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*Full Length Research Paper*

# Assessing the levels of trace metal from two fish species harvested from treated waste water stored in a manmade lake Pretoria, South Africa

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**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 and 4.41 µg/g for Cu, 16.45 and 72.23 µg/g for Zn, 1.92 and 4.71 µg/g for Cr, 2.45 and 5.65 µg/g for Ni, 10.23 and 44.31 µg/g for Mn, 9.67 and 46.59 µg/g for Fe and 0.12 and 0.56 µg/g 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 in *C. carpio* was in the order of liver > gill > muscle > bone and metal accumulation was in the order of Zn > Fe > Mn > Cr > Ni > Cu > Pb. The concentration of trace metals such as zinc, iron, chromium and nickel in fish samples were higher than the recommended legal limits for human consumption. The result reveals that properly treated waste water could be used for the purpose of aquaculture.**

**Key words:** Trace metals, waste water, pollution, lake, bioaccumulations inductive couple plasma-optical emission spectrometer (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; Allinor 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 associated with contamination of water which ultimately enters the food chain (Fytianos et al., 2010). 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 (Tunzen and Soylak, 2007). However, fish are constantly exposed

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**Table 1.** Mean of trace metal concentration of *C. gariepinus and C. carpio.*

| Species | | Metal | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pb | Zn | Cu | Cr | Mn | Fe | Ni |
| *C. 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 |
| *C. 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 |

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 water can be a useful tool for determining the level of bioaccumulation of trace metals in fish (Tarrio 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 long period of time. This may also be true with essential metals with an elevated intake in the body (Tuzen and Soylak, 2007). This study determined the level of trace metals of 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.

## MATERIALS 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 to October, 2010 within the University using gill nets (approval was received from the University authority before carrying out the experiment). Ten *C. gariepinus* with an average weight of 85.8 ± 0.35 g and ten *C. 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 prepared and samples were used to fill 50 ml glass bottles in which they were stored after grinding. Samples were stored in the freezer 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 to 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 in 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, 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 2005). The resulting solution was filtered and made up to volume by adding distilled 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 and water samples. The values obtained were presented as least significance differences (LSD) of means at $p<0.01$.

## RESULTS AND DISCUSSION

The results of the concentrations of trace metals from the fish species are presented in Table 1. Lead concentrations ranged between 0.12 ± 0.01 and 0.56 ± 0.01 µg/g (Table 1). The highest value recorded for this metal from the gill of *C. carpio* was slightly higher than the recommended limit of 0.50 µg/g (Woodward et al., 1997). Park and Presley (1997) determined trace metal levels in fish samples and their values for lead ranged between 1.95 and 4.79 µg/g. These values are higher than our lead values. The values reported for lead from the

**Table 2.** Mean of trace metal concentration of water samples.

| Station | Metal | | | | | | |
|---|---|---|---|---|---|---|---|
| | 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 |

present study are lower than those reported in literature (Tariq et al., 1994). Lead concentrations in the fish parts followed the same trend noted for all other metals especially for *C. 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 this metal over time.

The highest concentrations for all the metals were recorded for zinc from the gill of *C. carpio* with a mean value of 72.23 ± 0.89 µg/g (Table 1). The lowest value for this metal was recorded in the bone of *C. gariepinus* with a value of 16.45 ± 0.25 µg/g (Figure 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 long period of time and also as a result of the natural abundance of this metal in the environment (Olowu et al., 2009). 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 and 60.9 µg/g in literatures (Park and Presley, 1997; Mendil and Uluözlü, 2007). However, when compared with the WHO standard and reports from other countries, the concentration of zinc from the gill of *C. carpio* exceeded the permissible limit for human consumption (Woodward et al., 1997).

Copper concentrations ranged from 0.45 ± 0.03 to 4.45 ± 0.69 µg/g (Table 1). The trend in copper concentration showed that liver of *C. gariepinus* accumulated more of this metal than any other part. 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 µg/g for this element. The higher concentration recorded for copper in the liver of *C. 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 to 4.71 µg/g while Ni was from 2.45 to 5.65 µg/g (Table 1). The concentration of this element was clearly higher than the standard set for human consumption (Ekeanyanwu et al., 2011).

Manganese assists in reproduction and normal functioning of the nervous system. The concentrations of manganese in the fish species showed significantly higher concentrations for *C. carpio* over the *C. gariepinus* in all the fish parts ($p < 0.01$). The highest concentration of 44.31 ± 0.69 µg/g was recorded in the gill of *C. carpio* as against 19.04 ± 0.02 µg/g in the gill of *C. 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 to 72.9 µg/g.

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

The result of trace metals content of the treated waste sample collected from the three stations within the lake is presented in Table 2. The trace metal concentration in the treated waste water was in the order of 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 design used for the lake, as this happened to be the deepest part of the lake hence the accumulation of trace metals in 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 (Ekeanyanwu et al., 2011).

The pH of the treated waste water from the lake was in the range of 4.5 to 5.2. This indicates that 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 in fish harvested from an acidic lake.

The distribution pattern of trace metals from the different organs of *C. carpio* were in the order of gill > liver > muscle > bone. The distribution pattern in *C. gariepinus* followed the order of 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 gill or liver and the differences in the concentration were significant ($p < 0.05$). The gill 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 in 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 showed that metal accumulation was low in muscles of *C. 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 *C. carpio* could be attributed to the differences in feeding habit, *C. carpio* is a bottom feeder and as such, feed on bottom sediments containing higher concentrations of metals than that of overlying water (Adeniyi and Yusuf, 2007), and this might have contributed to the accumulation of these metals in *C. carpio*. The accumulation order may also be dependent on the body weight of the fish since *C. gariepinus* which has the higher 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 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 long period of time. Some parts of the fish (liver and gill) collected from the lake might not be good for human consumption as a result of the level of trace metals content recorded.

However, the gills and liver are parts that are less or not consumed by the people, so consumption of the fish collected from the lake might not pose a serious threat especially if *C. 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 will not become acidic, and hence will not only supply water for irrigation purposes but also fish could be harvested safely from the lake.

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