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Comparing the Levels of Trace Metal from Two Fish Species Harvested from Treated Waste Water in Pretoria, South Africa

[1]J.O. Olowoyo, [1]S.T.R. Mdakane and [2]O.O. Okedeyi
[1]Department of Biology, University of Limpopo, Medunsa Campus, South Africa
[2]Department of Chemistry, University of South Africa, South Africa

Abstract: The persistent problem of water scarcity with the ever increasing demand of water has necessitated the reuse of effluent in agriculture. The present study evaluated the reuse of treated waste water and bioaccumulation properties of two fish species from a manmade lake. Trace metals content of two fish species: *Clarias gariepinus* and *Cyprinus carpio* and levels of trace metals from waste water in the lake where the fish species were harvested were determined by Inductive Couple Plasma-Optical Emission Spectrometer (ICP-OES). The trace metal values from fish samples ranged between 0.45-4.41 $\mu g\ g^{-1}$ for Cu, 16.45-72.23 $\mu g\ g^{-1}$ for Zn, 1.92-4.71 $\mu g\ g^{-1}$ for Cr, 2.45-5.65 $\mu g\ g^{-1}$ for Ni, 10.23-44.31 $\mu g\ g^{-1}$ for Mn, 9.67-46.59 $\mu g\ g^{-1}$ for Fe and 0.12-0.56 $\mu g\ g^{-1}$ for Pb. The carp exhibited a significantly higher concentration for the trace metals for all the parts analyzed ($p<0.01$). The levels of trace metals concentration from *Cyprinus carpio* was in the order liver>gill>muscle>bone and metal accumulation was in the order Zn>Fe>Mn>Cr>Ni >Cu>Pb. The concentration of trace metals such as zinc, iron, chromium and nickel were higher than the recommended legal limits for human consumption. The result revealed that properly treated waste water could be used for the purpose of aquaculture. *Clarias gariepinus* bio accumulated more trace metals from the lake when compared with *Cyprinus carpio.*

Key words: Trace metals, waste water, pollution, lake, bioaccumulations, ICP-OES

INTRODUCTION

The pollution of water body and the persistent problem of water scarcity with an increased demand for water have necessitated the reuse of effluent in agricultural practices especially in developing countries (Marshall *et al.*, 2007; Alinnor and Obiji, 2010). The inability of different waste water methods to remove trace metals completely from treated waste water has been a source of concern because of the health risk via contamination of water which ultimately enters the food chain (Fytianos *et al.*, 2001). Heavy metal pollution of the aquatic environment (lake, river and sea) has been receiving worldwide attention due to the serious health risk it poses to humans via the intake of sea foods (Asegbeloyin *et al.*, 2010). Waste water may contain various trace metals depending on the source and these may include Zn, Cu, Pb, Mn, Ni, Cr and Cd. Metals may also enter the aquatic environment by atmospheric deposition, erosion of the geological matrix or through anthropogenic sources such as industrial effluents and mining wastes (Alam *et al.*, 2002; Olowoyo *et al.*, 2010).

Fish has been reported to accumulate large amounts of some metals from the water and are often at the top of the aquatic food chain (Mansour and Sidky, 2002). In general, fish is widely consumed by both the low and high income earners because of the easy accessibility through free fishing methods in open lakes and rivers. Fish has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Tuzen and Soylak, 2007). However, fish are constantly exposed to chemicals in polluted and contaminated waters. Fish living in polluted waters may accumulate toxic trace metals via their food chains. Fish living in polluted waters may accumulate toxic trace metals via their food chains. Fish living in polluted water can be a useful tool for determining the level of bioaccumulation of trace metals in fish (Tariq *et al.*, 1991; Mendil and Uluozlu, 2007).

Tuzen and Soylak (2007) reported on the toxicity of elements even at low concentration when consumed for a prolonged period of time. This may also be true with essential metals with an elevated intake within the body (Tuzen and Soylak, 2007). The study determined the level of trace metals from the treated waste water in the lake with a view to determining its overall effect on some of the fish species (*Clarias gariepinus* and *Cyprinus carpio*) present within the lake and to see whether these fish harvested within the lake are good for human consumption.

Corresponding Author: J.O. Olowoyo, Department of Biology, University of Limpopo, Medunsa Campus, South Africa

MATERIAL AND METHODS

Fish Sample collection and analysis: The fish samples used for this study were obtained from the Medunsa Lake (The initial purpose of the lake was to store recycled water that can be used for irrigational purposes within the university community) during the month of February-October 2010 within the University using gill nets (Approval was received from the University authority before carrying out the experiment). Ten *Clarias gariepinus* with an average weight of 85.8±0.35 g and ten *Cyprinus carpio* with an average weight of 12.4±0.23 g were used for the analysis. The fish samples were washed with the water from the lake as this was the usual practice of people harvesting fish from the lake and kept alive in water-filled buckets on the boat. They were transported to the laboratory where they were kept in a holding tank before the subsequent analysis for the trace metal contents. Fish were placed in a polypropylene dissection board and killed by cutting the spinal cord behind the head. Muscle, gill, liver and bones were removed from each fish sample. Each tissue was oven dried at 80°C for 48 h. Dried samples were standardized, enough to fill 50 mL glass bottles in which they were stored after grinding. Samples were stored in the fridge at temperature of -20°C until analysis. Digestion of fish samples was performed using 1.0 g of dried fish samples (muscles, gills, liver and bones) which were weighed in 100 mL Erlenmeyer flasks. 10 mL of HNO_3(65%) and 3 mL of H_2O_2 (65%) were added in each flask with each tissue type. The mixtures were heated up to 150°C for 4 h, after cooling, the mixtures were filtered in round bottom flasks, using a 0.45 µm Whatman filter paper and brought to a volume of 10 mL with deionized water. This procedure was repeated three times with a blank digest carried out the same way. The resulting solution was analyzed for trace metal contents using Inductive Couple Plasma-Optical Emission Spectrometer (ICP-OES) by flame absorption mode. The machine was calibrated over the relevant concentrations using individually certified standards obtained from Sigma-Aldrich, UK.

Water sample collection and analysis: Water sample were collected from three stations on the dam and later stored in the fridge prior to analysis. The analysis for trace metal concentration was performed by adding 1 mL of concentrated HNO_3 (65%) to the water sample of 50 mL on the field in order to prevent or stop microbial activity. In the laboratory, for the purpose of digestion, 9 mL of concentrated HNO_3 (65%) was further added to the solution and heated gently at 70°C until the solution became transparent (APHA, AWWA and WEF, 2005). The resulting solution was filtered and made up to volume by adding distill water and analyzed for trace metals content using ICP-OES.

Statistical analysis: Statistical analyses were carried out using SAS statistical package (SPSS 13.0). Student t-test was used to determine the differences in means recorded for the two fish species. The values obtained were presented as Least Significance Differences (LSD) of means at ($p<0.01$).

RESULTS AND DISCUSSION

The results for the concentrations of trace metals from the fish species are presented in Table 1.

The highest concentrations for all the metals were recorded for zinc from the gill of *Cyprinus carpio* with a mean value of 72.23±0.89 µg g^{-1} (Table 1). The lowest value for this metal was recorded from the bone of *Clarias gariepinus* with a value of 16.45±0.25 µg g^{-1}. Zinc is a component of more than fifty enzymes and required in large quantity. The high concentration of zinc in the gill could be associated with the bio accumulation of this metal from the treated waste water over a prolonged period of time and also as a result of the natural abundance of this metal in the environment (Olowu *et al*., 2010). The concentrations recorded for zinc in our study were higher than those reported in the literature. Zinc concentrations have been reported to be within the range of 45.0-60.9 µg g^{-1} in literatures (Park and Presley, 1997; Mendil and Uluozlu, 2007). However, when compared with the WHO standard and reports from other countries, the concentration of zinc from the gill of *Cyprinus carpio* exceeded the permissible limit for human consumption (Woodward *et al*., 1997).

The concentrations of iron ranged from 9.67±0.24 µg g^{-1} to 44.08±0.01 µg g^{-1} from the two fish species. The highest concentration was recorded from the liver of *Cyprinus carpio* while the lowest concentration was recorded from the bones of *Clarias gariepinus* (Table 1). Iron is required in the diet as prevention towards anemia which is often common among the low income earners. The level of iron from the liver and gill of these fish species were slightly higher than the recommended limit (Anonymous, 2002). However, the recorded concentrations for iron from the present study were lower than those reported by Karadede *et al*. (2004) and Chale (2002) of 200.86 µg g^{-1} and 125 µg g^{-1}, respectively.

Lead concentrations ranged between 0.12±0.01 µg g^{-1} and 0.56±0.01 µg g^{-1} (Table 1). The highest value recorded for this metal from the gill of *Cyprinus carpio* was slightly above the recommended

Table 1: Mean Trace metal concentration from Clarias gariepinus and Cyprinus carpio

	Metals						
Parts	Pb	Zn	Cu	Cr	Mn	Fe	Ni
Clarias gariepinus							
Gill	0.25±0.02	30.10±0.32	0.75±0.06	3.95±0.87	19.04±0.02	12.33±0.32	3.61±0.12
Muscle	0.12±0.01	17.36±0.12	0.65±0.01	3.88±0.65	13.75±0.01	10.81±0.25	3.34±0.32
Bone	0.15±0.01	16.45±0.25	0.62±0.01	1.92±0.25	10.23±0.03	9.67±0.24	2.56±0.02
Liver	0.23±0.02	53.40±1.23	4.45±0.69	4.15±0.36	16.81±0.01	26.59±0.26	5.65±0.05
Cyprinus carpio							
Gill	0.56±0.01	72.23±0.89	1.20±0.01	4.71±0.02	44.31±0.69	23.16±0.02	3.81±0.02
Muscle	0.35±0.02	30.10±0.02	0.92±0.01	4.50±0.01	36.65±0.32	14.13±0.02	3.45±0.01
Bone	0.22±0.01	26.65±0.12	0.45±0.03	2.43±0.01	23.35±0.23	14.05±0.03	2.45±0.05
Liver	0.29±0.01	32.54±0.65	1.05±0.01	3.56±0.01	23.05±0.12	44.08±0.01	2.89±0.01

Table 2: Mean trace metal concentration from water samples

	Metals						
Stations	Pb	Zn	Cu	Cr	Mn	Fe	Ni
1	0.15±0.01	3.25±0.14	5.40±0.08	0.90±0.02	3.55±0.21	10.50±0.08	0.65±0.01
2	0.25±0.02	5.75±0.03	5.60±0.12	11.20±0.03	8.10±0.24	18.50±0.12	1.50±0.00
3	0.21±0.01	5.55±0.09	5.80±0.23	2.55±0.02	4.05±0.08	14.50±0.07	1.05±0.05

limit of 0.50 $\mu g\ g^{-1}$. Park and Presley (1997) determined trace metal levels in fish samples and their values for lead ranged between 1.95-4.79 $\mu g\ g^{-1}$. These values are higher than our lead values. The findings reported by Tariq *et al*. (1994) showed that our lead values are lower than those reported in literature. Lead concentrations in the fish parts followed the same trend noted for all other metals especially for *Cyprinus carpio* with bone having the lowest concentration. Higher concentration of lead is known to inhibit active transport mechanisms involving ATP and may also suppress cellular oxidation-reduction reactions and even inhibit protein synthesis (Adeyeye *et al*., 1996). The level of lead from this study could not be said to pose any health risk since the values were within the permissible limit. The values recorded for lead were significantly higher ($p>0.01$) than those reported for the water samples (Table 2). These may be attributed to bioaccumulation efficiency of these metals over time.

Manganese assists in reproduction and normal functioning of the nervous system. The concentrations of manganese from the fish species showed significantly higher concentrations for *Cyprinus carpio* over the *Clarias gariepinus* in all the fish parts ($p<0.01$). The highest concentration of 44.31±0.69 $\mu g\ g^{-1}$ was recorded from the gill of *Cyprinus carpio* as against 19.04±0.02 $\mu g\ g^{-1}$ from the gill of *Clarias gariepinus* (Table 1). The result from our study showed that the concentrations of manganese are lower than those reported in the literature. Mendil *et al*. (2005) reported a range of 11.1 $\mu g\ g^{-1}$ -72.9 $\mu g\ g^{-1}$.

Copper concentrations ranged from 0.45±0.03 $\mu g\ g^{-1}$ to 4.45±0.69 $\mu g\ g^{-1}$ (Table 1). The trend in copper concentration showed that liver of *Clarias gariepinus* accumulated more of this metal than any other parts. The values recorded for this element from all the parts were clearly within the permissible limit set for human consumption. Demirak *et al*. (2006) reported a permissible level of 5 $\mu g\ g^{-1}$ for this element. The higher concentration recorded for copper from the liver of *Clarias gariepinus* may be attributed to its role as a protein carrier (Gbem *et al*., 2001).

The ranges for the concentrations of Cr were 1.92- 4.71 $\mu g\ g^{-1}$ while Ni was from 2.45-5.65 $\mu g\ g^{-1}$ (Table 1). The concentrations of these elements were clearly above the standard set for human consumption (Raphael *et al*., 2011).

The result of trace metals content from the treated waste sample collected from three stations within the lake is presented in Table 2. The trace metal concentration in the treated waste water was in the order Fe>Cr>Mn>Cu>Zn>Ni>Pb. The highest value for trace metals from the treated waste water was recorded for Fe with a value of 18.50±0.12. The lowest value was recorded for lead with a value of 0.15±0.01. From the result obtained for trace metal concentration from the treated waste water, it was gathered that station 2 presented the highest value for all these metals. This may be attributed to the nature of the construction of the lake, as this happened to be the deepest part of the lake hence the accumulation of trace metals from this area. When compared with result from literature, the level of trace metals form the treated waste water were comparable with those reported in literature (Raphael *et al*., 2011).

The pH of the treated waste water from the lake was in the range 4.5-5.2. This indicates the lake might be considered to be moderately acidic. This may account for the high concentration of trace metals such as Fe, Zn and Cd from some of the fish organs in this study. Water acidification affects metal accumulation rate by fish because it changes the solubility of metal compounds. Cogun and Kargin (2004) reported an increased concentration of cadmium and lead from fish harvested from an acidic lake.

The distribution pattern of trace metals from the different organs of *Cyprinus carpio* were in the order gill>liver>muscle>bone. The distribution pattern in *Clarias gariepinus* followed the order liver>gill>muscle>bone. From the two fish species, the values recorded for the muscle and bone were lower than the values recorded for either the gills or muscles and the differences in the concentration were significant ($p<0.05$). The gills and liver accumulated more of these trace metals. The accumulation of trace metals in the liver could be traced to the metabolic processes and enzyme catalyzed reaction taking place in the liver. The liver might have also played an important role in detoxification and this detoxification from the two fish species may be by sequestration rather than elimination by excretion (Gbem *et al*., 2001). In addition, the entry of metals into fish occurred either through the gill membrane or through ingestion and this may have accounted for the high concentration of trace metals in the gills of both fish species (Chatterjee *et al*., 2006).

The present study found metal accumulation to be low in muscles of *Clarias gariepinus*. This might be attributed to the growth factor as growth may dilute toxicant concentration if growth is faster than accumulation (Gbem *et al*., 2001). The other probable reason for the higher concentrations of trace metals from the *Cyprinus carpio* could be attributed to the differences in feeding habit, *Cyprinus carpio* is a bottom feeder and as such bottom sediments contain higher concentrations of metals than that of overlying water (Adeniyi and Yusuf, 2007) and this might have attributed to the accumulation of these metals in *Cyprinus carpio*. The accumulation order may also be dependent on the body weight of the fish since *Clarias gariepinus* which has the bigger body weight showed the lowest concentration of these heavy metals.

CONCLUSION

The pollution status of the fish immediate environment can accentuate its metal accumulation. From the results obtained from this study, the concentration of trace metals found in the water from the lake is low when compared with the level of trace metals from the fish species, this may be as a result of bioaccumulation over a prolonged period of time. Some parts of the fish (liver and gill) collected from the lake might not be good for human consumption going by the level trace metals content recorded. However, the gills and liver are parts that are less or not consumed by the people so the risk associated with the fish collected from the lake might not pose a serious threat especially if *Clarias gariepinus* is consumed. In view of water scarcity in the country, appropriate measure such as effective monitoring programme should be encouraged so that the lake does not become acidic and hence will not only supply water for irrigational purposes but also fish could be harvested safely from the lake.

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