significantly accumulated more amounts of zinc and cadmium in their gills compared to other benthic organism with a mean concentration of 0.076±0.002and 0.064±0.004, 4.100±0.945 and 2.487±0.455 for both heavy metal and benthic organism species respectively. Also, the crab and shrimp significantly accumulated more amounts of chromium in their gills compared to other invertebrates with a mean concentration of 1.060±0.020 and 1.020±0.020 respectively. On the other hand, the mudskipper significantly accumulated more amount of lead compared to other benthic organisms with mean concentration of 0.057±0.006.

As could be seen in Table 2, there was no statistical significant difference in the level of lead analyzed in the gills of the benthic organisms obtained from Sample point II. However, statistical significant difference is observed in levels of Zinc, Chromium and cadmium. The crab and periwinkle accumulated significantly more zinc, chromium and cadmium in their gills compared to other invertebrates with a mean concentration of 0.067±0.015and 0.053±0.004 for zinc respectively, 0.610±0.075 and 0.353±0.035 for chromium while 4.507±0.075 and 2.160 ± 0.111^{b} of cadmiumwas recorded for both species respectively.

TABLE 2
Mean concentrations of heavy metals in gills of benthic organism obtained from Sample point II

	Zinc	Lead	Chromium	Cadmium	Arsenic
Mudskipper	0.006 ± 0.001^{c}	0.017 ± 0.003^{a}	0.140 ± 0.020^{c}	0.650 ± 0.141^{c}	ND
Crab	0.067 ± 0.015^{a}	0.038 ± 0.004^{a}	0.610 ± 0.075^{a}	4.507 ± 0.075^{a}	ND
Periwinkle	0.053 ± 0.004^{a}	0.021 ± 0.003^{a}	0.353 ± 0.035^{b}	2.160 ± 0.111^{b}	ND
Shrimp	0.030 ± 0.010^{b}	0.037 ± 0.015^{a}	0.120 ± 0.035^{c}	0.467 ± 0.045^{c}	ND

*Means with the same superscript are not significant different at P>0.05 and those with different superscripts are significantly different at P<0.05. * ND=Not detected

3.2 Correlation between concentrations of heavy metals in benthic organism's gills And in surface water across all spatial gradients.

Correlation analysis was conducted to ascertain the relationship between levels of heavy metals accumulated in the gills of the invertebrate analyzed and concentrations present in the surface water sampled. The result from the Pearson's correlation analysis conducted for samples obtained within Station I is presented in Table 3.correlation coefficient values of -0.989, 0.554, -.444 and 0.249 was obtained for zinc concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively. Also, the correlation coefficient values of -0.954, -0.929, -0.434 and -0.189 was obtained for lead concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively.

The correlation coefficient values of -0.822, -0.500, 0.000 and -0.115 was obtained for chromium concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively while the correlation coefficient values of 0.125, 0.215, -0.600 and -0.314 was obtained for cadmium concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively.

The result from the Pearson's correlation analysis conducted for samples obtained within Station II is presented in Table 4.

TABLE 3
Result of correlation analysis between heavy metals concentration in benthic organism's gills and in surface water obtained in Sample point I

		Surface water			
		Zinc	Lead	Chromium	Cadmium
Gills	Mudskipper	-0.989	-0.954	-0.822	0.125
	Crab	0.554	-0.929	-0.500	0.215
	Periwinkle	-0.444	-0.434	0.000	-0.600
	Shrimp	0.249	-0.189	-0.115	-0.314

***Exactly** –**1.** A perfect negative *–**0.70** A strong negative, *–**0.50** A moderate negative, *–**0.30** A weak negative, ***0.** No linear relationship, ***+0.30** A weak positive, ***+0.50** A moderate positive, ***+0.70** A strong positive, ***Exactly +1** A perfect positive

TABLE 4
Result of correlation analysis between heavy metals concentration in invertebrate gills and in surface water obtained in Station II

		Surface water			
		Zinc	Lead	Chromium	Cadmium
Gills	Mudskipper	-0.793	1.000**	-0.610	-0.902
	Crab	0.212	0.500	0.866	0.682
	Periwinkle	0.741	-1.000**	-0.945	0.629
	Shrimp	0.854	0.918	-0.866	0.847

***Exactly** –**1.** A perfect negative *–**0.70** A strong negative, *–**0.50** A moderate negative, *–**0.30** A weak negative, ***0.** No linear relationship, ***+0.30** A weak positive, ***+0.50** A moderate positive, ***+0.70** A strong positive, ***Exactly +1** A perfect positive

As shown on Table 4. the correlation coefficient values of -0.793, 0.212, 0.741 and 0.85 was obtained for zinc concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively. Also, the correlation coefficient values of -1.000, 0.500, -1.000 and 0.918 was obtained for lead concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively.

The correlation coefficient values of -0.610, 0.866, 0.945 and -0.8 was obtained for chromium concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively while the correlation coefficient values of -0.902, 0.682, 0.629 and 0.847 was obtained for cadmium concentration in surface water correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively.

3.3 Correlation between concentrations of heavy metals in benthic organism's gills and in sediment across all spatial gradients

Correlation analysis was also conducted to ascertain the relationship between levels of heavy metals accumulated in the gills of the invertebrate analyzed and concentrations present in the sediment sampled. The result from the Pearson's correlation analysis conducted for samples obtained within Station I is presented in Table 5. Correlation coefficient values of -0.522, -0.673, 0.590 and -0.478 was obtained for zinc concentration in sediment samples correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively.

TABLE 5
Result of correlation analysis between heavy metals concentration in invertebrate gills and in sediment water obtained in Sample point I

		Sediment			
		Zinc	Lead	Chromium	Cadmium
Gills	Mudskipper	-0.522	-0.303	0.058	-
	Crab	-0.673	0.656	0.982	-
	Periwinkle	0.590	0.303	-0.929	-
	Shrimp	-0.478	0.977	-0.327	-

***Exactly** –**1.** A perfect negative *–**0.70** A strong negative, *–**0.50** A moderate negative, *–**0.30** A weak negative, ***0.** No linear relationship, ***+0.30** A weak positive, ***+0.50** A moderate positive, ***+0.70** A strong positive, ***Exactly +1** A perfect positive.

Also, the correlation coefficient values of -0.303, 0.656, 0.303 and 0.977 was obtained for lead concentration in sediment samples correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively. The correlation coefficient values of 0.058, 0.982, -0.929 and -0.327 was obtained for chromium concentration in sediment samples correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively. However, correlation coefficient for cadmium could not be computer because the concentration values obtained in sediments were constant.

The result from the Pearson's correlation analysis conducted for samples obtained within Station II is presented in Table 6.

According to Table 6, the correlation coefficient values of -0.145, 0.843, 0.843 and -0.929 was obtained for zinc concentration in sediment samples correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively. Also, the correlation coefficient values of -0.756, -0.898, -0.040 and -0.115 was obtained for lead concentration in sediment samples correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively.

The correlation coefficient values of 0.839, -0.771, 0.925 and 0.999 was obtained for chromium concentration in sediment samples correlated with its accumulation levels in the gills of the mudskipper, crab, periwinkle and shrimp samples respectively. However, correlation coefficient for cadmium could not be computer because the concentration values obtained in sediments were constant.

TABLE 6
Result of correlation analysis between heavy metals concentration in invertebrate gills and in sediment obtained in Station II

		Sediment			
		Zinc	**Lead**	**Chromium**	**Cadmium**
Gills	Mudskipper	-0.145	-0.756	.0839	-
	Crab	0.843	-0.898	-0.771	-
	Periwinkle	0.843	-0.040	0.925	-
	Shrimp	-0.929	-0.115	0.999	-

***Exactly** –**1.** A perfect negative *–**0.70** A strong negative, *–**0.50** A moderate negative, *–**0.30** A weak negative, ***0.** No linear relationship, ***+0.30** A weak positive, ***+0.50** A moderate positive, ***+0.70** A strong positive, ***Exactly +1** A perfect positive.

IV. DISCUSSION

Pollution is the introduction of contaminant into the environment by man through his daily activities resulting in a measurable effect, a major contributor which is the lack of care and proper management for natural resources. Proper pollution management thus require a good background knowledge of the type, level and source of pollution. The Great Kwa River is faced with this challenge considering its location and position which exposes it to the accumulation of human and industrial waste from heavy rain falls and lack of sewage facility in the surrounding community. It is in view of this that benthic organisms were used in this study to serve as bio-indicator in water pollution evaluation. Bio-indicators have shown ability in giving an insight on the condition of the environment they are found as a result of the simple correlation between the contaminant concentration in the environment and in their tissue.

The result of benthic composition in the study compared favourably with those reported by [26, 27 and 28]. The dominance of Decopoda in the macro benthic community assayed has also been documented by [26 and 28]. The comparable higher number of benthic species censored in Station II as against Station I may be related to spatial differences in oxygen availability, sediment structure and point sources of organic matter. Across temporal gradients, sediment samples collected during the dry season had greater macro benthic population compared to those obtained in the wet season. This result compared favorably with [29]. The phytoplankton detritus accumulated in the sediments during the dry season and the benthic organisms must have responded to this store of food, leading to their massive proliferation. However, during the wet season when temperature is lower, the stored detritus must have been exhausted and the benthic organisms declined. All diversity indices (Shannon Weiner, Evenness and Margalef) employed to investigate benthic organism diversity across temporal and spatial gradients revealed the dry season and Station II to be more diverse and stable in macro benthic composition than wet season and Station I respectively. [30], also reported a higher diversity and stability of benthic organisms in dry season compared to the wet season. The comparable high diversity and stability in benthic composition in Station II may due to differences in the physicochemical characteristics in sediments of the two locations.

The gills of four selected benthic organism species were analysed for the accumulation of five heavy metal species. Gills are of major concern of heavy metal toxicology as it shows significantly high bioaccumulation factor owing to the fact that gills have larger surface area and comes in direct contact with heavy metal laden water [31]. Arsenic concentration was below detection limit in gills of all benthic organism species analysed. With the exception of lead, statistical significant differences in metal concentrations were recorded in the gills of the benthic organism species analyzed across all temporal gradients. Studies on bioaccumulation of benthic organism species in sediments are scarce. However, variations in gill size have been reported to be the major determining factor for differences in heavy metal accumulation among fish species [32]. The spatial distribution of metals in the gills of the studied benthic organism species is mainly due to anthropogenic input of metals as it is not close to any industrial location. The concentrations of heavy metals in the studied benthic organism species from across spatial gradients fell below the permissible limit for human consumption recommended by FAO with the exception of lead in Mudskipper samples collected from Station I. This species is found to be unsafe for consumption in Station I. This result compared favorably with those in six Mediterranean fish species reported by [33]. Young children are vulnerable to toxic effects of lead and can suffer profound and permanent adverse health effects, particularly the development of brain and nervous system.

In Station II, a strong positive relationship existed for zinc concentrations in sediment and in the gills of the crab and periwinkle. Similar result was obtained for chromium analysed in sediment and in shrimp, periwinkle and mudskipper. All other correlations showed negative or no relationships. In Station I, a strong positive relationship existed for chromium concentrations in sediment and in the gills of the crab samples. A moderate positive relationship existed for zinc concentrations in sediment and in the gills of the periwinkle. Similar result was obtained for lead analysed in sediment and in crab and mudskipper while a weak positive relationship existed for lead concentration in sediment and periwinkle and for chromium in sediment and mudskipper. All other correlations showed negative relationships. Studies on the subject for benthic organism species are scare. In Station II, a moderate positive relationship existed for lead concentrations in surface water samples and in the gills of the crab, and for cadmium in the periwinkle sample. A strong positive relationship existed for cadmium, lead and zinc concentrations in surface water samples and in shrimp, for zinc and cadmium in periwinkle, and for chromium and cadmium in crab. This is in agreement with the work of [34 and 35]. Hence suggest bioaccumulation of metals in gills to be concentrated from water. A perfect positive relation existed for lead concentrations in surface water and in mudskipper while a weak positive relationship for zinc concentration existed between surface water samples and crab. All other correlations showed negative or no relationships. On the other hand, in Station I, a weak positive relationship for cadmium concentration existed between in surface water and in mudskipper and crab, for zinc in shrimp. Also, a moderate positive relationship existed for zinc in surface water and in crab, all other correlations showed negative or no relationships. This is in accordance with the work of [36] who suggested that the gill size could be the reason for negative correlation since a negative correlation is linked with different adsorption rate through various body organs. The fact that the main route of adsorption is through the gills, with its size relative to the organisms' size could also be the reason for negative correlation between the concentration of the element in its surrounding and the gill size.

V. CONCLUSION

This study on the evaluation and correlation analysis of heavy metals concentration in gills of benthic organisms in Great Kwa river revealed that the correlation coefficient between physicochemical parameters, phytoplankton and zooplankton showed negative correlation. Also, analysis on bioaccumulation of heavy metal showed gills of mudskipper (*Periophthalmus koelreute*re) in sample point 1 among the benthic organism sampled to have high lead concentration. All benthic organisms encountered were somewhat pollution tolerant and pollution tolerant species. This suggests that the river could be under pollution stress as moderate benthic diversity indices were revealed. It is therefore recommended that laws are enforced to control the disposal of effluent and other waste products.

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