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Heavy metal concentrations in environmental samples and amphibian tissues in the Niger Delta, Nigeria: correlations with parasite species

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ABSTRACT:
Soil samples and amphibian host specimens were collected monthly from Agbada, an oil flow station in the Niger Delta region of Nigeria, for heavy metal analysis and also for the recovery of the helminth endo-parasites of the amphibians. Collection of samples was done monthly for a period of twelve months and heavy metal analysis was done by wet digestion of environmental and biological samples using atomic absorption spectrophotometer. Parasites were collected using standard protocols. Both environmental and biological samples accumulated varying concentrations of the heavy metals. All the concentrations of the metals in environmental samples were lower than the target values, whereas concentrations of all the metals in the biological samples exceeded the permissible limits. Seventeen parasite species were recovered from infected hosts and included nematodes, trematodes, cestodes, pentastomids, and acanthocephalan cysts. Pearson's correlation coefficients were computed for pairs of heavy metal concentrations in the environmental and biological samples with the parasite burdens and the results revealed among other findings that the lung trematodes, *Haematoloechus exoterorchis* and *H. micrurus*, correlated significantly positively with Cu in the soil samples and negatively with Pb and Cd in lungs and Cr in soil. This reflects the conditions that favour and hinder the ability of the parasites to establish infection in their host specimens.
Key words: Heavy metals, Environmental samples, Amphibians, Parasites, Correlation, Niger Delta.

INTRODUCTION

Amphibians are recognised as good bio-indicators of environmental health [1], and many researchers have used them, both at the larval and adult stages, to demonstrate the levels and effects of heavy metals in animal tissues in laboratory and field conditions [2, 3]. Related studies in Nigeria include those of [4-7].

Research into the parasitic helminths of amphibians in Nigeria has also progressed relatively although research in the oil-rich Niger Delta region of the country is scanty [8,9]. In some of these researches, observations of the influence of certain environmental differences on the parasitic fauna of the amphibian hosts were made. For instance, the acidic nature of a mangrove environment in the Niger Delta was found to be unconducive for the free-living larval stages of some parasite species [10].

However, the individual environmental factors responsible for the prevalence patterns of particular parasites have not been elucidated. It was in an effort to provide some background information on the possible relationships between heavy metal concentrations in environmental samples and organs of host specimens and their helminth endo-parasites that this research was carried out.

MATERIALS AND METHODS

Amphibian host specimens were collected from Agbada (E 4° 55′ 57.006″, N 7° 1′ 13.692″), an oil flow station located in the Niger Delta area of Nigeria. They were collected using visual encounter and acoustic survey methods and triplicate top soil samples (up to 15cm deep) were collected using a spade. Collections were made monthly for a period of twelve months (July, 2015 to June, 2016), and ten amphibians were collected on each visit. The amphibian specimens and soil samples were transported in plastic containers to the parasitology laboratory in the Department of Animal and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt for dissection and other analysis.

The top soil samples were composited, ground to powdery form and 2.0g of the composite was digested using 25ml nitric acid according to APHA 301A [11] and used for the analysis of heavy metals (lead Pb, cadmium Cd, chromium Cr and copper Cu) using the Buck Scientific, Atomic Absorption Spectrophotometer (AAS), Model 210 VGP, and the concentrations were expressed in mg/kg.

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Organs and muscle tissues of the amphibian, *Hoplobatrachus occipitalis*, were used for the heavy metal analysis. The host specimens were longitudinally dissected and the liver, lungs, gastro-intestinal tract and muscle tissues were excised, weighed and analysed for heavy metals as described above.

For parasite collection, the amphibian species were dissected and the gut excised from the viscera. The gut was severed into the following sections: esophagus, stomach, small intestine, and large intestine/rectum. The lungs, urinary bladder and body cavity were also examined for parasites. Each section was placed separately in a Petri dish containing 0.72% saline solution for examination and collection of parasites. Nematodes were fixed in hot 70% alcohol and preserved in fresh 70%alcohol. Trematodes and cestodes were flattened under a cover slip on a microscope slide and fixed in 5% formol saline. The fixed specimens were preserved in the same medium (fixative). Pentastomids were preserved in 70% alcohol saline

Permanent mounts were made in Canada Balsam after the worms were washed to remove the fixative, stained in acetocarmine for 6 hours, dehydrated in alcohol series (50%, 70%, 90% and 100%) and cleared in Xylene (50% and 100%). Nematodes were cleared in lactophenol before examination.

Amphibians were identified [12] and the parasites were identified according to standard protocols [13-15]. Prevalence and mean intensity of parasitic infection were computed [16].

RESULTS AND DISCUSSION

Results of the heavy metal analysis revealed that the organs and muscle tissues of the amphibian host specimens accumulated the heavy metals at varying concentrations. In the soil samples taken from this site, lead values ranged from <0.001 to 0.3mg/kg; chromium from 0.0 to 0.78mg/kg; cadmium 0.03 to 0.34mg/kg, and copper from 0.00 to 0.18mg/kg. The pattern of metal accumulation was Cr > Cd > Pb > Cu.

In the thigh muscle, lead ranged from <0.001 to 1.60 mg/kg. Chromium ranged from <0.001 to 1.39 mg/kg. Cadmium ranged from 0.01 to 0.3mg/kg, while copper ranged <0.001 to 0.2mg/kg. The pattern of accumulation was Pb > Cr > Cd > Cu.

In the gastrointestical tract (GIT), lead ranged from <0.001 to 0.01mg/kg. Lead was very negligible in this organ. Chromium values were also mostly negligible, being found to be <0.001mg/kg in all the months except for November, 2015 when a value of 0.220mg/kg was obtained. Cadmium values ranged from 0.00 to 0.3mg/kg, and copper values from <0.001 to 0.5mg/kg. The pattern of accumulation here was Cu > Cd > Cr > Pb.

In the lungs, lead ranged from 0.00 to 0.90mg/kg. Chromium was more or less absent from the lungs. The values for chromium were <0.001mg/kg throughout the sampling period. Cadmium ranged from 0.00 to 0.32mg/kg, while copper values ranged from <0.001 to 0.60mg/kg. The pattern of metal accumulation in the lungs was Pb > Cu > Cd >Cr.

Lead values in the liver ranged from 0.00 to 0.14mg/kg. Here again, chromium was mostly negligible; values of <0.001mg/kg were obtained for all the months except for November, 2015, when a value of 0.40 mg/kg was obtained. While cadmium ranged from <0.001 to 0.38mg/kg, copper values ranged from <0.001 to 2.53mg/kg. The pattern of metal accumulation in the liver was Cu > Cd > Pb >Cr.

Generally, the muscle and liver accumulated higher concentrations of the heavy metals than the other organs, followed by the lungs and then the GIT. The soil did not have values, for any of the heavy metals, which were higher than those observed in any of the tissues. Thus indicating that the heavy metals actually bio-accumulated in the organs of the anuran species to levels higher than environmental concentrations, and also suggesting that some of the metals could have been absorbed by the anuran tissues through the skin.

Some of the heavy metal concentrations were found to be above permissible limits for food substances which are 0.01mg/kg, 0.07mg/kg, 0.05mg/kg and 2.0mg/kg for lead, chromium, cadmium and copper, respectively [17]. The soil concentrations of the metals were all lower than the target values; 0.8mg/kg, 100.0mg/kg, 36.0mg/kg, and 85.0mg/kg for cadmium, chromium, copper and lead, respectively [18].

Seventeen parasite species accounting for a total of 1,395 parasites were recovered from the 120 hosts

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captured from Agbada within the period of study. The parasites included nematodes (*Amplicaecum africanum*, *Oswaldocruzia hoepplii, Chabaudus leberrei, Cosmocerca ornata, Rhabdias* sp. and encysted larval ascaridoids), trematodes (*Mesocoelium monodi, Diplodiscus fischthalicus, Prosotocus exovitellosus*, *Ganeo africana* and *Polystoma* sp.), cestodes (*Baerietta sp., Cephalochlamys compactus* and an unidentified cestode) and Acanthocephalan cysts. The gastrointestinal parasites recovered were *C. leberrei, C. ornata, A. africanum, O. hoepplii, M. monodi, G. africana, P. exovitellosus, D. fischthalicus, C. compactus* and *Baerietta* sp., while the lung parasites included *Rhabdias* sp., *H. exoterorchis* and *H. micrurus*. *Polystoma* species were recovered from the urinary bladder of the host specimens while encysted larval ascaridoids and acanthocephalan cysts were recovered from the body cavity. The prevalence and mean intensity of the parasites in infected host specimens are shown in Table 1.

Pearson's correlation coefficients were calculated for pairs of heavy metal variables and parasite species recovered monthly. The pairs with significant coefficients ($r_{0.05\ (2)39}$=0.308; $r_{0.01(2),39}$=0.398) are presented in Table 2. The results revealed an interesting pattern for the lung helminth parasites. The lung helminths, especially the trematodes, *H. exoterorchis* and *H. micrurus*, were found to have very similar patterns of correlation with the metal variables. For instance, both were significantly negatively correlated with Pb and Cd in the lungs, Cr in the soil, liver and GIT, and with Cu in the GIT. Both together with *Rhabdias* sp., the lung nematode, had significant negative correlation coefficients with Cu in lungs and GIT, and with Cr in soil samples, while *H. micrurus* and *Rhabdias* sp. were significantly negatively correlated with Cd in the lungs.

The same lung trematodes correlated significantly positively with Cu in soil, Cd in muscle and liver and Pb in GIT. Together with *Rhabdias* sp., they correlated significantly positively with Cd in muscle and Pb in GIT of host specimens.

There were significant positive correlation coefficients between Pb in GIT and acanthocephalan cysts, the intestinal parasites (*Baerietta* sp., *C. leberrei, D. fischthalicus, G. africana*) and larval ascaridoids. Another intestinal parasite, *A. africanum*, had significant positive correlation coefficients with Cd in GIT, and Pb and Cu in soil, whereas the *O. hoepplii* (also an intestinal nematode parasite) had significant negative correlation coefficients with Pb, Cd and Cu in the soil samples and liver of host specimens.

Several events can occur when parasites invade organisms which are facing other environmental stressors [19]. For instance, some stressors may make hosts more susceptible to infection or reduce host immunity in such a way that parasites that occur in low numbers in normal individuals increase in population, thereby causing disease. Some other stressors (like high acidic habitats) [10] may kill parasites by being incapable of supporting the free-living stages of the parasites, or by reducing the population of the parasite's intermediate host. Parasites may also increase in population if the environmental stressor (such as nutrient enrichment) increases the population of the intermediate hosts. On the other hand, parasitic infections may reduce the immune status of host organisms thereby making them more adversely affected by toxic chemicals in their environment.

The combined effects of pesticides and trematode infections on the tree frog, *Polypedates cruciger*, was examined [20] and it was found that while pesticides (chlorpyrifos, dimethoate, glyphosate, propanil) did not induce mortality in the cercaria of the digenetic trematode *Acanthostomum burminis*, both pesticide and cercarial exposure, in isolation, significantly decreased frog survival, development, and growth, and increased developmental malformations, such as scoliosis, kyphosis, and also edema and skin ulcers. The combination of cercariae and pesticides generally posed greater risk to frogs than either factor alone by decreasing survival or growth or increasing time to metamorphosis or malformations.

Toxic chemicals and parasites can cause death in amphibians but may also affect amphibian populations through sub-lethal effects that can reduce host fitness such as reduced size at metamorphosis and longer time needed to metamorphose [21] and smaller metamorphs die off easily in terrestrial environments and are not as fecund as bigger ones [22].

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Table 1: Prevalence (%) and mean intensity () of helminth parasites of amphibian host specimens from Agbada

Parasite	Host species										
	Ps n=2	Po n=14	Ho n=35	Pb n=12	Ac n=4	Am n=2	Pp n=12	St n=9	Pm n=24	Hg n=5	Hfb n=1
Nematodes											
A. africanum	50.00 (1.0)	21.43 (3.0)	20.00 (1.9)	-	25.00 (2.0)	-	8.33 (2.0)	-	4.17 (1.0)	-	-
O. hoepplii	50.00 (1.0)	21.43 (1.7)	2.86 (1.0)	25.00 (3.3)	-	-	25.00 (3.0)	-	8.33 (1.0)	-	-
C. leberrei	-	14.29 (5.5)	25.71 (4.0)	-	-	-	-	-	4.17 (1)	-	-
C. ornata	-	-	14.29 (4.0)	50.00 (6.8)	25.00 (1)	100.00 (35.5)	41.67 (6.2)	11.11 (2.0)	33.33 (5.1)	20.00 (1.0)	-
Rhabdias sp.	-	35.71 (4.2)	2.86 (1.0)	-	-	-	8.33 (1.0)	-	4.17 (3.0)	20.00 (13.0)	-
Enc. Larv. Ascaridoid	-	14.29 (5.5)	22.86 (2.0)	-	-	-	-	-	-	-	-
Trematodes											
M. monodi	50.00 (26.0)	42.86 (34.7)	2.86 (8.0)	50.00 (8.5)	-	100.00 (31.0)	33.33 (13.0)	-	25.00 (10.7)	60.00 (15.3)	-
D. fischthalicus	-	-	14.29 (2.8)	-	-	-	8.33 (2.0)	-	4.17 (1.0)	-	-
G. africana	-	-	8.57 (2.7)	-	-	-	-	-	-	-	-
P. exovitellosus	-	-	8.57 (2.0)	-	-	-	-	-	-	-	-
Polystoma sp.	-	-	-	16.67 (1.0)	-	-	8.33 (1.0)	-	4.17 (1.0)	-	-
H. micrurus	-	-	34.29 (4.9)	-	-	-	-	-	-	-	-
H. exoterorchis	-	-	37.14 (28.4)	-	-	-	-	-	-	-	-
Cestodes											
Baerietta sp.	-	-	-	-	-	-	-	-	12.50 (7.0)	-	-
C. compactus	-	-	11.43 (1.3)	-	-	-	-	-	-	-	-
Unidentified cestode	-	-	-	-	-	-	-	-	4.17 (1.0)	-	-
Acanthocephala											
Acanthocephalan cysts	-	-	-	-	-	-	-	-	12.50 (3.7)	20.00 (1.0)	-

Key: Ps = *P. schubotzi*; Po = *P. oxyrhynchus*; Ho = *H. occipitalis*; Pb = *P. bibroni*; Ac = *A. camerounensis*; Am = *A. maculatus*; Pp = *P. pumilio*; St = *S. tropicalis*; Pm = *P. mascareniensis*; Hg = *H. galamensis*; Hfb = *Hyp. fusc. burtoni*.

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Table 2: Parasite species showing significant positive and negative correlation coefficients with heavy metal variables

Variable	Parasite species	
	Significant positive correlation	Significant negative correlation
Soil Pb	*A. africanum* *H. micrurus* *M. monodi* *G. africana*	*C. ornata* *O. hoepplii* *Polystoma* sp.
Soil Cu	*A. africanum* *H. exoterorchis* *H. micrurus*	*O. hoepplii*
Soil Cd	-	*O. hoepplii* *Rhabdias sp.* *G. africana*
Soil Cr	-	Larval ascaridoids Acanthocephalan cysts *D. fischthalicus* *H. exoterorchis* *H. micrurus* *Rhabdias sp.* *Polystoma sp*.
GIT Pb	Acanthocephalan cysts Larval ascaridoids *H. exoterorchis* *H. micrurus* *Rhabdias sp.* *Baerietta sp.* *C. leberrei* *D. fischthalicus* *P. exovitellosus* *G. africana*	-
GIT Cd	*A. africanum* *H. micrurus*	-
GIT Cr	-	*H. exoterorchis* *H. micrurus*
GIT Cu	-	*H. exoterorchis* *H. micrurus* *Rhabdias sp.* *Polystoma sp* *D. fischthalicus*
Lungs Pb	-	*H. exoterorchis* *H. micrurus*
Lungs Cd	*A. africanum* *C. leberrei*	*H. micrurus* *Rhabdias sp.*
Lungs Cu	-	*A. africanum* *H. exoterorchis* *H. micrurus* *Rhabdias* sp
Lungs Cr	-	-
Muscle Pb	*C. compactus* *O. hoepplii*	*A. africanum* *D. fischthalicus* *H. micrurus*

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Muscle Cd	*H. exoterorchis* *H. micrurus* *Rhabdias sp* *A. africanum* *D. fischthalicus* *G. africana* *Baerietta sp*. Acanthocephalan cysts	*C. compactus*
Muscle Cr	*Polystoma* sp.	*A. africanum* *D. fischthalicus* *C. leberrei* *M. monodi*
Muscle Cu	*P. exovitellosus*	*D. fischthalicus* *O. hoepplii* *Polystoma sp.* *Rhabdias sp.*
Liver Pb	*C. compactus* *Larval ascaridoids* *H. mirurus* *P. exovitellosus*	*O. hoepplii* *Polystoma sp.*
Liver Cd	*H. exoterorchis* *H. micrurus*	*O. hoepplii* *Baerietta sp.*
Liver Cu	Larval ascaridoids	*O. hoepplii*
Liver Cr	-	*H. exoterorchis* *H. micrurus*

CONCLUSION

In conclusion, the results presented in this report have revealed the correlation between parasite species and heavy metal concentrations in soil samples and organs of amphibian host specimens. Though not all-inclusive, it shows certain conditions in the organs or tissues of amphibian hosts that support or enhance and hinder the establishment of certain parasite species. More research is however solicited in order to have a comprehensive record of the specific habitat characteristics and how they affect amphibian parasites.

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