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Full Length Research Paper

Comparative Analysis of Heavy Metal Accumulation in Biomarkers of *Clarias gariepinus* Subjected to Chemical Additive Contaminated Effluent

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The toxicity of Sublethal concentrations of chemical additives effluents were investigated on African catfish *Clarias gariepinus* using a renewable static bioassay. The trend of bioconcentration of metals in the gut, liver, gills and kidney of the test organisms differs significantly ($p < 0.05$) and it followed the order, liver> gill >gut > muscle. The result revealed that the liver had Ni concentration of 0.0046 mg/L and 16.1208 mg/L of magnesium as the highest. In the muscle, Ni was not bioaccumulated (0.0000 mg/L) while the highest magnesium concentration of 10.7345 mg/L was recorded. The gill had the least concentration of 0.0010 mg/L for Cu while the highest concentration recorded for Mg was 12.6797 mg/L. The gut had Mn concentration of 0.0401 mg/L and Mg concentration of 14.5001mg/L. It was revealed that fish can bioaccumulate heavy metals from a polluted environment, which may result in reduction or impairment of natural population size and could be a risk to consumers. Consumption of fish from polluted environment should be discouraged.

Keywords: Bioaccumulation, Chemical additive, Concentration, Environment, Heavy metals.

INTRODUCTION

Fish constitutes an important aspect of human food due to the high level of quality protein and essential amino acids for the proper growth and functioning of body muscles and tissues. *Clarias gariepinus* inhabit freshwater, it's suitable species for aquaculture because it grows fast and feeds on a large variety of agricultural by-products and can tolerate adverse water quality conditions. Fish are commonly situated at the top of the food chain and therefore, they can accumulate large amount of toxicants (Yilmaz *et al*., 2007). Fish are also considered as one of the most susceptible aquatic organisms to toxic substances present in water (Alibabic *et al*., 2007). Since the fish meat represents a major components of human diet, the presence of heavy metals in the aquatic environment and their accumulation in fish call for concern (Erdogrul and Erbilir, 2007; Alibabic *et al*., 2007; Keskin *et al*., 2007).

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades. Among the various toxic pollutants, heavy metals are particularly severe in their action due to persistence in biological amplification through the food chain (Adami *et al*., 2002; Waqar, 2006; Vutukuru, 2005; Olojo *et al*., 2005; Erdogrul and Erbilir, 2007; Senthil *et al*., 2008; Honggang *et al*., 2010). Heavy metals have long been recognized as serious pollutants of the aquatic system because contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosylene and Jankaite, 2006; Farombi *et al*., 2007).The heavy metals that are toxic to many organisms at very low concentrations and are never beneficial to living beings are Hg, Cd and Pb (Dural *et al*., 2006). Mercury is classified as one of the most toxic metals, which are introduced into the natural environment by human interference (Ishikawa *et al*., 2007). The main sources of

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heavy metal pollution are the agriculture, industry and mining activities (Kumar *et al*., 2007). Organisms develops a protective defense against the deleterious effects of essential and unessential heavy metals and other xenobiotics that produces degenerative changes like oxidative stress in the body (Filipovic and Raspor, 2003; Abou EL-Naga *et al*., 2005). As a result of metal absorption, regulation, storage and excretion mechanisms, the tissue differ in bioaccumulation rates and their roles in these processes (Storelli *et al*., 2006). Due to the presence of metal-binding proteins in some tissues, such as metallothioneins in the liver, they can bioaccumulate significantly higher metal concentrations than other organs (Ploetz *et al*., 2007; Uysal *et al*., 2009). High metal concentrations in the gills can point out the water as the main source of contamination (Bervoets and Blust, 2003). Total metal level in gills have been observed to be influenced by absorption of metals onto the gill surface, and also through complexion with the mucous (Rashed, 2001; Storelli *et al*, 2006; Dural, 2006; Erdogrul and Erbilir, 2007). Production of wholesome aquatic foods demands adequate management of the aquatic environment through effective screening for toxicants for corrective actions.

The objective of this research therefore was to determine different bioaccumulative pattern of some metals in *Clarias gariepinus* as a prelude to advice on the need for effective hazard analysis critical point control application in aquaculture and waste management.

MATERIALS AND METHODS

The Test Chemical

The effluent used for the toxicity test was collected from discharge point of a company that produces chemical additives and emulsions. The collections were made bi-monthly between June 2010 to July 2011, and between the hours of 8.00 am to 9.00 am on the days of sample collection. The samples were kept in the refrigerator to avoid further activities of microorganisms before the experiment commenced. The waste waters were then pooled together to avoid variability in concentration.

The Test Organism

The test organism; *Clarias gariepinus* at their juvenile stage were purchased from a commercial Agricultural farm in Nigeria and transported in a big bowl to the Laboratory. The test organisms were almost of the same size and weight since variability in size may lead to different responses to the effluent of the same concentration.

The test organisms were kept in a large plastic container that has already been washed and rinsed with 5% potassium trioxonitrate to remove any adhered metals and thereafter acclimatized for a period of fourteen days. During this period of acclimatization, renewal bioassay was employed and fish were fed twice daily (12 hourly) with an already formulated fish feed (Copens) with about 40% crude protein content.

The Physico-Chemical Analysis

The physico-chemical analysis of the effluent was carried out prior to the laboratory experiment and it is to quantify the concentrations of the metals and other parameters in the effluent of study using the APHA/AWWA/WEF (1995) Standard method for examination of water and waste waters.

Toxicity Test

After the acclimatization period, range finding test using the ASTM, (2007) method was carried out to determine the definitive concentrations to be used for the evaluation. Renewal bioassay test was employed in the experimental set up.Ten *C. gariepinus* each was placed in six different plastic containers containing well aerated bore-hole water. The fishes were then exposed to chemical additives effluent at concentrations of 0.00 (control), 0.30 mg/L, 0.40 mg/L, 0.50 mg/L, and 0.60 mg/L for 42 days. All the experiments were set up in two replicates. Careful observations were then made to note the number of mortalities of the test organisms.

Digestion of specimen

The specimens were dissected to remove the various organs, which were then kept in the freezer prior to analysis. The dissected parts were oven dried at 70-73°C until constant weight was obtained. The specimens were then grounded to fine powder and stored in desiccators in order to avoid moisture accumulation before digestion. The digestion procedure was carried out as described by Kotze *et al*., (2006). Twenty ml of concentrated nitric acid (55%) and 10ml of perchloric acid (70%) were added to approximately 1g tissue (dry mass) in a 100ml Erlenmeyer flask. The digestion was done on a hotplate (200 to 250^{O}C) until the solutions were clear (Van Loon, 1980). The solutions were then filtered through an acid resistant 0.45mm filter paper and made up to 50ml each with distilled water. The samples were stored in clean glass bottles prior to the determination of the metal concentration using a PYE UNICAM Atomic Absorption Spectrophotometer (AAS). A standard sample, consisting of tuna homogenate (sample IAEA-350) from the International Atomic Energy Agency Marine Environment Laboratory, was prepared and use as a control in

Table 1. Physicochemical Parameters of Chemical Additives Effluent.

Parameters	Chemical Additives Effluent (mg/L)	F. E. P. A. 1991 Specification (mg/L)
Ph	6.7	6.9
DO	2.6	5.0
BOD	0.4	5.0
Total suspended solid	72	30
Oil & Greece	12.5	10.0
Alkalinity	65.0	45.0
Iron	0.6	1.0
Cadmium	ND	<1.0
Chromium	0.05	<1.0
Sulphide	0.25	0.2
Nitrate	3.3	20
Cyanide	ND	20
Lead	9.6	<1.0
Total hardness	52.0	-
Total solid	396	-
Magnesium	0.59	-
Nickel	1.01	-
Copper	0.08	<1.0
TDS	324	-

KEY ND: Not Detected

accordance with the above-mentioned procedures with every set of samples, to ensure accuracy of data through comparison. Analytical standards were prepared from Holpro stock solutions. Prior to use all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 h, rinsed in distilled water, acid-washed in 1 m HCL for another 24 h and rinsed again in distilled water (Giesy and Wiener, 1977)

Statistical Analysis

The obtained data were statistically analyzed by using one way analysis of variance (ANOVA) followed by Duncan multiple range tests as a post-hoc test, with the aid of SPSS 10 computer statistical software package.

RESULTS

Physicochemical Characteristics of Chemical Additives Effluent

The physicochemical analysis parameters of the chemical additives effluent used in this research are shown in table 1. The result of the analysis showed that the effluent is unsafe and deleterious to aquatic organisms when compared with Federal Environmental Protection Agency (FEPA, 1991) standard specifications.

Behavioural Responses

Distress behavioural responses such as erratic swimming, gasping for breath, frequent surfacing, ventral surface turned upward were noticed, these behavioural changes increases as the concentration increases. As the experiment progressed, the test organisms were seen to get weaker, and those that couldn't tolerate the concentrations went into comatose. Normal ehavior was however observed in the control.

Concentration of metals in the organs

The highest concentrations of most of the analyzed metals were recorded in the liver (Table 2), while the lowest ones were in the muscle (Table 3). A significantly higher level of Cu was found in the liver than in other fish organs. This study revealed high levels of Fe in liver while Zinc and Nickel had the highest concentration in the gill (Table 4) than in liver. Manganese and Magnesium were found to reach their maximum level of bioaccumulation in the liver. Accumulation of metals in the gut was also observed to be concentration dependent as in other organs (Table 5).

Table 2. Bioaccumulation of metals in the liver of *Clarias gariepinus* at sub-lethal concentration (+se)

	Metals (mg/L)					
Conc.(%)	**Nickel**	**Copper**	**Zinc**	**Magnesium**	**Manganese**	**Iron**
Control	ND	ND	ND	ND	ND	ND
0.30	0.3002 +0.3002^{a}	0.1072 +0.1073^{a}	5.1460 +0.6100^{a}	16.1208 +0.1612^{a}	1.3075 +0.1307^{a}	8.1812 +3.8181^{a}
0.40	0.1909 +0.1909^{a}	0.4003 +0.2003^{a}	4.2311 +0.4231^{a}	16.0112 +0.4213^{a}	1.2982 +0.1787^{a}	8.6214 +0.0467^{a}
0.50	0.1801 +0.1801^{ab}	0.1865 +0.1865^{a}	4.2142 +0.4214^{a}	16.0064 +0.4213^{a}	1.3201 +0.2914^{a}	10.0859 +1.7123^{a}
0.60	0.1782 +0.1781^{ab}	0.1132 +0.1122^{a}	3.2492 +1.5214^{a}	14.2141 +1.1829^{a}	0.9921 +0.4294^{a}	9.1200 +2.1140^{a}

Table 3. Bioaccumulation of metals in the muscle of *Clarias gareipinus* at sub-lethal concentration (+se)

	Metals (mg/L)					
Conc. (%)	**Nickel**	**Copper**	**Zinc**	**Magnesium**	**Manganese**	**Iron**
Control	ND	ND	ND	ND	ND	ND
0.30	0.5362 +0.5361^{a}	0.0010 +0.0010^{a}	1.4417 +0.1446^{ab}	9.6585 +6.1504^{a}	1.1321 +0.1132^{b}	6.8713 +2.6840^{a}
0.40	0.5993 +0.5993^{a}	0.0022 +0.0012^{a}	4.8559 +1.8556^{a}	7.5444 +3.3526^{a}	1.3101 +0.1327^{b}	8.0015 +0.0840^{a}
0.50	1.0819 +0.1081^{a}	0.3958 +0.0395^{a}	2.0496 +0.2049^{ab}	9.8567 +0.9530^{a}	0.6429 +0.1218^{b}	4.8337 +1.4213^{ab}
0.60	0.8883 +0.8820^{a}	0.2900 +0.1000^{a}	4.6256 +2.3112^{a}	10.7345 +1.7670^{a}	0.9071 +0.0969^{a}	9.4083 +1.1242^{ab}

Means within column having the same alphabet(s) are not significantly different ($P > 0.05$).
Se = Standard error, ND**=** not detected

Table 4. Bioaccumulation of metals in the Gills of *Clarias gariepinus* (+se)

	Metals (mg/L)					
Conc. (%)	**Nickel**	**Copper**	**Zinc**	**Magnesium**	**Manganese**	**Iron**
Control	ND	ND	ND	ND	ND	ND
0.30	1.9016 +0.1016^{a}	0.0010 +0.0000^{a}	9.4218 +0.0421^{ab}	10.3485 +3.1513^{a}	0.0021 +0.0663^{ab}	6.6713 +2.0793^{a}
0.40	2.1193 +0.1935^{a}	0.0010 +0.1000^{a}	12.0552 +1.0556^{a}	10.5424 +3.3506^{a}	0.0100 +0.0121^{b}	7.9015 +0.2784^{a}
0.50	2.4814 +0.0481^{a}	0.3955 +0.0391^{a}	12.1826 +0.5600^{ab}	11.1570 +0.8143^{a}	0.0426 +0.0218^{b}	9.0137 +1.6253^{a}
0.60	3.7883 +0.0378^{a}	0.3308 +0.3308^{a}	11.1252 +1.1012^{a}	12.6797 +1.5081^{ab}	0.1011 +0.1009^{a}	9.7013 +3.0210^{a}

Table 5. Bioaccumulation of heavy metals in the Gut of *Clarias gariepinus* (+se).

	Metals (mg/L)					
Conc. (%)	**Nickel**	**Copper**	**Zinc**	**Magnesium**	**Manganese**	**Iron**
Control	ND	ND	ND	ND	ND	ND
0.30	0.0864 +0.1064^{a}	0.2300 +0.0230^{a}	1.9403 +0.0196^{ab}	11.1235 +2.1204^{a}	0.0921 +0.0162^{b}	4.6713 +2.0193^{a}

Table 5. Cont.

0.40	0.1230 ±0.1935[a]	0.3100 ±0.1390[a]	2.0439 ±1.2048[ab]	10.7444 ±2.3526[a]	0.2001 ±0.1021[b]	5.9015 ±0.2181[a]
0.50	0.1281 ±0.1281[a]	0.1215 ±0.0695[a]	1.0886 ±0.5835[ab]	13.7417 ±0.7431[a]	0.0401 ±0.7018[b]	5.8337 ±1.6223[a]
0.60	0.1001 ±0.2801[a]	0.1708 ±0.1708[a]	4.0201 ±2.4310[a]	14.5001 ±1.5081[a]	0.1014 ±0.9640[a]	4.4083 ±3.1210[a]

Means within column having the same alphabet(s) are not significantly different (P > 0.05). Se = Standard error, ND= not detected

DISCUSSION

The physico-chemical characteristics of the effluent revealed that there were high total suspended solids, high pH level, high total solids, high total hardness and low dissolve oxygen content. This might have resulted from the organic loads in the effluent, which serves as a suitable medium for microorganisms that competes with the test organisms for the utility of the limited available oxygen. Most of the parameters investigated in the physico-chemical characteristics of the effluent showed deviation from the Federal Environmental Protection Agency (1991) safe limit for waste discharge into water bodies.

In this study, the fish exposed to chemical additives effluent were observed to display abnormal responses like erratic swimming, water surface frequently with their opercula and mouths moving rapidly. Activities of test organisms like swimming and feeding reduced drastically and they became very weak since they could no longer feed well. Oxygen depletion in the medium must have been caused by toxic effect of the effluent. The mucus covering the entire body of the test organisms might have resulted from the excretion of some accumulated metals in their tissues and organs.

In the present study, the highest concentrations of most of the analyzed metals was recorded in the liver, while the lowest ones were in the muscle. Such pattern has been observed in a number of other studies, covering several fish species (Rashed, 2001; Dural *et al*., 2006; Storelli *et al*., 2006; Ploetz *et al*., 2007; Pyle *et al*., 2006; Agah *et al*., 2009). Muscle is generally considered to have a weak accumulating potential (Bervoets and Blust, 2003; Erdogrul and Erbilir, 2007; Uysal *et al.*, 2009). High accumulating ability of the liver is a result of the activity of metallothioneins, the proteins that can be binded to some metals, such as Cu, Cd and Zn, thus reducing their toxicity and allowing the liver to accumulate high concentrations (Wu *et al*., 2006; Ploetz *et al*., 2007; Uysal *et al*., 2009). Due to the above discussed reasons, liver has been recommended by many authors as the best environmental indicator of both the water pollution and chronic exposure to heavy metals (Dural *et al.*, 2006; Agah *et al*., 2009; Messaoudi *et al*., 2009).

A significantly higher level of Cu was found in the liver than in other fish tissues which has has also been observed by other authors (Rashed, 2001; Wu, *et al*., 2006; Storelli *et al*., 2006; Farag *et al*., 2007;Yilmaz *et al*., 2007; Uysal *et al*., 2009). According to Pyle *et al*. (2006), the liver Cu concentrations are usually regulated by a homeostatic control below 50 µg g−1 dw, and can exceed this threshold only if the control mechanisms are overloaded. High Cu levels found in the present study might imply loss of regulatory control of liver Cu (Pyle *et al*., 2006).The present study revealed high levels of Fe in liver. Fe has been found to reach maximum concentrations in liver (Dural *et al*., 2006;Yilmaz *et al*., 2007; Uysal *et al*., 2009),. Zinc reached higher levels in the gill than in liver, although Rashed (2001) presented opposite finding. Several studies have determined the highest Zn concentrations in gills (Dural *et al*., 2006;Yilmaz *et al*., 2007). Nickel had the highest concentration in the gill, which agrees with findings of other studies, suggesting the gills as the centre of their accumulation (Rashed, 2001; Storelli *et al*., 2006). Gills could be important as a site of direct metal uptake from water (Storelli *et al*., 2006). High metal concentrations in gills can point out the water as the main source of contamination (Bervoets and Blust, 2003). According to Dural *et al.* (2006) and Erdoğrul and Erbilir (2007), total metal levels in gills can be influenced by absorption of metals onto the gill surface, but also through the element complexion with the mucous, that is very difficult to remove from lamellae prior to the analysis. Manganese and Magnesium were found to reach their maximum level of bioaccumulation in the liver suggesting the liver as the major site for their bioaccumulation. Most of the metals were found in this study to have the least bioaccumulation in the muscle. This is in contrast to the findings of Kotze *et al*., (2006) and Senthil *et al.,* 2008 who reported significant bioaccumulation of metals in fish muscle

It was observed in this study that accumulation of heavy metals in the liver followed the order of Mg >Fe > Zn >Mn> Cu>Ni. In the case of the muscle, the order was Mg > Fe > Zn >Mn> Ni >Cu >.In the gill, the order was Mg >Zn>Fe>Ni>Cu >Mn while in the gut, the order was found to be Mg > Fe > Zn > Cu >Mn> Ni. In all the metals analysed, the bioaccumulation of magnesium, iron and zinc proportion was significantly increased in the liver, gill and gut of *Clarias gariepinus*. The result conformed closely with the work done by Vinodhini and

Narayanam, (2008) where they carefully observed the trend of bioaccumulation of heavy metals in various organs of the fresh water fish *Cyprinus carpio* (common carp) exposed to heavy metal contaminated water system.

The recorded significant differences in the bioconcentration of metals in the fish under study may be attributed to the observed differences in the behavioural and metabolic responses of the fish to the effluent; these differences can also be attributed to the differences in the physiological role of each tissue. It can be conclusively deduced from this study that fish has the tendency to bioaccumulate metals in a polluted environment. Thus the indiscriminate consumption of fish from a polluted water body should be discouraged. Federal government should enact laws that will ensure industries make use of standard waste treatment plants for the treatment of their wastes.

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