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Effects of selected heavy metals on morphology of *Oreochromis niloticus* and *Clarias gariepinus* along Ruiru River, Kenya

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Abstract. *Ouma KO, Otieno SA, Sharma RR 2019. Effects of selected heavy metals on morphology of Oreochromis niloticus and Clarias gariepinus along Ruiru River, Kenya. Bonorowo Wetlands 9: 92-107.* The objective of this study was to determine the levels of heavy metals in tilapia and catfish species along the Ruiru River. Sediments, water, and fish samples were collected using systematic random sampling techniques. Three sites were established downstream of the river, 1000 meters from Ruiru Town. The other three were established upstream of the river, 1000 meters from Ruiru Town. Fish samples were transported to the National Museums of Kenya laboratory for identification. An atomic absorption spectrophotometer was used to examine metals. Fish gills and livers were also examined for histopathological changes. Using one-way analysis of variance, researchers analyzed data on heavy metal levels in the water, sediments, fish gills, livers, and muscles. Correlation coefficients were also calculated to assess the relationship between fish length and weight and metal concentrations in fish liver, gills, and muscles and between levels of heavy metals in water and sediments. In April, 0.167±0.014 mg/L, August, 0.054±0.003 mg/L, and December, 0.222±0.101 mg/L ($F = 2.10$, $p = 0.202$), mean chromium levels in water were not statistically significant, but were statistically significant at the downstream sites 0.236±0.019 mg/L, 0.058±0.001 mg/L, and 0.222±0.101 mg/L during the three months ($F = 125.63$, $p<0.001$). Significant positive correlations between the levels of iron in sediments and water were found at both upstream and downstream sites, with $r = 0.7319$, $p = 0.025$, and $r = 0.8506$, $p = 0.0037$, respectively. The linkage between lead levels in sediments and water at upstream sites was not significant ($r = 0.343$, $p = 0.366$), while it was significant ($r = 0.7523$, $p = 0.019$) at downstream sites. Chromium levels in sediments and water at upstream sites indicated a positive but non-significant connection ($r=0.5339$, $p=0.138$). There was a substantial positive correlation between chromium levels in sediments and water ($r = 0.9787$, $p<0.001$). Metals accumulated in fish tissues in the following order: liver>gills>muscle, and *Clarias gariepinus* tissues from downstream sites exhibited higher amounts of metals than *Oreochromis niloticus* tissues from the same sites. Both kinds of fish exhibited higher iron levels than lead in all tissues, with chromium being the lowest. The liver and gills of fish from the upstream research sites had normal features on histology. Hepatocytes with larger nuclei were found in fish livers from downstream sites. The secondary lamellae of the gills had degenerated and fused. According to the study, metal levels in sediments were greater than in water. Fish from the downstream sites also showed morphological alterations in the liver and gills. The study's findings suggest that people who eat such fish may be exposed to metal poisoning. According to the report, the National Environment Management Authority should implement measures to reduce industrial trash flow into the Ruiru River.

Keywords: *Clarias gariepinus,* heavy metals, morphology, *Oreochromis niloticus,* Ruiru River

INTRODUCTION

Ruiru Town is located on the banks of the Ruiru River, which originates on the slopes of the Aberdare Ranges. Along the channels and in the catchments, it is exposed to pollutants and experiences various anthropogenic consequences (Bundambula and Mwachiro 2006). Intensive agriculture, animal husbandry, silviculture, horticulture, charcoal burning, and quarrying are the principal human activities in the catchment (UNEP 2001). Large amounts of contaminants are carried downstream by water drainage from upstream districts (Mwenda and Guthiga 2010). Agricultural runoff containing organic wastes, fertilizers, pesticides, and weed killers is thrown into the river, posing a concern (UNEP 2001). The river continues to be polluted as it passes through the highly populated Ruiru Municipality, which lacks sufficient sewage infrastructure, a wastewater treatment facility, and solid waste disposal systems (UNEP 2001). Residential areas, industrial zones, the marketplace, and the commercial central business district are the primary wastewater sources in Ruiru Town (CBD). In addition to agricultural operations, Ruiru town has several factories, including Devki Steel Mills, Spinners and Spinners Garment Factory, and Ruiru Feeds (EIA Report 2011). Most of the town's wastewater is dumped into the Ruiru River with little or no treatment. In aquatic ecosystems, the presence of heavy metal pollutants over natural loads has become a growing concern (UNEP 2001). The rapid growth of populations, industrial development and the discharge of untreated industrial wastes, growing urbanization, expansion of natural resources, irrigation expansion, and contemporary agricultural methods have all contributed to this predicament (UNEP 2001). Concerns regarding the health of aquatic species as a result of pollution have grown in recent years. Pollution of rivers has been caused by industrial effluents, raw sewage, and trash from human settlements along rivers, posing a health risk to those communities and putting a strain on the aquatic ecosystem (UNEP 2001).

The global food crisis has impacted Kenya and other African countries. According to a Food and Agriculture Organization report, high food costs are a global issue (Ministry of Agriculture 2009). The urban poor and small-scale farmers have been identified as two of the most vulnerable populations. Fish like tilapia, catfish and common carp have been reported in the Ruiru River (UNEP 2001). Anecdotal evidence suggests that fishing occurs on occasion along the river. Fish in the aquatic food chain bioaccumulate and store organic and inorganic contaminants in organs like the liver, gills, and kidneys, which can then be transferred down the food chain to other organisms consuming fish, such as birds. Fish play a crucial role in maintaining the biological balance of plankton colonies and other aquatic invertebrates. They can concentrate huge amounts of metals from the water (Abdulali et al., 2011).

The aims of this study were (i) To determine the concentration of lead, iron, and chromium in water and sediments in Ruiru River, Kenya (ii) To determine lead, iron, and chromium concentrations in the gills, liver, and muscles of *Clarias gariepinus* and *Oreochromis niloticus* in the Ruiru River, (iii) To assess the morphological effects of lead, iron, and chromium on the gills and liver of tilapia and catfish in Ruiru River.

MATERIALS AND METHODS

Study area

The research was conducted near the Ruiru River in Kiambu County, Kenya. During the investigation, six major sampling locations along the river were sampled. The river rises on the Aberdare Range's foothills and passes past various coffee farms before reaching Ruiru Town. Ruiru Town is situated in a transitional zone on the border between the Upper Athi Basin and the Kikuyu Plateau. The topography is undulating in general, with a drainage system that leads to the Athi river basin. The township is split in two by the Ruiru River. The town's geography is mainly hilly to the north-west, and the Mukuyu and Ruiru Rivers cut through it (EIA Report 2011). These areas are characterized by housing and industrial growth in addition to agriculture activities (EIA Report 2011). Despite the enormous population, many informal settlements along the river with no adequate sewage or solid waste disposal infrastructure. The river also runs through locations where certain industries pour their waste (UNEP 2001). As a result, human activities along the Ruiru River impact wildlife living in the river's downstream zones. Longitudes 36055'52” and 3701'18” and latitudes 107'54” and 109'50” were used to sample the area (Figure 1). The climate can be classified as tropical. The climate and temperatures within the research region are influenced by height because these sites are located on the slopes of the Aberdare Range, with colder zones to the north and drier zones to the south. The average annual rainfall ranges from 600mm to 1100mm. Temperatures are generally hot, with an average yearly temperature of 18°C to 20°C. The landscape in the research region is very gentle, with a general descent towards the Athi River.

However, the higher hillnorthwestorth west has a deeply dissected topography with numerous streams and ridges, while the lowlands to the southeast have fewer streams, shallower, and broader valleys. The average elevation is around 1520 meters above sea level. Agriculture is an important source of income in Ruiru. The county government's agricultural activities include crop and animal husbandry, livestock sale yards, county abattoirs, plant and animal disease management, and fisheries (Kiambu County Government Report 2014). Climate conditions are favorable for important cash crops such as coffee and horticulture (Makokha et al. 2001). Crops are farmed for both personal and commercial interests. The main cash crop cultivated in this area is coffee, which is processed locally, and the excess is exported. Horticulture and floriculture farming, which is mainly done in greenhouses, are also conducted by some farmers. Red Lands Roses, for example, based in Ruiru since 1996, specializes in cultivating and exporting T-Hybrid and spray roses in over 100 kinds in greenhouses. Cereals like maize and beans are mainly used for household consumption, but surpluses are frequently shipped to surrounding areas like Nairobi and Thika. Dairy cattle are raised to produce milk and other dairy products. Following the government's Economic Stimulus Program (ESP), fish aquaculture has developed in this area (The Star 2011). Ruiru is well-covered by industries that have supplied labor and contributed to the town's economic development. There are currently various flower businesses and coffee factories in operation, which both commercial enterprises and cooperative societies hold. The majority of these factories are located around the Ruiru River. Textiles, plastics, chemicals, food processing, and steel industries are the key industries of Ruiru. Study design

The research design used in this study was comparative. Gill nets were used to catch the fish, and plastic bottles collected water samples. Lead, iron, and chromium levels in water and sediment samples obtained from sampling locations along the Ruiru River's downstream were compared to levels in samples collected from three study sites upstream of Ruiru Town, which served as a control. The amounts of lead, iron, and chromium in the gills, liver, and muscles of *O. niloticus* and *C. gariepinus* were determined. Fish tissues and organs were compared to those from reference sites on the upstream parts of the river that were not exposed to high amounts of pollution. The morphology of the gills and liver of fish sampled from the downstream components of the river was evaluated, and a comparison was performed with fish sampled from the reference sites.

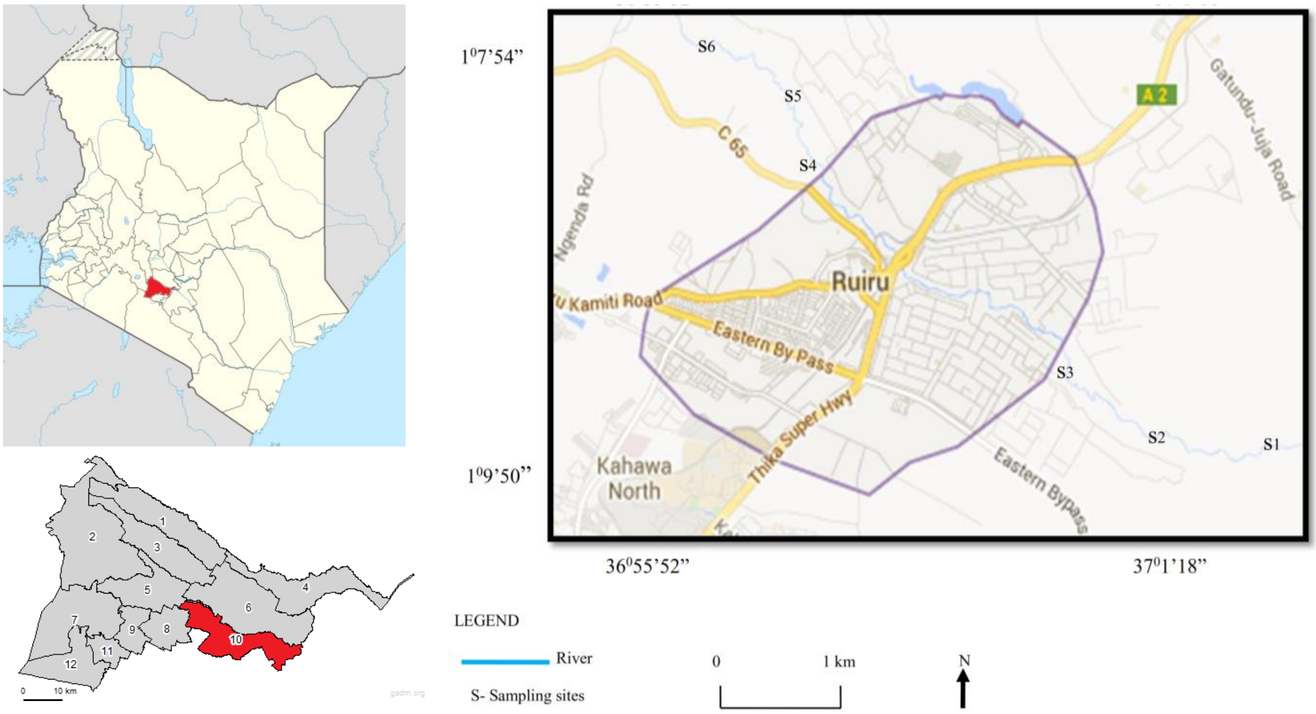

Figure 1. Map showing sampling sites along the course of Ruiru River in Kiambu County, Kenya

Sampling and sample size

Concerning Ruiru Town, the course of the Ruiru River was partitioned into downstream and upstream areas, with three sampling sites chosen in each area, for a total of six sampling sites along the river, S1, S2, S3, S4, S5, and S6 (Figure 1). The first, second, and third sampling sites, designated as S1, S2, and S3, were positioned 1000 meters downstream of Ruiru Town. In contrast, the three control sites, designated as S4, S5, and S6, were located 1000 meters upstream of Ruiru Town. The sampling points were separated by 100 meters. Locations of effluent discharge into the river, ecosystem types, points of confluence between the Ruiru River and other rivers, and sample site proximity to settlements were all considered while selecting sampling sites. The physical appearances of the river water, land-use patterns, economic activity, and the physical look of the river water were all considered. Each sampling location measured 100 meters in length.

The distance between sampling sites was set at 100 meters before the survey began, based on the levels of pollution, the geology of the area, and the processes that influence the current sediment distribution, such as erosion, transportation, and accumulation. During the preliminary studies, sites with sufficient samples were purposefully identified. During each month of the study, six water samples, six composite sediment samples, and eighteen samples of each fish species were taken from the river.

During December 2012, April 2013, and August 2013, samples were collected once a month. The three months were chosen to account for seasonal changes in iron, lead, chromium levels, sediments, and fish tissues investigated in water, sediments, and fish tissues.

Water quality parameters

During each sampling water and sediments session, water quality parameters were determined at each sampling site. Hanna's digital meter was used to measure temperature, pH, and electrical conductivity.

Collection of water samples

Each sample bottle was dipped 30 centimeters below the water surface, and the open end of the container was projected against the flow direction during sampling. Water samples from each site were collected in clean 250-milliliter plastic bottles for heavy metals analysis.

Concentrated nitric acid was applied to lower the pH to 2 and preserve the water samples until they were examined for iron, lead, and chromium at Kenyatta University's Department of Chemistry. Collection of sediment samples

The Riverbank sampled sediments at the water sampling locations. These were taken directly from the surface (5–20 cm) with a hand-held trowel. At each sampling site, two equal amounts of sediment were obtained and homogenized thoroughly in a plastic container to create a combination representative of the area tested, yielding a total of six composite samples from the downstream and upstream research sites throughout the Ruiru River's course. Surface water was decanted from the sample during the operation while keeping the fine sediment component. The composite sediment samples were then transferred to designated plastic containers using a plastic laboratory spoon.

Sampling of fish

Fish samples were collected using gill nets. The nets were 20 feet long and 5 feet high, with 3-inch mesh, and were made with floats on the top horizontal line and weights on the bottom horizontal line. The nets were dropped down at the sampling sites at 9.00 a.m. and picked up at 3.00 p.m. Fish samples were sent to Kenya's National Museums for identification. *O. niloticus* and *C. gariepinus* were the targeted fish. During each month of sampling, three fish of each species were collected from each of the six sampling sites, for a total of eighteen samples of each fish species. The total length of each fish was measured in centimeters, and its wet body weight was determined in grams using a digital electronic balance immediately after collection. The studied fish ranged from 15 to 30 centimeters in length and weighed between 30 and 180 grams.

Preparation and analysis of heavy metals in water samples

To ensure that metals do not attach to the container's walls, a preservative, nitric (V) acid (HNO_3), was added to the original sample. After vigorous shaking to guarantee suspension of any materials that may have settled, sample aliquots for digestion were obtained. On a heated plate, water samples were digested with hydrochloric acid (HCl) and HNO_3 in a volume: volume ratio (1%: 0.5%). At Kenyatta University's Department of Chemistry, digested samples were filtered, diluted to 100 cm3, and then evaluated for lead, iron, and chromium using a Buck Scientific Atomic Absorption Spectrophotometer model 210 VGP. Each sampling station provided three water samples, which were evaluated. Preparation and analysis of heavy metals in sediment samples

In the laboratory, samples were air-dried at room temperature. After air-dried, sediment samples were pulverized and sieved using a 160 m sieve. The sediment samples were then weighed and placed in digestion flasks with 10 milliliters of HNO_3/HCl (1:3 v/v) and digested in the fume chamber on a hot plate. At Kenyatta University's Department of Chemistry, samples were evaluated for lead, iron, and chromium using a Buck Scientific Atomic Absorption Spectrophotometer model 210 VGP.

Preparation and analysis of heavy metals in fish tissues

Muscle, gills, and liver were removed from the fish and dried separately in an oven at 80°C for two days to achieve a constant dry weight. These were pulverized with a porcelain pestle and mortar (Poldoski, 1980). Half a gram of powdered muscle and gill samples, as well as 0.1 gram of dry weight liver samples, were digested with 3 milliliters of HNO3 (65%) and 1-milliliter hydrogen peroxide (35 %). The samples were then transferred to volumetric flasks and diluted with deionized water to 50 milliliters for muscle and gills and 25 milliliters for liver samples due to the smaller quantity of liver samples, then filtered with Whatman filter paper. Using an Atomic Absorption Spectrophotometer, the concentrations of lead, iron, and chromium were determined. The amounts of these metals in water, sediment, and fish tissues were compared in the downstream and upstream sections of the Ruiru River and the maximum allowable levels set by the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA).

Histological procedures

Fish were dissected, and sections of gills and liver were removed and fixed for 48 hours in Bouin's solution. The tissues were dehydrated in four steps using a graded series of ethanol solutions in glass vials: 50 %, 70 %, 90 %, and two changes of 100 % ethanol. Xylene was used to remove ethanol from the tissues. Tissues were then paraffin-infiltrated, embedded in paraffin wax blocks, and sectioned at 5 microns using a microtome. To remove creases, cut sections were floated in a water bath kept at 56°C, then picked up on glass microscope slides. Paraffin wax was removed from the tissue by running the slides through xylene before staining. Tissues were then hydrated in increasing ethanol concentrations, stained with hematoxylin and eosin stains, and viewed under a light microscope at magnifications of 150 and 400.

Control

Comparison of levels of iron and lead in water and sediments collected from the downstream sections of the Ruiru River with levels in samples obtained from the control sections of the river identified as S4, S5, and S6 (Figure 1) based on the assumption that they were not contaminated with lead, iron, and chromium arising from industrial wastes and human activities in Ruiru Town. Fish liver and gill histology sections from downstream river sections were compared to those from the control sites.

Data analysis

One-way analysis of variance was used to examine lead, iron, and chromium levels in water, sediments, fish gills, liver, and muscles. The correlation coefficient was also performed to analyze the relationship between biotic parameters such as fish weight and length and metal concentrations in the gills, liver, and muscles of the fish. It was also utilized to establish a link between heavy metal concentrations in water and sediments and fish gills, liver, and muscles.

RESULTS AND DISCUSSION

Water quality parameters

The lowest temperature values along the Ruiru River were reported in April at upstream study sites (20.33 ± 0.14°C) and downstream study sites (21.22 ± 0.17°C) (Table 1). There was no statistically significant variation in water temperature between the upstream and downstream study locations along the river during this month, $p = 0.001$. Temperature values were found to be substantially lower at upstream study sites (20.89 ± 0.18°C) than downstream study sites (21.67 ± 0.12°C) throughout August, $p = 0.0025$. The highest temperatures were reported at the upstream (23.33 ± 0.12°C) and downstream (24.22 ± 0.17°C) study locations during December (Table 1). During December, the temperature differences between the two research sites were likewise statistically significant (p = 0.0005). At both the upstream (F = 112.54, p < 0.001) and downstream (F = 110.78, p < 0.001) study sites, the mean differences in temperature values throughout the three months were found to be statistically significant (Table 1).

The pH of the water in the Ruiru River was found to be mildly acidic, with the lowest mean values recorded in April at the upstream (5.97 ± 0.02) and downstream (5.87 ± 0.02) research sites. The pH difference between the two study sites was statistically significant (p = 0.0026). The greatest values were found at the upstream (6.79 ± 0.01) and downstream (6.53 ± 0.02) research sites along the river during August. In August, the changes in pH mean values were likewise considerably different (p < 0.001). In December, the pH value at the downstream study site (6.10 ± 0.07) was found to be substantially lower than at the upstream study site along the river (6.51 ± 0.07), p < 0.001). Seasonal fluctuations in pH mean values were statistically significant at both the upstream (F = 99.58, p < 0.001) and downstream (F = 59.14, p < 0.001) study sites along the river during the three months (Table 1).

The lowest mean electrical conductivity (EC) values in Ruiru River were obtained in April at upstream study sites (424.00 ± 0.88 S/cm) and downstream research sites (592.11 ± 1.72 S/cm). Differences in electrical conductivity mean values were statistically significant (p 0.001). The mean electrical conductivity at upstream study sites along Ruiru River was 761.11 ± 8.80 S/cm in August, while the value at downstream study sites was 1003.56 ± 11.57 S/cm. The mean electrical conductivity differences between the two sites were found to be statistically significant (p < 0.001) (Table 1). During December, the downstream sites had the highest electrical conductivity values. The mean values of electrical conductivity recorded at the upstream (839.78 ± 10.91 S/cm) and downstream study locations (1162.00 ± 10.31 S/cm) were significantly different in December (p < 0.001). There were also significant differences in mean electrical conductivity values at upstream study sites (F = 741.83, p < 0.001) and downstream study sites (F = 1067.07, p < 0.001) along the river throughout April, August, and December (Table 1).

The mean total dissolved solid (TDS) value recorded at upstream study sites in April (34.67 ± 0.94 mg/L) was statistically lower than the values recorded at downstream study sites (72.11 ± 1.48 mg/L) (p < 0.001). TDS mean values at the upstream (38.89 ± 1.21mg/L) and downstream (56.11 ± 1.27mg/L) research locations were likewise statistically significant (p < 0.001) in August. The mean TDS values at the downstream study sites (68.00 ± 1.41 mg/L) were substantially higher in December than at the upstream study sites along Ruiru River (54.89 ± 0.75 mg/L), p < 0.001. Seasonal fluctuations in mean TDS values in Ruiru River throughout the three months differed considerably both upstream (F = 117.16, p < 0.001) and downstream (F = 35.69, p < 0.001) study sites (Table 1).

Levels of iron in sediments along Ruiru River

Between April and December, there was an overall increase in iron levels in sediments at both upstream and downstream study sites along the river. In April, August, and December, the differences in mean iron values in sediments at the upstream and downstream research locations were not statistically significant. However, across the three months, the amounts of iron in sediments were considerably higher in December, followed by August and then April, both upstream (F = 399.84, p < 0.001) and downstream (F = 574.95, p < 0.001) along Ruiru River (Figure 2).

Levels of iron in the water

The mean amounts of iron in water samples collected from downstream sampling sites were greater in April and August than in samples collected from upstream sampling sites, albeit the differences were not statistically significant. The mean levels of iron in water samples from downstream research sites along Ruiru River (4.26 ± 0.21mg/L) were substantially higher in December than the mean levels of iron observed at upstream study sites along the river (2.81 ± 0.21mg/L), (p = 0.007). These were the highest iron levels found in water samples taken along the river. At the upstream study sites (F = 10.17, p = 0.012), and also at the downstream study sites along the river (F = 141.90, p < 0.001), levels of iron in water were substantially higher in December, followed by April, then August (Figure 3).

Table 1. Mean values (±SD) for water quality parameters at the upstream and downstream sampling sites along Ruiru River during April, August, and December.

		Temp (°C)	pH	Electrical conductivity (µS/cm)	TDS (mg/L)
April	Upstream	20.33±0.14	5.97±0.02	424.00±0.88	34.67±0.94
	Downstream	21.22±0.17	5.87±0.02	592.11±1.72	72.11±1.48
	P value	0.001	0.0026	<0.001	<0.001
August	Upstream	20.89±0.18	6.79±0.01	761.11±8.80	38.89±1.21
	Downstream	21.67±0.12	6.53±0.02	1003.56±11.57	56.11±1.27
	P value	0.0025	<0.001	<0.001	<0.001
December	Upstream	23.33±0.12	6.51±0.07	839.78±10.91	54.89±0.75
	Downstream	24.22±0.17	6.10±0.07	1162.00±10.31	68.00±1.41
	P value	0.0005	<0.0008	<0.001	<0.001

Note: TDS: Total dissolved solids

Levels of lead in sediments

Between April, August, and December, the lead levels in sediments at both upstream and downstream study locations along the Ruiru River increased. In April, August, and December, the differences in mean lead levels in sediments at the upstream and downstream research sites were not statistically significant. However, lead levels in sediments were found to be considerably higher in December, followed by August, and then April at both upstream (F = 43.10, p < 0.001) and downstream (F = 41.84; p < 0.001) study sites along Ruiru River (Figure 4).

Levels of lead in water

In April and August, the mean lead levels in water variations between the upstream and downstream research locations were not statistically significant. The mean levels of lead in water at downstream sampling sites along Ruiru River (4.07 ± 0.53mg/L) were considerably higher in December than the mean levels of lead in water at upstream sampling sites (1.88 ± 0.54mg/L), (p = 0.044) (Figure 5). According to this study, the changes in lead levels in water over the three months of April, August, and December were not statistically significant at the upstream study locations along Ruiru River (F = 1.501, p = 0.296). However, lead levels in the water were considerably higher in December at the downstream study sites, followed by April and August (F = 33.199, p = 0.0006) (Figure 5).

Levels of chromium in sediments

Chromium levels were lower in sediments from upstream sections along the Ruiru River than in samples from downstream sections. In April, chromium levels in sediments at downstream study sites along the river (3.27 ± 0.25mg/kg) were substantially higher than chromium levels in sediments at upstream study sites along the river (1.86 ± 0.24mg/kg), (p = 0.015) (Figure 6). In August and December, the differences in mean chromium levels in sediments at the upstream and downstream study sites were not statistically significant. However, when chromium levels in sediments were compared by month of sampling, it was found that levels were considerably higher in December, followed by April, and then August at upstream study sites (F = 14.48, p = 0.0051), as well as downstream study sites (F = 64.20, p = 0.0001) (Figure 6).

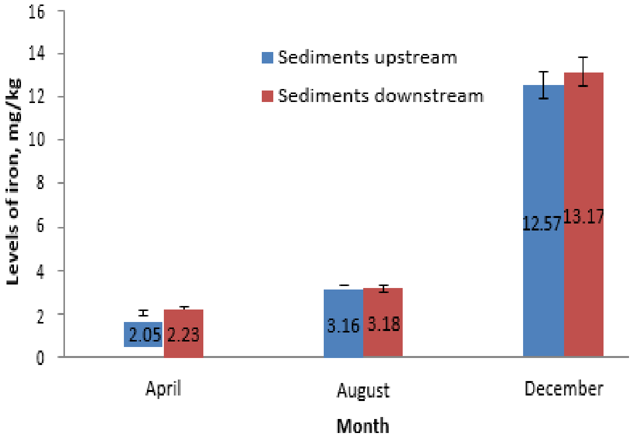

Figure 2. Levels of iron in sediments (mg/kg) during April, August, and December at the upstream and downstream study sites along Ruiru River.

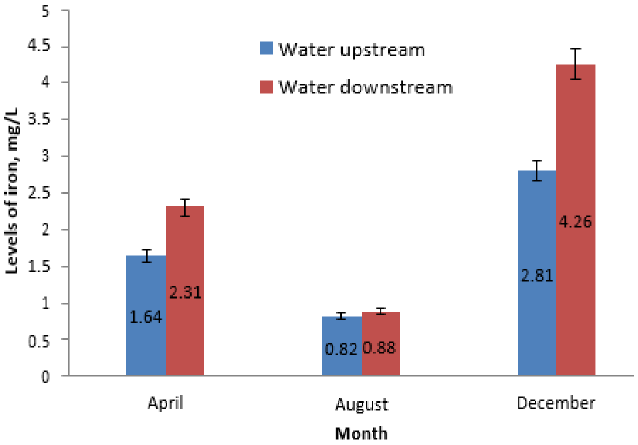

Figure 3. Levels of iron in the water (mg/L) during April, August, and December at the upstream and downstream study sites along Ruiru River.

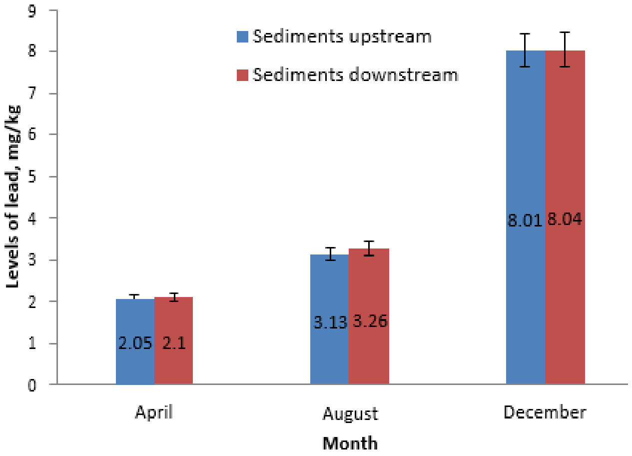

Figure 4. Lead levels in sediments (mg/kg) during April, August, and December at the upstream and downstream study sites along Ruiru River.

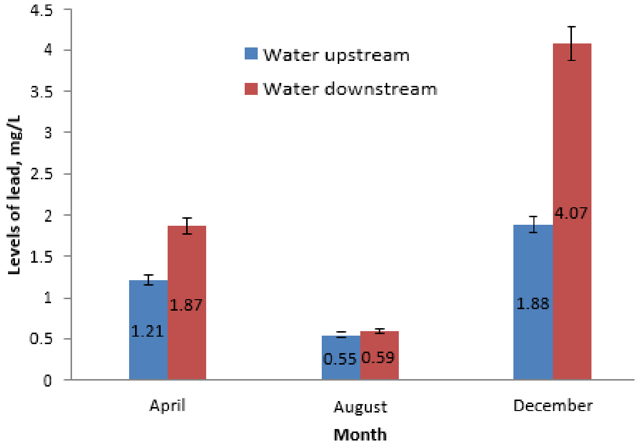

Figure 5. Levels of lead in water (mg/L) during April, August, and December at the upstream and downstream study sites along the Ruiru River

Levels of chromium in water

The downstream study sites along the Ruiru River had greater chromium levels than the upstream sampling sites. In April, the levels of chromium in water at the downstream study sites (2.36 ± 0.19 mg/L) were substantially higher than the levels of chromium in water at the upstream research sites (1.67 ± 0.14 mg/L) ($p = 0.045$) (Figure 7). In December, researchers discovered that the differences in mean chromium levels in water at the upstream study site (2.22 ± 1.01 mg/L) and downstream study sites (5.30 ± 0.31 mg/L) were statistically significant ($p = 0.043$). At the downstream study sites, chromium levels in the water were considerably higher in December, followed by April, and then August ($F = 125.63$, p 0.001) (Figure 7).

Correlation between levels of iron, lead, and chromium in sediments and water during the three months of sampling

During April, August, and December, there was a significant positive correlation between iron levels in sediments and water at the upstream study sites along the Ruiru River ($r = 0.7319$, $p = 0.025$) (Table 2).

There was also a significant positive correlation ($r = 0.8506$, $p = 0.0037$) in the downstream sites along the Ruiru River, indicating that high iron levels in the sediments grew as metal levels in water increased (Table 2). At the upstream study sites, there was no significant correlation between lead levels in sediments and water ($r = 0.343$, $p = 0.366$) (Table 2). However, there was a significant correlation ($r = 0.7523$, $p = 0.019$) at the downstream study sites (Table 2).

The levels of chromium in sediments and water along the Ruiru River exhibited a positive correlation ($r = 0.5339$, $p = 0.138$); however, it was not statistically significant (Table 2). The value of r was 0.9787 in the downstream study sites along the river. This is a substantial positive correlation, indicating that greater chromium levels in sediments increased as chromium levels in water increased ($p < 0.001$) (Table 2).

Levels of metals in the tissues of *C. gariepinus* and *O. niloticus were* sampled from the upstream and downstream study sites along Ruiru River.

Although the differences were not significant, upstream research sites along the Ruiru River, *C. gariepinus*, had greater mean levels of the three metals than *O. niloticus* (Table 3).

The findings also demonstrate that all *C. gariepinus* tissues collected from downstream research sites along the Ruiru River had higher amounts of all the examined metals than *O. niloticus* tissues collected from the same sites. Both investigated fish species exhibited higher iron levels than lead in all tissues. Chromium has the lowest concentration of all the metals tested. Furthermore, both fish species collected heavy metals in their tissues in a similar pattern. The liver carries higher levels of metals than the gills and muscles containing the lowest levels (Table 3).

Table 2. Relationship between levels of metals in sediments (mg/kg ± SD) and in water (mg/L ± SD) at the upstream and downstream study sites along Ruiru River during April, August, and December.

	Sediments	Water	r	p
Upstream				
Iron	5.81±1.62	1.75±0.32	0.731	0.025
Lead	4.39±0.94	1.21±0.33	0.3429	0.366
Chromium	2.52±0.60	1.47±0.38	0.534	0.138
Downstream				
Iron	6.19±1.75	2.48±0.49	0.8506	0.0037
Lead	4.46±0.94	2.17±0.53	0.7523	0.01935
Chromium	3.58±0.68	2.74±0.69	0.9787	0.000

Note: Correlation value is significant at $P \leq 0.05$, Pearson linear correlation, $\alpha = 0.05$

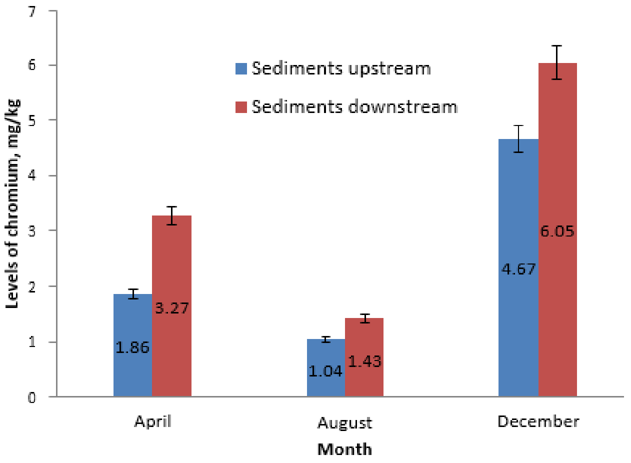

Figure 6. Levels of chromium in sediments (mg/kg) during April, August, and December at the upstream and downstream sampling sites along the Ruiru River

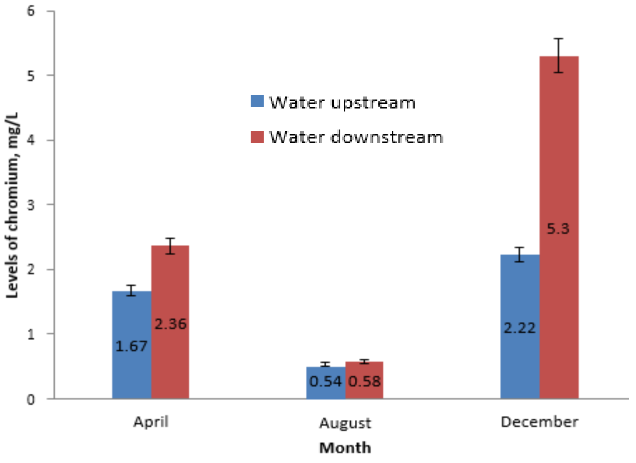

Figure 7. Levels of chromium in water (mg/L) during April, August, and December at the upstream and downstream study sites along Ruiru River.

Table 3. Mean levels of iron, lead, and chromium (mg/kg ± SD) in the tissues of *Clarias gariepinus* and *Oreochromis niloticus* sampled from the upstream and downstream study sites along Ruiru River.

Part of the body	Fish species	Fe (mean±SE) mg/kg	Pb (mean±SE) mg/kg	Cr (mean±SE) mg/kg
Upstream				
Liver	*C. gariepinus*	3.67±0.54	0.84±0.30	0.62±0.18
	O. niloticus	2.71±0.44	0.555±0.21	0.50±0.14
p-value		0.1792	0.4834	0.619
Gills	*C. gariepinus*	2.73±0.54	0.26±0.12	0.27±0.1
	O. niloticus	1.85±0.30	0.26±0.10	0.15±0.04
p-value		0.165	0.9974	0.295
Muscles	*C. gariepinus*	2.11±0.46	0.25±0.07	0.16±0.06
	O. niloticus	1.38±0.23	0.15±0.05	0.13±0.04
p-value		0.166	0.278	0.679
Downstream				
Liver	*C. gariepinus*	5.24±0.86	1.36±0.46	0.68±0.19
	O. niloticus	3.55±0.58	0.69±0.28	0.56±0.15
p-value		0.112	0.225	0.631
Gills	*C. gariepinus*	3.17±0.50	0.75±0.26	0.33±0.11
	O. niloticus	2.08±0.30	0.44±0.14	0.17±0.06
p-value		0.072	0.306	0.211
Muscles	*C. gariepinus*	2.34±0.42	0.46±0.15	0.20±0.06
	O. niloticus	1.68±0.25	0.29±0.09	0.15±0.04
p-value		0.183	0.349	0.5619

Table 4. Mean levels of iron, lead, and chromium (mg/kg ± SD) in tissues of *Clarias gariepinus* and *Oreochromis niloticus* from upstream and downstream study sites along Ruiru River during April, August, and December.

		Liver	Gills	Muscles
Clarias gariepinus				
Iron	Upstream	3.67±0.55	2.73±0.54	2.11±0.46
	Downstream	5.24±0.87	3.17±0.50	2.34±0.42
	p	0.132	0.277	0.718
Lead	Upstream	0.84±0.3	0.26±0.12	0.25±0.07
	Downstream	1.36±0.4	0.75±0.26	0.46±0.15
	p	0.352	0.099	0.227
Chromium	Upstream	0.62±0.18	0.27±0.1	0.16±0.06
	Downstream	0.68±0.19	0.33±0.11	0.20±0.06
	p	0.812	0.670	0.697
Oreochromis niloticus				
Iron	Upstream	2.71±0.44	1.85±0.30	1.38±0.23
	Downstream	3.55±0.58	2.09±0.30	1.68±0.25
	p	0.254	0.592	0.398
Lead	Upstream	0.55±0.21	0.26±0.10	0.15±0.05
	Downstream	0.69±0.28	0.44±0.14	0.29±0.09
	p	0.694	0.329	0.202
Chromium	Upstream	0.50±0.14	0.15±0.04	0.13±0.04
	Downstream	0.56±0.15	0.17±0.06	0.15±0.04
	p	0.771	0.823	0.761

Table 5. Relationship between iron levels (mg/kg) in selected tissues and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites.

	Upstream		Downstream	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Clarias gariepinus				
Liver	r = 0.151	r = 0.171	r = 0.153	r = 0.147
	p = 0.452	p = 0.393	p = 0.466	p = 0.465
\Gills	r = 0.249	r = 0.354	r = 0.364	r = 0.269
	p = 0.210	p = 0.070	p = 0.062	p = 0.174
Muscles	r = 0.266	r = 0.361	r = 0.317	r = 0.194
	p = 0.180	p = 0.064	p = 0.107	p = 0.332
Oreochromis niloticus				
Liver	r = 0.535	r = 0.358	r = 0.510	r = 0.618
	p = 0.004**	p = 0.067	p = 0.007**	p = 0.001 **
Gills	r = 0.504	r = 0.343	r = 0.519	r = 0.633
	p = 0.007**	p = 0.080	p = 0.006**	p = 0.001**
Muscles	r = 0.452	r =0.296	r = 0.438	r = 0.507
	p = 0.018**	p = 0.134	p = 0.022*	p = 0.007**

Note: * Correlation value is significant at $p \leq 0.05$, Pearson linear correlation, $\alpha = 0.05$

Levels of iron, lead, and chromium in tissues of *Clarias gariepinus* and *Oreochromis niloticus* from the upstream and downstream sites along Ruiru River.

All three heavy metals were found in higher concentrations in the tissues of *C. gariepinus* fish obtained from downstream study sites than in fish collected from upstream study sites along the river. However, the variations in the mean were not statistically significant (Table 4). The mean levels of iron, lead, and chromium in the liver, gills, and muscles of *O. niloticus* taken from upstream and downstream research sites along the Ruiru River made no significant change (Table 4).

Relationship between levels of iron and fish morphometrics in *Clarias gariepinus* And *Oreochromis niloticus*

In *C. gariepinus* sampled from both upstream and downstream study sites along the Ruiru River, the correlation between iron levels in the fish liver, gills, and muscles and fish length and weight indicated favorable relationships. The correlations, however, were not statistically significant (Table 5).

In *O. niloticus* sampled from both upstream and downstream study sites along the Ruiru River, the linkage between iron levels in the fish liver, gills, and muscles and fish length and weight indicated positive relationships. There was a significant positive correlation between the levels of iron in fish liver and the length of fish in *O. niloticus* sampled from upstream study locations (r = 0.535, p = 0.004). Although there was a positive correlation between fish liver iron levels and fish weight from the same research site (r = 0.358, p = 0.067), the correlation was not statistically significant. There were substantial positive correlations between the levels of iron in fish liver and both length (r = 0.510, p = 0.007) and weight (r = 0.618, p = 0.001) of fish taken from the downstream research sites (Table 5).

The levels of iron in the gills of *O. niloticus* captured from upstream research locations were significantly correlated to length (r = 0.504, p = 0.007), but not to weight (r = 0.343, p = 0.080). However, there were substantial positive relationships between the levels of iron in *O. niloticus* gills and both length (r = 0.519, p = 0.006) and weight (r = 0.633, p = 0.001) in fish obtained from downstream research locations along the Ruiru River (Table 5).

The findings also show a substantial positive correlation between iron levels in *O. niloticus* muscles and fish length (r = 0.452, p = 0.018) in fish acquired from upstream study sites, whereas a positive but not significant correlation between levels of iron in the muscles and fish weight. In fish gathered from downstream research locations, significant positive correlations were found between levels of iron in the muscles of *O. niloticus* and length (r = 0.438, p = 0.022) and weight (r = 0.507, p = 0.007) (Table 5).

Relationship between levels of lead and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites

The lead levels in the liver, gills, and muscles of *C. gariepinus* were positively associated with both length and weight in fish sampled from upstream study sites and length and weight in fish taken from downstream research sites in this investigation. However, the connections were not statistically significant (Table 6). Relationship between levels of lead and fish morphometrics in *O. niloticus* at the upstream and downstream study sites

The lead levels in fish liver, gills, and muscles were positively correlated to fish length, and weight in *O. niloticus* sampled from upstream study sites along Ruiru River; however, the correlations were not statistically significant (Table 6).

Relationship between levels of chromium and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites

Similarly, levels of chromium in the tissues of *C. gariepinus* and *O. niloticus* in fish samples from upstream and downstream study sites were positively correlated with both fish length and weight. The correlations, on the other hand, were not statistically significant. Morphological effects of iron, lead, and chromium in the liver and gills of *C. gariepinus* and *Oreochromis niloticus.*

The levels of iron, lead, and chromium in water and sediments in the Ruiru River were connected to morphological alterations in the gills and liver of *C. gariepinus* and *O. niloticus* in the current study. Fish liver histology at the upstream research site revealed normal liver structure with no pathological abnormalities. A continuous mass of huge hexagonal hepatic cells makes up the liver of a fish. Hepatic cells were polygonal, with spherical nuclei either exocentric or slightly centrally located (Figures 8 and 9).

Table 6. Relationship between levels of lead (mg/kg) in selected tissues and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites.

	Upstream		**Downstream**	
	Length (cm)	**Weight (g)**	**Length (cm)**	**Weight (g)**
Clarias gariepinus				
Liver	r = 0.241	r = 0.156	r = 0.068	r = 0.021
	p = 0.225	p = 0.437	p = 0.708	p = 0.916
Gills	r = 0.157	r = 0.191	r = 0.097	r = 0.129
	p = 0.435	p = 0.340	p = 0.631	p = 0.522
Muscles	r = 0.264	r = 0.306	r = 0.211	r = 0.182
	p = 0.184	p = 0.121	p = 0.291	p = 0.365
Oreochromis niloticus				
Liver	r = 0.209	r = 0.008	r = 0.288	r = 0.31
	p = 0.295	p = 0.970	p = 0.145	p = 0.115
Gills	r =0.16	r = 0.153	r = 0.137	r = 0.237
	p = 0.408	p = 0.445	p = 0.495	p = 0.235
Muscles	r = 0.248	r = 0.116	r = 0.317	r = 0.288
	p = 0.212	p = 0.563	p = 0.107	p = 0.146

Note: * Correlation value is significant at $p \leq 0.05$, Pearson linear correlation, α = 0.05

Hepatocytes with loss of normal arrangement were found in the livers of *C. gariepinus* and *O. niloticus* from the downstream regions of the Ruiru River. Fish livers showed larger hepatocytes with expanded nuclei (Figures 10 and 11).

Clarias gariepinus and *O. niloticus* gills collected from upstream study sites along the Ruiru River have normal gill structures. Each arch featured two rows of primary gill filaments connected at the base by a gill septum. Primary gill lamellae featured central supporting axes and rows of secondary gill lamellae on both sides (Figures 12 and 13). Fish gills from downstream areas of the river, on the other hand, showed proliferation of interlamellar epithelial cells, as well as hyperplasia and fusion of the secondary lamellae (Figures 14 and 15).

Permissible values of iron, lead, and chromium in fish

According to the findings, in comparison to O. niloticus, C. gariepinus showed higher levels of the examined metals. Fish from the downstream study sites, on the other hand, accumulated larger levels of metals than those from the upstream study sites. The results also suggest that lead and chromium levels in both fish species' tissues were greater than the allowed values in fish, as shown in Table 7. The permitted limits for iron set by WHO (2011) and FAO (2011) are 43 mg/kg, 0.2 mg/kg for lead set by EU (2001), and 0.15 mg/kg for chromium set by WHO (1985) and FEPA (2003).

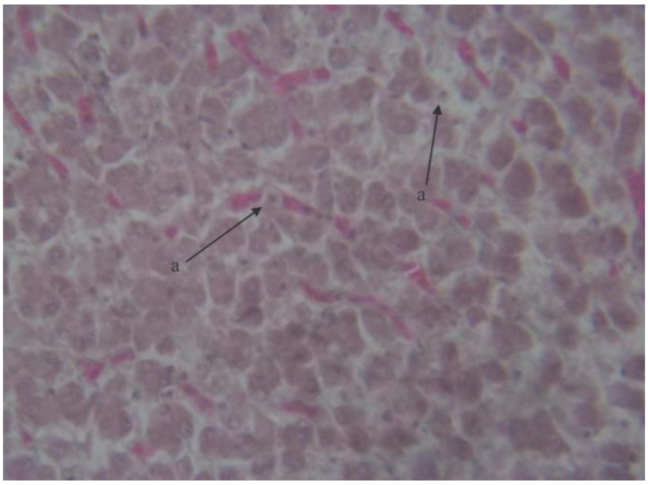

Figure 8. Liver of *Clarias gariepinus* obtained from the upstream study sites, showing normal hepatocytes with nuclei (a) (Haematoxylin and Eosin, × 400)

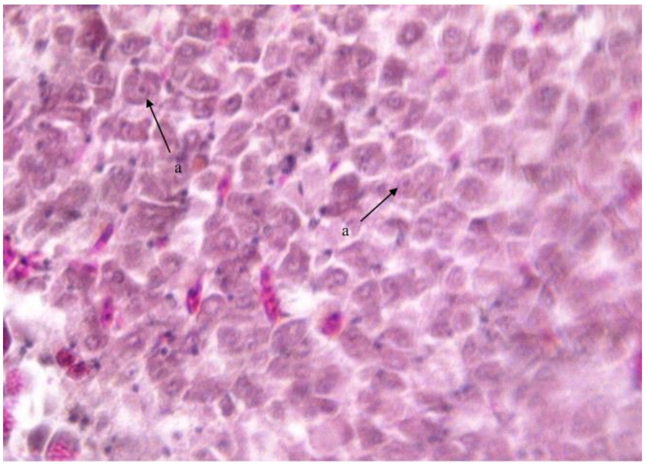

Figure 9. Liver of *Oreochromis niloticus* obtained from the upstream study sites, showing normal hepatocytes with nuclei (a) (Haematoxylin and Eosin, ×400).

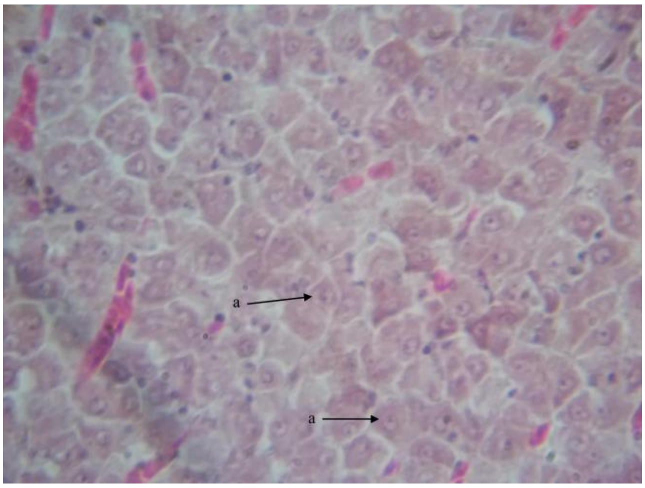

Figure 10. Liver of *Clarias gariepinus* obtained from the downstream study sites, showing enlarged hepatocytes (a) (Haematoxylin and Eosin, ×400).

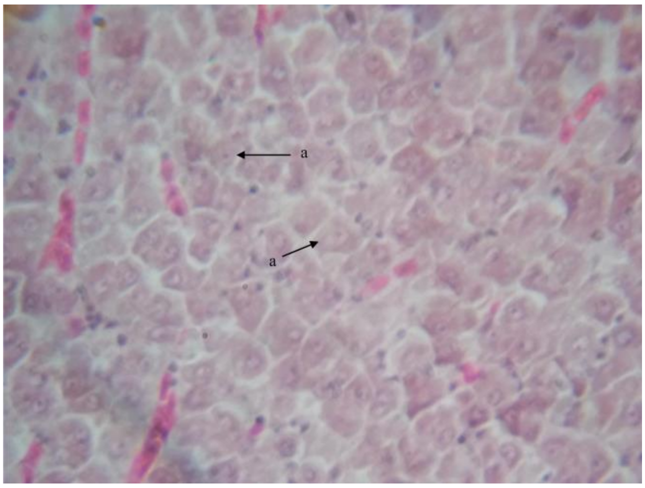

Figure 11. Liver of *Oreochromis niloticus* obtained from the downstream study sites, showing enlarged nuclei (a) (Haematoxylin and Eosin, × 400).

Figure 12. Gills of *Clarias gariepinus were* obtained from the upstream study sites, showing gill filaments and lamellae (b) (Haematoxylin and Eosin, × 150).

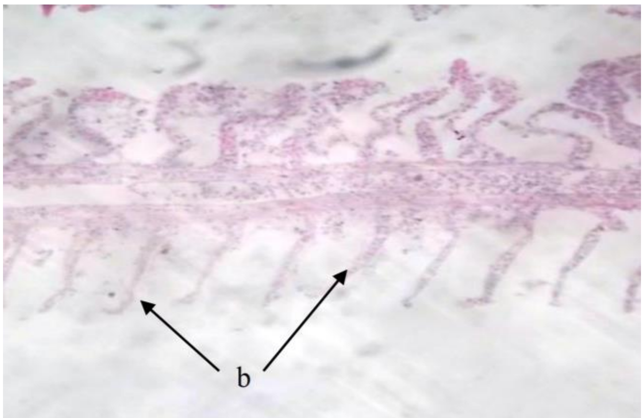

Figure 13. Gills of *Oreochromis niloticus were* obtained from the upstream study sites, showing gill filament and lamellae(b) (Haematoxylin and Eosin, × 150).

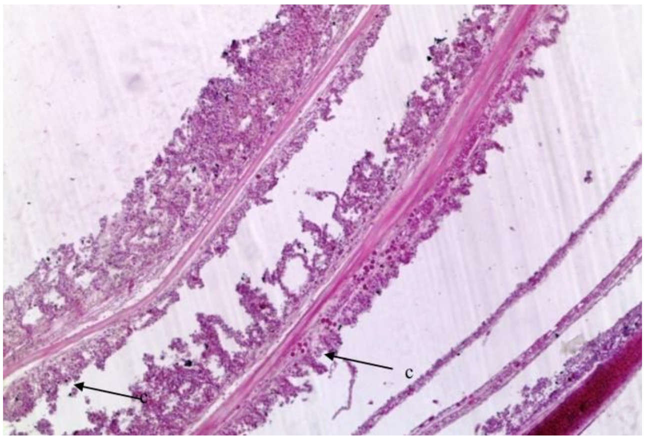

Figure 14. Gills of *Clarias gariepinus* were obtained from the downstream study sites, showing proliferation of interlamellar epithelial cells, which also show hyperplasia (c) (Haematoxylin and Eosin, × 150)

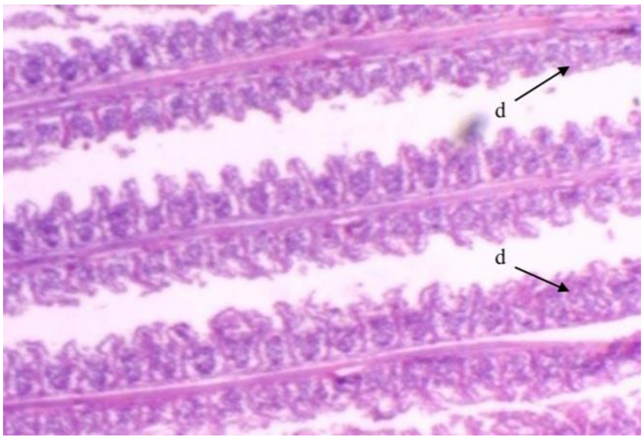

Figure 15. Gills of *Oreochromis niloticus were* obtained from the downstream study sites, showing fusion of secondary lamellae and hyperplasia due to increased multiplication of the epithelial cells lining the secondary lamellae and primary lamellae (d) (Haematoxylin and Eosin, × 150).

Table 7. Comparison of levels of iron, lead, and chromium in fish tissues, sediments, and water against permissible levels

Levels in mg/kg	Upstream study sites	Downstream study sites	P	Permissible values (mg/kg dry weight)	References
Iron in tissues of *Clarias gariepinus*	2.84±0.30	3.58±0.38	0.018	43	WHO 2011, FAO 2011
Iron in tissues of *Oreochromis niloticus*	1.98±0.20	2.44±0.25	0.011	43	
Lead in tissues of *Clarias gariepinus*	0.45±0.11	0.86±0.18	0.000	0.2	European Union 2001
Lead in tissues of *Oreochromis niloticus*	0.33±0.08	0.48±0.11	0.016	0.2	
Chromium in tissues of *Clarias gariepinus*	0.35±0.07	0.41±0.08	0.178	0.15	FEPA 2003
Chromium in tissues of *Oreochromis niloticus*	0.26±0.05	0.29±0.06	0.183	0.15	
Iron in sediments	0.58±0.16	0.62±0.18	0.112		
Iron in water (mg/L)	0.18±0.03	0.25±0.05	0.018	20	FEPA 1999
				0.2	NWQMS 2000
Lead in sediments	0.44±0.09	0.45±0.09	0.110	50	NWQMS 2000
Lead in water (mg/L)	0.12±0.03	0.22±0.05	0.059	0.1	FEPA 1999, 2006
				2	NWQMS 2000
Chromium in sediments	0.25±0.06	0.36±0.07	0.002	80	NWQMS 2000
Chromium in water (mg/L)	0.14±0.04	0.28±0.07	0.044	<1	FEPA 1999
				0.1	NWQMS 2000

Discussion

Levels of iron, lead, and chromium in sediments and water along Ruiru River

Higher levels of metals in sediments at downstream study sites than upstream study sites could be attributed to their role as reservoirs for pollutants coming from the surrounding areas and important sinks for various pollutants such as trace metals. Under the right conditions, they can also help with trace metal remobilization in aquatic systems (Aderinola et al., 2009; 2012; Uzairu et al., 2009; Osman et al., 2012). They increased total dissolved metal ions in water in the Ruiru River. Thus, an elevated level of metal ions that may have caused an increase in metals acquired can be linked to increased levels of iron, lead, and chromium in water and sediments observed from April to December. The elevated levels of chromium in water and sediments in December compared to April and August. The metals could have come from industrial, municipal, urban, and agricultural pollutants that runoff into the river. During wet seasons, lower levels of heavy metals in water may result from a dilution effect generated by additional water in the river due to rains (Damodharan and Vikram 2013). Organic substances from nearby farms may have contributed to the occurrence of heavy metals in the upstream study locations (Emere and Dibal 2013). Higher levels of metals in Ruiru River water during December, as observed in this study, could be linked to industrial effluents and human activities within Ruiru town, as well as evaporation of water due to increased temperatures associated with the dry spell, which typically begins in September and lasts through October and December, resulting in higher levels of metal residues in the river water. Significantly greater amounts of chromium documented in sediments at the downstream study sites and iron, lead, and chromium in water at the same sites, p 0.05, can be attributable to higher temperatures at the downstream study sites over the three-month investigation (p 0.05). Warmer water speeds up the breakdown of wastes and the dissolution of chemicals in the water (Isyagi et al., 2009). As a result, greater temperatures at the downstream study sites along the Ruiru River may contribute to higher levels of the examined metals observed at these sites, as well as in the tissues of the fish found there. Furthermore, significant positive correlations between levels of iron, lead, and chromium in sediments and water samples collected from downstream study sites suggest that there was a strong tendency for high levels of metals in sediments to increase in tandem with increased levels of metals in water.

The proportional increase in temperature of water at downstream study sites along the Ruiru River has the potential to impact water's oxygen retention capacity, as temperature affects the quantities of dissolved oxygen in the water column, which is inversely related to temperature. Furthermore, the entry of surplus organic matter along the Ruiru River's downstream sections may result in a fall in oxygen levels, especially when the conditions are warm. Fish require oxygen for metabolism, and dissolved oxygen is required to convert potentially hazardous metabolic wastes into less toxic forms, such as ammonia (NH_3) to nitrite (NO_{2-}) and finally nitrate (NO_3) (NO_{3-}). The amount of dissolved oxygen in the water impacts the compounds in the water. Metals harden and precipitate out of water in the presence of oxygen. These metals dissolve into the water significantly more hazardous to aquatic species when there is no oxygen present (CWT 2004). Temperatures in the extreme top range make maintaining dissolved oxygen concentrations more difficult, according to research (Dennis et al., 2009).

Levels of iron, lead, and chromium in the liver, gills, and muscle of Clarias gariepius and Oreochromis niloticus.

Elevated amounts of these metals in sediments and river water have been correlated with the presence of iron, lead, and chromium in the liver, gills, and muscles of *C. gariepinus* and *O. niloticus*. Based on the current data, the presence of iron, lead, and chromium in the liver, gills, and muscles of fish can be attributed to high amounts of metals in river water. In addition to anthropogenic activities inside the Ruiru Municipality, higher levels of iron, lead, and chromium in the surface waters at sampling sites downstream and upstream in the river could be attributable to agricultural operations along the river banks and industrialization in Ruiru town. In this study, *C. gariepinus* obtained from both upstream and downstream sites along Ruiru River had higher iron, lead, and chromium levels in the gills, liver, and muscles than *O. niloticus*, albeit the differences in mean levels of metals in the fish tissues were not significant. As a result, metal accumulation in fish gills, liver, and muscle can be related to metal concentrations in water (Damodharan and Vikram 2013; Hmoud et al. 2013; Kpobari et al. 2013).

Statistically significant differences in the levels of chromium in sediments at the upstream and downstream study sites and iron and lead levels. Chromium in water at the two sites during the sampling occasions could contribute to differences in the levels of metals accumulated in the two fish species investigated, resulting in higher levels of metals in the tissues. Differential metabolic demand varies between species and is responsible for fish's metal accumulation variations. Even when the metal concentration is relatively low, chronic metal exposure leads to increased accumulation and enhanced toxicity in fish. Organisms exposed to pollutants develop mechanisms to sequester and excrete them, but chronic exposure over a long period leads to increased accumulation, allowing metals to reveal their toxic effects (Paulami and Samir 2012).

The lipid content of the tissue also determines metal levels in fish, but the distribution of metals in fish tissues is determined by the metabolic demands of a specific tissue (Paulami and Samir 2012). The fish environment and biological factors also influence metal excretion in fish, and the final level in fish is determined by the fish's ability to regulate the metal (Ishaq et al., 2011; Paulami and Samir, 2012).

This study revealed that the levels of iron, lead, and chromium in the liver and gills of fish were higher than in the muscles. The liver and gills acquire the most metals among fish organs, emphasizing their usefulness as bioindicators for studying heavy metal levels in general. At the same time, the muscles contain the least amount of heavy metals (Edem et al., 2009).

Lower levels of metals in the muscles of fish compared to the gills and liver reported in this study are comparable to earlier work by Ebenezer and Eremasi (2012), who found lower levels of copper, lead, cadmium, and nickel in the muscles of *Tilapia zilli* compared to its gills, and Javed and Usmani (2011), who found a similar order of heavy metal accumulation in the tissues of different fishes, with least accumulation of metals in the muscles. They also discovered that chromium was the metal with the least accumulation in all tissues investigated. Crafford and Avenant (2011) found greater levels of chromium, copper, iron, and manganese in the liver and gills of *C. gariepinus,* which was validated by the findings of this study.

Metal uptake also occurs through food; therefore, the metal accumulating in fish is influenced by their location, feeding habits, and trophic level. Most fish are at the top of the aquatic food chain and can acquire many metals even in low-pollution situations (Kpobari et al., 2013). In the current study, discrepancies in iron, lead, and chromium levels in *C. gariepinus* and *O. niloticus* might be associated with various factors, including feeding patterns, habitats, ecological demands, metabolism, biology, and fish physiology. The way fish are fed has a significant impact on the amount of heavy metals they accumulate (Ishaq et al., 2011). *O. niloticus* is adapted to feeding on a low trophic level and does not normally feed in deep waters (Robert 2001). They are primarily herbivorous and have a well-balanced diet, with healthy vegetable and animal components. Plankton, green leaves, benthic creatures, aquatic invertebrates, larval fish, debris, and decaying organic materials are all eaten by *O. niloticus*. Although adult *O. niloticus* are not usually piscivorous, youngsters eat larval fish.

Clarias gariepinus, on the other hand, is euryphagous and is thought to be an opportunistic, omnivorous predator. *C. gariepinus* is primarily omnivorous, eating on detritus, invertebrates, and small fishes, and can transition between alternate food sources such as plants and detritus when prey animals become rare (FAO 2014). *C. gariepinus* is a bottom feeder by nature; however, they can modify their eating habits and filter feed in groups above the water surface occasionally. Because the fish has physical adaptations for piscivorous and filter-feeding (largemouth, marginal and pharyngeal teeth, robust and muscular stomach, and short intestine), it can shift from one feeding habit to the other on the availability and emergence of some food organisms (Elias 2009).

Compared to O. niloticus, C. gariepinus may consume mud-burrowing organisms to complement their diet, resulting in unintentional silt consumption and increased metal loading. Bottom-feeding fish come into more contact with sediments containing higher amounts of heavy metals; therefore, eating sediments while digging for food exposes the fish to additional metals. As a result, bottom-feeding fish have greater levels of heavy metals in their tissues (Ling et al., 2013). *O. niloticus* and *C. gariepinus* have different feeding patterns yet share the same habitat. The findings demonstrate that the investigated tissues of the omnivore fish *C. gariepinus* accumulated larger amounts of heavy metals than those found in the herbivorous fish *O. niloticus*.

The current study discovered positive correlations between iron, lead, and chromium levels in the fish liver, gills, muscles, and fish length and weight. The correlations, however, were not statistically significant. Heavy metal levels in fish tissues have also been shown in previous studies related to the fish's age and thus to the fish's size and length (Damodharan and Vikram 2013). Metals can accumulate in fish tissues faster than the metal's rate of excretion as the fish grows, and the accumulation is organ and species-specific (Indrajith et al., 2008). The relationship between tissue metal levels and fish size has not been studied in the Ruiru River fish population. Iron, lead, and chromium concentrations in the liver, gills, and muscles of *O. niloticus* and *C. gariepinus* were studied about the fish length and weight. According to the results, there was a positive correlation between metal concentrations and both fish length and weight. It has been found that metal accumulation in fish is stabilized at a certain age, indicating that the concentrations of metals are regulated and maintained at certain levels (Yi and Zhang 2012). However, suppose metal concentrations in the surrounding water are higher than the capacity of these factors. In that case, the dilution of tissue metal concentrations associated with growth and lowered metabolic activity in older individuals may not be seen. Here, metals may continue to build up, with a positive relationship between the size of the animals and metal concentrations in tissue (Yi and Zhang 2012).

Previous studies have shown positive and negative relationships between heavy metal levels in the tissues and fish length and weight. Usha and Vikram (2013) found that heavy metal levels in fish species decreased as fish size increased. In contrast, Canli and Atli (2003) found that zinc and lead accumulation in fish gills increased as fish size increased. That negative correlations between metal levels and size may be due to differences in metabolic activity between younger and older fish. A similar relationship has been observed between fish age, length, and weight and the accumulation of heavy metals in all tissues. Younger fish show higher nickel, lead, and chromium levels due to higher metabolic rates (Sahar et al., 2014).

As the length and weight of *O. niloticus* increase, the levels of copper, iron, lead, manganese, and zinc increase, while aluminum, cadmium, and mercury levels decrease (Authman 2008). According to research, zinc and lead levels in fish increase with body weight, particularly in the liver, gills, and kidneys (Paulami and Samir 2012). According to research, fish size and age are crucial characteristics when addressing metals accumulation in fish. Some elements' concentrations remain constant while others grow as fish size and age rose (Naeem et al. 2011). Luciobarbus xanthophores fish showed a positive relationship between heavy metal accumulation in muscle and size (Dusukcan et al., 2014). According to the researchers, there was a strong positive correlation between fish age and muscle heavy metal concentrations. There was a positive correlation between levels of metals in the studied fish tissues and both length and weight of fish, which could be an indication of possible acquisition and accumulation of metals in fish from the surrounding water, and could also point to the observed morphological changes that higher levels of metals present in the tissues of fish living in the affected areas downstream along Ruiru River.

Morphological effects of iron, lead, and chromium in liver and gills of C. gariepinus and O. niloticus.

There were structural differences between the liver and gill tissue of both C. gariepinus and O. niloticus caught from downstream and upstream study sites. There is a possibility that the metals present in the water are responsible for the observed morphological effects. In the current study, iron, lead, and chromium levels were significantly lower in the relatively unpolluted upstream study sites along Ruiru River than in the downstream study sites. In comparison to the downstream sections of the river, the upstream sections are comparatively free of pollution from industries, irrigation, and domestic effluents. Hepatocyte cytoplasm vacuolation, necrotic regions, and thickening of hepatocyte cells found in fish from downstream study sites along the river can be connected to toxic liver damage caused by river pollution. Heavy metals produced changes in the liver of *O. niloticus* living in the Nile River, according to Atif et al. (2009). Their histological study revealed several pathological alterations in the livers of *O. niloticus* living in the studied stations, including fatty degeneration, necrosis, and edema, and they explained that the discharge of various types of wastes, including heavy metals, degraded the water quality in the river, affecting aquatic inhabitants. Hepatocyte injury was also shown in Deore and Wagh's (2012) study. They also discovered that the severity of damage depended on both the dose and the duration of metal exposure. Heavy metal salts enter cells by quickly crossing cell membranes, where they interfere with the cell's enzyme systems, causing morphological damage (Bhatkar 2011). In hepatocytes, heavy metals promote degeneration and vacuole necrosis, as well as hemolysis and hemosiderin pigmentation (Seham and Soad 2005). The accumulation of hemosiderin pigments in the liver tissue is caused by the rapid and ongoing destruction of erythrocytes due to increased hemolysis and disruption to the iron metabolism (Seham and Soad 2005). Large amounts of iron may be absorbed through the intestinal mucosa, resulting in aberrant hemosiderin buildup in the liver. Lysosomal membranes, which are particularly sensitive to numerous pathogenic stimuli, are disturbed, and their enzymes are released, resulting in liver cell degeneration and vacuolation (Seham and Soad 2005).

Previous studies reported histopathological changes happened in the muscles, liver, gills, kidneys, and intestine liver of *Tilapia zillii* and *Solea vulgaris* from Lake Qarun. They suggested that the pathological changes in the tissues of both studied fish could directly result from heavy metals, pesticides, fertilizers, salts, and sewage that enters the lake via the drainage water (Fatma 2009). The liver, the primary organ of metabolism, is constantly exposed to xenobiotics absorbed from the environment, and liver diseases are strongly associated with aquatic pollution (Fatma 2009). Hepatocyte degeneration, nuclear pycnosis, cellular edema, and blood vessel congestion were also seen in the livers of *O. niloticus* subjected to zinc (Abdel et al., 2011).

The histology of fish gills from the downstream parts of the Ruiru River revealed proliferation of the interlamellar epithelia, secondary lamellae degeneration, club-shaped primary lamellae, secondary lamellae hyperemia, and secondary lamellae fusion in this study. These impacts can be attributed to much greater metal levels recorded at these research sites than at the upstream study sites. Hexavalent chromium, which may easily pass through gill membranes and concentrate at higher amounts in various organs and tissues, can have a harmful effect both internally and on the gill surface. Chromium is particularly harmful since it may accumulate in many organisms at levels up to 4000 times higher than the ambient level (Avenant and Marx 2000).

The gills, which are involved in various critical processes in the fish, including breathing, osmoregulation, and excretion, stay in close contact with the water. They are sensitive to changes in water quality and are considered the pollutants' principal target (Jalaludeen and Arunachalam 2012). The response they elicit can range from almost non-existent to severe and widespread, resulting in lesions and tissue damage, and side effects like hyperplasia. Hyperplasia, fusion, and necrosis are the most prevalent types of lesions. Hyperplasia - aberrant increases in the amounts of cells in the gill epithelium – is the most common response to stimulation (Fitzroy Report 2012).

The gills regenerate uncontrollably due to continued deadly lead exposure, and the pillar cells become haphazardly placed. As a result, the space between adjacent secondary lamellae is nearly completely filled with polygonal epithelium, and the gill filaments appear as a solid mass of cells. The tips of adjacent main lamellae (PL) also fuse, leaving no gap between them. The secondary lamellae become multi-layered thick due to uncontrolled hyperplasia of the respiratory epithelium's polygonal cells. The secondary lamellae's free surface is wholly gone, and the secondary lamellae appear as a solid mass. By raising the blood oxygen barrier distance, the respiratory of fish efficiency is reduced. However, extending the diffusion distance of xenobiotics through the gill epithelium also slows xenobiotic penetration. Extracellular vacuolization between polygonal cells is common, and these polygonal cells appear to be metabolically active (Parashar and Banerjee 2002). The much greater levels of iron in water measured at the downstream study sites can also be linked to the morphological abnormalities found in the liver and gills of fish in this study. The presence of ferrous iron (Fe II) and the oxidation of ferrous to ferric (Fe III) iron are the main causes of iron toxicity. Furthermore, Fe (II) can induce tissue damage by forming free radicals and causing lipid peroxidation. Temperature and pH are significant iron modifiers toxicity, and a combination of low temperature and low pH can result in relatively high levels of poisonous Fe (II) (Nicolas and Martin 2003). Fish can be poisoned by the transition of iron from Fe (II) to Fe (III) and the creation of Fe (III) precipitates. The production of solid, or colloidal, precipitates is related to a decrease in Fe (II) and an increase in Fe (III), and a change from low molecular mass forms to higher molecular mass forms of iron. These changes in iron behavior are accompanied by a rise in gill iron concentrations (Adam et al., 2011).

Dilation and wall thickening of blood vessels in the liver, which becomes clogged with blood cells, cytoplasmic vacuolation of hepatocytes with profoundly colored nuclei, and cytoplasmic vacuolation of hepatocytes with deeply stained nuclei are all observed in tilapia treated with lead nitrate (Bothaina et al. 2012). During ten days of exposure, the toxic effects of chromium on the histology of gill and liver of *Labeo rohita* fingerlings cause modest histological changes. After thirty days, however, gill lamellae fusion, hypertrophy, and epithelial degradation are visible (Muthukumaravel and Rajaraman 2013). Vacuolation, hepatocyte degeneration, and hepatocyte cell boundary disintegration are examples of liver pathologies caused by metal exposure. Because the liver is the primary organ for detoxifying, changes in the liver may be linked to the direct harmful effects of contaminants on hepatocytes (Mohammad et al., 2013). Chromium, nickel, and zinc chlorides cause degenerative histopathological changes in Labeo rohita's liver, including larger nuclei, condensation of cytoplasm, and hepatic cords' disorder and blood congestion in sinusoids, vacuolation of hepatocytes, and necrosis (Bhatkar 2011).

Higher chromium levels in the water at the downstream study sites along the river may have contributed to the structural changes seen in the fish. The effects of chromium in the gills of *Cyprinus carpio* were dosage dependant, with disintegrating gill mucosal epithelium, basement membrane, and submucosa cells shown at lower doses and hyperplasia seen at higher doses (Solangi et al. 2012). According to the researchers, disintegrated primary and secondary lamellae were also detected at lower chromium concentrations, followed by hyperplasia at higher concentrations. Total mean levels of iron, lead, and chromium in fish

Iron had the highest levels of metals in the tissues of the fish species investigated, whereas chromium had the lowest. Compared to *O. niloticus*, *C. gariepinus* had consistently high amounts of iron, lead, and chromium. The liver of *C. gariepinus* has the highest amounts of all metals. Ekpo et al. (2013) found high amounts of zinc, manganese, copper, lead, cadmium, and chromium in the liver and gills of *Heterotis niloticus*, *O. niloticus*, and *C. gariepinus* collected from Akampa Local Government Area, while muscles accumulated the least quantities of metals.

The liver is a detoxifying organ that is responsible for the metabolism and excretion of toxins in the body. All hazardous chemicals are likely to be processed in this organ because it is the center of metabolic processes (Ali and Shaakori 2011; Ekpo et al. 2013). The liver is prone to toxins because of its location and proximity to the venous drainage of the digestive tract; the high metabolic activity of hepatocytes makes them vulnerable, and toxins can quickly affect them (Yousuf et al., 2013). Higher levels of metals in the liver have also been linked to the fact that it is an organ most connected with detoxification and biotransformation processes, as well as one of the organs most affected by toxins in the water due to its function, position, and blood supply (Fatma 2009). Metal levels in fish tissues were found to be higher than those set for fish by international regulations and guidelines such as EC (2001), WHO (2011), FEPA (2003, 1999, and 2006), and FAO (2001). (2011). Metals in the tissues of *C. gariepinus* and *O. niloticus* observed in the current study surpassed the permitted limits defined for heavy metals.

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