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Assessing Uptake Kinetics and Natural Depuration of Lead and Cadmium in African Catfish Juvenile

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ABSTRACT: This study was carried out to assess the uptake kinetics of two heavy metals lead and cadmium in African catfish juveniles and their ability to depurate during subsequent exposure to clean water. Catfish juveniles with average weight and length of 5.3g and 5.8cm respectively were randomly selected and acclimatized for two weeks. A 24-hour range finding test was carried out. In the definite test, the fish were stocked in 24 tanks containing 5litres of water with lead and cadmium treatment separately labeled according to predetermined concentrations 5, 10, 15, 20, 25 mg for lead and cadmium with 4 fish in each treatment. The water and fish were analyzed for the metals after 5 days to show percentage uptake of the metals from the water. The fish were transferred back into clean water to access the rate of depuration and water was changed continuously. After 4 days the fish were analyzed again to see if they were purged of the heavy metals. Based on these findings catfish have shown to be good accumulators of heavy metals but can be depurated if allowed to swim in clean water. Uptake percentage after the period of exposure to 5mg, 10mg, 15mg, 20mg, 25mg concentrations were 18.68, 10.17, 5.95, 1.41, 8.26 for cadmium and 20.47, 10.17, 8.76, 9.92, 10.08 for lead; Percentage of metals depurated after 4 days were 40.00, 80.70, 90.00, 77.78, 45 for cadmium and 73.045, 20.413, 41.425, 37.580, 61.141 for lead. Lead was readily taken but cadmium was readily depurated.

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Heavy metals are trace components of the aquatic environment, whose level have been reported to be on the increase in recent times due to pollution from industrial wastes, changes in geochemical structure, agricultural and mining activities (Singh *et al.,* 2007; Sprocati *et al.,* 2006). Heavy metals unlike organic contaminants are not degraded with time, but concentration can only increase through bio-accumulation (Aksoy, 2008). The ubiquitous presence and use of heavy metals has not been without significant consequences (UNEP-CEP, 2008). Industrial and agricultural applications of heavy metals have added large amount of these metals, making the fishes unsafe for consumption (Castro-González and Méndez-Armenta, 2008). The concentrations of these toxic substances, however, in rivers, lakes, and oceans are very low but they can accumulate to very high concentration in fishes and other marine organisms by bioaccumulation and biomagnification mechanisms resulting to high occurrences of cardiovascular, inflammatory and associated diseases. Various disease associated with consumption of seafood has prompted investigation into alternative measures or methods of purifying contaminated fish to render them fit for consumption. Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake eventually resulting in physiological and histopathological changes. These changes can also be altered by water physico-chemistry such as pH, alkalinity, increase total dissolved solid, conductivity salinity etc. and this has general effect on growth and development of fishes. With fish constituting an important link in the food chain, its contamination by toxic metals causes a direct threat, not only to the entire aquatic environment, but also to humans that utilize it as food. From a human health perspective cadmium and lead have been identified as primary contaminants of concern with cumulative effect (Cunningham and Saigo, 1997). Lead is the number one environmental poison amongst the toxic heavy metals all over the world, causing serious health hazards to humans, especially to young children. Over exposure to Pb causes damage to foetal nervous system, increasing the risk of premature birth or low birth weight. Other effects associated with consumption of foods containing high level Pb contents include: inhibition of biosynthesis of haem, severe vomiting, intestinal cramps, circulatory disorder, madness and death (Cunningham and Saigo, 1997). Chronic exposure to cadmium is associated with heart disease, anaemia, skeletal weakening,

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depressed immune system response and kidney and liver diseases (Burger *et al.,* 2007). These metals have no bio-importance in human biochemistry and physiology, but its consumption, even at very low concentration can be toxic (Young, 2005). Even for metals with bio-importance such as zinc, nickel and chromium (Abduljaleel and Shuhaimi-Othman, 2011), dietary intake has to be maintained at the regulatory limits, as excesses will result in poisoning or toxicity (Young, 2005). This is because they combine with body biomolecules such as metal-binding protein and enzymes to form stable biotic compounds, thereby mutilating their structures and hindering them from performing the designated functions within the body system (Duruibe *et al.,* 2007). This study was carried out to assess the uptake kinetics of two heavy metals lead and cadmium in African catfish juveniles and their ability to depurate during subsequent exposure to clean water

MATERIALS AND METHOD

Sample Collection: 140 samples of *Clarias gariepinus* of average weight 5.3g and length 5.8cm were purchased from a local culture pond at Nkpolu. Fish were transferred to University of Port Harcourt toxicology laboratory at the Department of Animal and Environmental Biology and kept for 2 weeks for acclimatization under laboratory conditions 25+1°C with light ventilation and water quality parameters (TDS=8.92 ppm, PH 8.6) Temperature 26.2°C, salinity 0.01 ppt, conductivity 17.80 us. DO 48mg/L were measured during the experiment.

Range Finding Test (First Exposure): Rage finding testing of cadmium and lead toxicity were performed using different concentrations of Pb and Cd (100 mg, 250 mg, 500 mg, 750 mg, and 1000 mg/l both with 2 replicates and each concentration containing 4 fish with (4 fish in each control). The lead salt was insoluble, so it was dissolved using the magnetic stirrer hot plate to obtain a uniform solution. The concentrations of each heavy metals concentration caused 100% mortality in fish within 12- 15 hours and the second exposure was conducted using lower concentration.

Definitive Testing: Second exposure (Sublethal concentration): The concentrations of metals were reduced to 5, 10, 15, 20, and 25mg/l for both lead and cadmium with 4 fishes in each tank. Aerators were fixed to supply more oxygen to each tank and allow to stand for 5 days after which the samples were taken to the lab for analysis and number of death recorded each day. Only one fish from each duplicate was taken and sacrificed for analysis at initial treatment.

Depuration Period: The remaining fish were placed into clean tanks filled with clean water which allows normal biological activities. The fishes were fed and water was changed continuously to determine rate at which it will take to purge it of its contaminants (Pb and Cd).

Sample Digestion (Biota) and Analysis: The biota (fish)were dried to remove H_2O content and crushed using the crucible to expose a better surface area to obtain the analytes.10ml of digestion mixture (sulphuric, nitric and pechnoric acid) were added in the ratio of 2:2:1 respectively. Samples were heated using the heating mantel until a colour change was obtained. Sample was allowed to cool and then filtered using Whatman's filter paper into a 100ml standard volumetric flasks. Sample was made up with distilled water to calibration mark and poured in a poly bottle and sent for heavy metal analysis.

Digestion for the water Sample and Analysis: 50ml of the water sample was measured and digested with 3ml of nitric acid, it was heated using heating mantle for 15 mins. Filter sample to avoid impurities in to 50ml standard volumetric flask. Poured sample in a poly bottle and taken to AES (model Agilent technologies 4210 MP-AES) for analysis.

Statistical Analysis: Data obtained were subjected to t-test to determine the difference in the heavy metals concentrations between the two toxicants Pb and Cd salt with statistical significant level set at $p<0.05$ using ASSISTAT Version 7.7 (2017).

RESULTS AND DISCUSSION

The result obtained after 14 days of acclimation show mortality rate of <1% as shown in Table 1. In 24 hours, percentage death rate was 25, 50, 25, 50 % at 10, 15, 20 and 25 mg/l respectively for cadmium and 25, 25, 50, 50 % at 5, 10, 15, and 25 mg/L for lead respectively. The uptake, depuration, percentage depurated of cadmium and lead after 5 days is shown in the table 2 and figures 2 and 3. At 5 mg/L concentration, mean metal uptake of cadmium was 0.055 ± 0.038, at 10 mg/L = 0.057 ± 0.016, 15 mg/L = 0.058 ± 0.029, 20 mg/L = 0.018 ± 0.024 and 25 mg/L = 0.12 ± 0.084. Mean uptake for lead was 5 mg/L=0.3005 ± 0.219, 10 mg/L=0.1935 ± 0.088, 15 mg/L=0.1895 ± 0.076, 20 mg/L=0.2355 ± 0.201 and 25 mg/L=0.2805±0.087. Percentage uptake is represented in table 3 and after depuration period, percentage loss figure 1. This study was done to assess the potential uptake kinetics of heavy metals (Cadmium and Lead) in the African Catfish. Uptake rate and depuration ability through exposure to different concentration of introduced heavy metals were assessed. Both metals displayed characteristic of being toxic by increasing mortality rate at short period of fish exposure (48h) (El-Shenawy. 2004) causing more than 1% death rate compared to period of 2weeks of acclimation. Comparison of death rate between both treatment showed lethality in order of Pb>Cd, this implies that lead was more toxic. Accumulation of metals in fish is dependent on metals concentration and period of exposure (Dublin-Green *et al*., 1994; Alves *et al*., 2006). Other factors such as water salinity, pH, hardness and temperature, size age (young fish tend to be more susceptible) ecological need and feeding habit play significant role too, this can explain reason for irregular values in concentration 25 mg/l of lead and 20 mg/l of cadmium accumulation is not only determined by concentration and exposure but metals are also preferentially accumulated by different organs of the body (Bilgrami *et al*., 1996; *Rauf et al.,* 2009). Most metals taken up by organism do not bioaccumulate but instead are processed internally and excreted (Wallace *et al*., 2003). Chronic toxicity of cadmium is a function of total amount of metal accumulated (Borgmann *et al.,* 2001). Uptake percentage of cadmium 5 mg/l is 1.9E+01, 10 mg/l=1.0E+01, 15 mg/l=6.0E+00, 20 mg/l=1.4E+00, 25 mg/l=8.3+00. For 5 mg/l is 2.0E+01, 10 mg/l=1.0E+01, 15 mg/l=8.8E+00, 20 mg/l=9.9E+00, 25 mg/l=1.0+01. Accumulation rate is Pb>Cd this implies that lead is preferably taken in by *Clarias gariepinus.* Bioaccumulation is dependent on structure and function of different tissues (Kotze, 1997). It is also known ingested portion of metals do not accumulate in tissues and are excreted after passing through digestive system (Glover & Hogstand, 2002). This could explain variation in concentration 25mg/l of lead and 25mg/l of cadmium with uptake percentage of 8.3E + 00 and 1.0E+01 respectively. Percentage is low despite high concentration of lead.

Table 1: Fourteen Days Acclimation Period, 13 of 140 samples died before 14 days of acclimation

Duration (hour)	24	48	72	96	120	144	168	192	216	240	240-312
Mortality	4	2	0	3	0	2	1	1	0	0	0

Table 2: Treatment Mortality Rate, 18 of 80 samples died within four days in treatment

		Percentage Mortality (%)					
		Concentration (mg/L)					
	Hours	**Control**	**5.00**	**10.00**	**15.00**	**20.00**	**25.00**
Cadmium	**24**	0	0	13	25	13	25
	48	0	0	14	0	0	17
	72	0	0	0	0	0	0
	96	0	0	0	0	0	0
Lead	**24**	0	13	13	25	0	25
	48	0	0	0	0	13	33
	72	0	0	0	0	0	0
	96	0	0	0	0	0	0

Table 3: The concentration of Cadmium (Cd) and Lead (Pb) in mg/kg at Uptake and After Depuration

Conc.	**Cadmium (Cd)**			**Lead (Pb)**		
	Uptake	**After Depuration**	**Percentage Uptake**	**Uptake**	**After Depuration**	**Percentage Uptake**
5	0.055±0.038[a]	0.0335±0.0007[a]	18.68	0.3005±0.219[a]	0.0815±0.0007[a]	20.47
10	0.057±0.016[a]	0.0105±0.0007[a]	10.17	0.1935±0.088[a]	0.19355±0.0007[a]	10.17
15	0.058±0.029[a]	0.00575±0.0007[a]	5.95	0.1895±0.076[a]	0.1115±0.0007[a]	8.76
20	0.018±0.024[a]	0.018±0.0007[a]	1.41	0.2355±0.201[a]	0.1465±0.0007[a]	9.92
25	0.12±0.084[a]	0.0665±0.0007[a]	8.26	0.2805±0.087[a]	0.1085±0.0007[a]	10.08s

[a-b] Different letters in the same row indicate significant difference (P<0.05)

Table 4: Summary of Percent lost to water after depuration

Initial Conc.	**Cadmium (Cd)**		**Lead (Pb)**	
	Depurated	**Percentage**	**Depurated**	**Percentage**
5	0.0215	40.00	0.3005	73.045
10	0.0465	80.70	0.1935	20.413
15	0.5215	90.00	0.1895	41.425
20	0.05215	77.78	0.2355	37.580
25	0.0535	45	0.2805	61.141

Depuration: *Clarias species* have shown ability to depurate / eliminate heavy metals but tend to be slow, (El-Shenawy., 2004). This can be attributed to reduction in physiological processes such as reduction in respiratory rate and movement. This is because the organism tend to survive through avoidance mechanism and this reduces rate of elimination of contaminants because increase in ammonia and faecal excretion is associated with an increase in respiration rate (El-Shenawy., 2004). Closure in clam (*corbicula flominea*) in response to water borne metals was reported in previous work by Chung-Min *et al*., (2004). Uptake percentage was high in lead but cadmium was preferably depurated, depuration is also related to availability of water Okereke *et al*., (2017) reported that depuration of contaminants on shellfish (*Tympanatonus fuscastus)* was faster during rainy season than dry season, this means that for effective depuration to occur there is need for availability of source that is water which attained the safety standard. Cadmium was preferably depurated compared to lead due to this could be due to insolubility nature of lead in water under normal condition or affinity for tissues. This indicates that lead take longer period to depurate from the fish tissues, this insoluble nature can interfere with respiratory function of the gills resulting in acute respiratory stress and death by suffocation. The good news is that due to insoluble nature of lead in water, they are most times adsorbed on to sediment and suspended particles hence reducing its availability to marine organisms but the organisms at the bottom is more likely to be exposed.

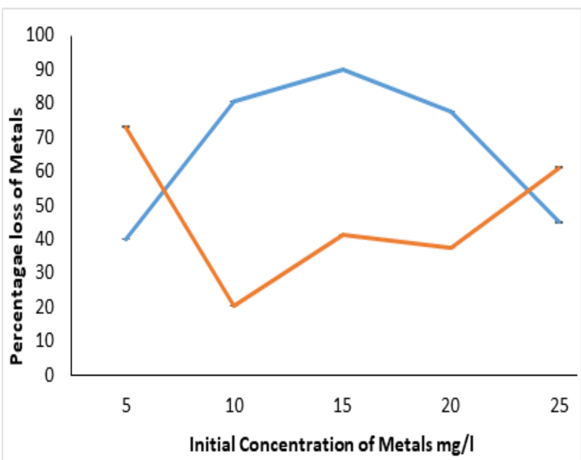

Fig 1: Percentage loss to water of lead and cadmium n = 2

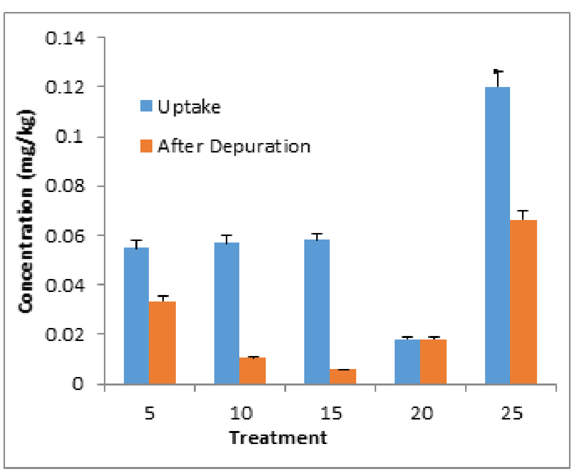

Fig 2. The Concentration of Cadmium at uptake and after depuration. n = 2

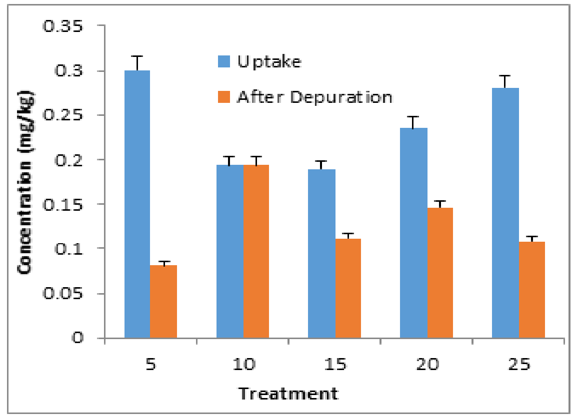

Fig 3: The Concentration of Lead at uptake and after depuration. N = 2

Conclusion: This study revealed that Heavy metals can be eliminated from fish by transferring them into clean water before consumption but this process of natural depuration tends to be slow as shown in this research work. Based on these findings catfish have shown to be good accumulators of heavy metals but can be depurated if allowed to swim in clean water. Depuration is not a substitute for pollution control but a process to enhance healthy living by improving safety consumption of sea food. Farmers and consumers should depurate fishes before consumption or storage

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