

*Original Research*

# Accumulation of Trace Metal(loid)s in Fish Muscle Tissue From the Groot Letaba River, South Africa

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## Abstract

Many elements become toxic when they exceed certain concentrations in the environment. In the aquatic ecosystems, they can be accumulated in the biota such as fish and may be toxic to humans upon consumption. This study assessed bioaccumulation of the following elements; arsenic, cadmium, chromium, copper, lead and Zinc in the muscle tissue of ten common consumable fish species from Groot Letaba River in South Africa and assessed the potential human health risks. In the water, only zinc exceeded the recommended safe limit. However, most of the elements were found to be significantly accumulated in the sediments and the muscle tissue of the fish species. The elements were more accumulated in the omnivorous and some predatory fish species than the herbivores. Concentrations of As, Cd, Cr and Zn in the fish muscle tissue were above the recommended food safety guideline values, indicating a potential health risk to consumers, especially As at Site 1 and Zn at Site 4. There is a need therefore for continuous monitoring of trace metals and metalloids in the river in order to ensure the safety of aquatic organisms and humans who rely on the river.

**Keywords:** bioaccumulation, fish muscle tissue, Groot Letaba River, human health

## Introduction

Globally, fish consumption is increasing due to the increasing demand of good food for healthy living. They are considered as an integral component of a well-balanced diet worldwide because they provide energy, proteins, vitamins, essential minerals, and unsaturated fatty acids [1]. In the rural areas of many developing

countries, freshwater fish are the main source of animal protein, however, due to environmental pollution, freshwater fish are accumulating micro pollutants including metals and metalloids from the water, sediments and food that may make them to be toxic for human consumption.

The eminent increase in human population is putting severe pressure on the environment especially freshwater ecosystems. Freshwater ecosystems are being polluted mainly by industrial, agricultural and mining activities, and the disposal of partially treated and untreated

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effluents containing organic and inorganic contaminants [2, 3]. Metal(loid)s from natural and anthropogenic sources continuously enter the aquatic environment where they pose a serious threat to ecological health of freshwater ecosystems and humans, owing to their toxicity, long persistence, bioaccumulation, and biomagnification in the food chain [4]. Trace metals may accumulate in aquatic life, enter the food chain and cause serious harm to human health [5].

The distribution of trace metal(loid)s in water and sediments contribute significantly in freshwater pollution [5]. Health risks arising from the toxicity of metals and metalloids mainly include kidney and skeletal damages, neurological disorders, endocrine disruption, cardiovascular dysfunction, and carcinogenic effects [6].

Fish usually accumulate elements from their environment (water and sediments), and food [7]. They are good bioindicators because they are easily identifiable, easily sampled and are species-rich [8]. They are also on top of the aquatic food chain and can be indicative of the bioavailability of pollutants, such as trace metal(loid)s in the aquatic environment [9, 10].

The Groot Letaba River catchment in South Africa is characterised mainly by agricultural activities. The increase in commercial farming of citrus and other fruits, has resulted in increasing use of chemical fertilizers pesticides and fungicides,, often fortified with specific concentrations of elements to enhance the genetic, physical and physiological quality of the crop [11, 12]. The use of chemicals can lead to undesirable effects on the natural environment and human health [13, 14]. Adequate research on the fate, occurrence and impact of agrochemicals in the aquatic ecosystems in South African is lacking [15, 16]. Information on the eco-toxicological status of the Groot Letaba River between Tzaneen town and the Kruger National Park (KNP) is necessary to provide an indication of the extent to which the agricultural activities have affected the water quality and aquatic biota in the river. The main objectives of the present study were to assess trace metal(loid)s (As, Cd, Cr, Cu, Pb, and Zn) in the muscle tissue of commonly consumed fish species in the river and to determine which fish species pose a higher health risk upon consumption. The metals and metalloids in this study are found in agricultural products such as fertilizer, pesticides and fungicides, and are toxic to humans, even the essential metals such as Cu and Zn at high concentrations [17]. Furthermore, these are the main metals and metalloid that have been reported to be of concern in the area [18-20].

## Materials and Methods

### Study Sites and Description

The Groot Letaba River falls within the Olifants River Basin Water Management Area (WMA).

The sub-catchment drains a surface area of about 13,670 km<sup>2</sup> [21]. The river is about 461 km long. The mean width of the study area is between 10 and 12 m. The mean discharge during raining season is about 35.2 m<sup>3</sup>s<sup>-1</sup>. The Groot Letaba River is a perennial river contributing over 50% of the downstream flow of the Olifants River into the Kruger National Park (KNP). The KNP is a conservation tourism protected area in South Africa, hence proper management of water flow of the Groot Letaba River is of great importance for sustainable natural resource utilization in the park.

The Groot Letaba River catchment between Tzaneen Dam and the Kruger National Park (KNP) was selected for the study. The area was situated between two large impoundments, Tzaneen Dam and the Nondweni Weir, where intensive agricultural activities take place in the catchment and may have impact on the water quality of the river. The area is dominated by commercial agriculture (citrus, mango, avocado, bananas, cotton, maize and vegetables), of which more than 42% of this agricultural land use is under irrigation [22]. The study sites illustrated in Fig. 1 were selected based on ease of access and safety, since some sections of the river are infested with crocodiles and hippopotamus. Site 1 was situated upstream, just below Tzaneen Dam and is characterised by riparian and in-stream vegetation. However, the vegetation is mostly invasive plants including lantana, castor-oil plant, bugweed, large cocklebur and peanut butter cassia. The substrate consists mainly of cobbles (20%), gravel (30%), sand (20%), silt (20%) and clay (10%). Site 2 was situated midstream, near 400 ha citrus farm, called Letaba Estate and it is characterised by mainly rock (10%), cobbles (10%), gravel (20%), sand (30%), silt (20%) and clay(10) with small channels and islands in between. The riparian vegetation included large trees and reeds. Other anthropogenic activities, besides citrus farming are a golf course and human settlements in Nkowankowa Township. Site 3 was also situated midstream, surrounded by many small farms and canning warehouses. The substrate consists mainly of rocks (5%), cobbles (10%), gravel (20%), sand (30%), silt (25%) and clay (10%). Site 4 was downstream, at a weir and near small farms, human settlements and a waterworks facility. The substrate at this site consists mainly of pebbles (10%), sand (30%), silt (40%) and clay (20%).

### Field Data Collection and Laboratory Analysis

Sampling was carried out twice a year (during low and high flow of 2015 and 2016). Water samples were collected in acid-washed sampling bottles. A composite sampling technique was used to take water samples at three points (two littoral zone edges and main channel centre) of the river at each site for chemical analysis. Surface sediment samples were collected at a depth of about 5-10 cm using a hand shovel, after removing

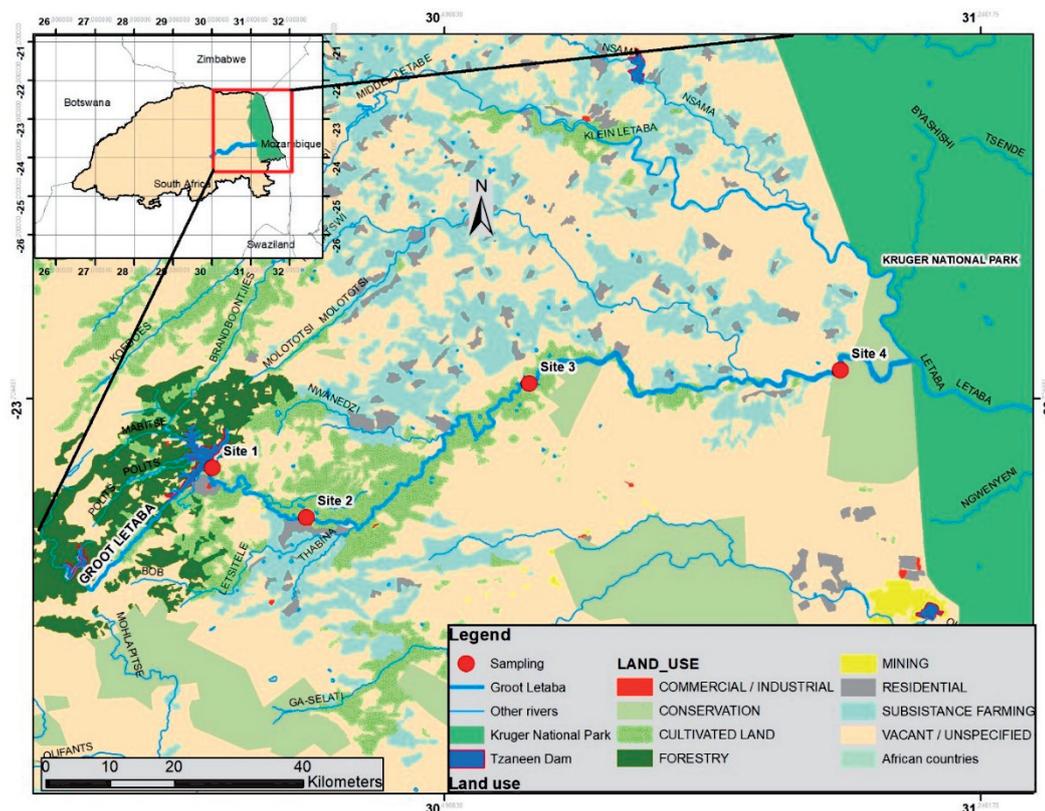


Fig. 1. The selected study sites between Tzaneen Dam and KNP of the Groot Letaba River.

the overlaying debris. In collecting the sediments, where possible, large stones were shifted to collect samples beneath them. At each site, five sub-samples were mixed together, forming a composite sample [23]. The samples were placed in 10% nitric acid pre-treated polyethylene ziplock bags, transported to the laboratory and were frozen ( $-20^{\circ}\text{C}$ ) prior to chemical analysis. Water and sediment samples were analysed for elements at an accredited (ISO 17025) chemical laboratory (WATERLAB (PTY) LTD in Pretoria, South Africa). The samples were put in acid-washed polypropylene pre-weighed vials and dried at  $60^{\circ}\text{C}$  for 24 h. They were then digested according to the methods of Bervoets and Blust [23] (2003) and analysed for metals (As, Cd, Cr, Cu, Pb and Zn) using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer, Optima 2100 DV). Concentrations of the metals in the sediments were calculated and expressed as mg/kg dry weight. Analytical accuracy was determined using certified standards (De Bruyn Spectroscopic Solutions 500 MUL20-50STD2) and recoveries were within 10% of certified values.

The various fish species collected in the Groot Letaba River and their habits are shown in Table 1. These fish species were selected as they are common species consumed in the area and consists of the various feeding groups. Fish were collected biannually at the four selected sites using gill nets (20 m long and a depth of 1.5 m with different mesh sizes ranging from 1.5x1.5 cm to 4x4 cm, set overnight).

A maximum of 20 adult fish per species were collected where possible. Each fish was then sacrificed by severing the spinal cord behind the head with sharp scissors [24, 25]. Muscle tissue (10 g) was removed for metal(loid)s analysis. The samples were snap frozen in liquid nitrogen and transported to laboratory at the Department of Biodiversity, University of Limpopo to be stored at  $-80^{\circ}\text{C}$  refrigerator until analysis. The muscle tissue samples were dried, digested and analysed for metals [23], using inductively coupled plasma-optical emission spectrometry (Perkin Elmer, Optima 2100 DV). Because of insufficient samples of the fish species, the samples of the seasons were pooled for further analysis.

### Data Analysis

The mean and standard deviation of metal concentrations in the sediment and fish muscle tissue samples were calculated. Analysis of variable (ANOVA) was used to test for significant differences in the concentrations of trace metal(loid)s in the samples among the sites, using SPSS software version 25. For comparison of means, ANOVA test was used. The results of the test were considered significant if the calculated p-values were  $\leq 0.05$ . Spearman correlation was used to assess the relationship between the elements in the fish species.

Table 1. Fish species from the Groot Letaba River and their habits [49] (Skelton, 2001)

| Fish species                      | Common names                   | Food items   | Habits   |
|-----------------------------------|--------------------------------|--|--|
| <i>Oreochromis mossambicus</i>    | Mozambique tilapia             | Algae, especially diatoms and detritus. Large fish, insects and other invertebrates.                                     | Inhabits reservoirs, rivers, creeks, drains, swamps and tidal creeks; often in well-vegetated areas. Normally not found at high altitudes. Highly euryhaline. Tolerates low dissolved oxygen levels and can utilise atmospheric oxygen when water oxygen levels drop. Mainly diurnal, omnivorous/ herbivore.   |
| <i>Schilbe intermedius</i>        | Silver catfish (butter barbel) | Fish, insects, shrimps, snails, plant seeds and fruit  | Reported to be a pelagic species, generally abundant in open water, in both lacustrine and fluvial conditions, often showing shoaling habits; Occur mainly on shallow waters. Migrate to the surface at night. Surface feeder; nocturnal, omnivorous, voracious and predaceous.  |
| <i>Clarias gariepinus</i>         | Sharptooth catfish             | Fish, birds, frogs, small mammals, reptiles, insects, shrimps, snails, crabs, other invertebrates, plant seeds and fruit | Adults occur mainly in quiet waters, lakes and pools and prefer rather shallow and swampy areas with a soft muddy substrate and calmer water, also occur in fast flowing rivers and in rapids. Widely tolerant of extreme environmental conditions. The presence of an accessory breathing organ enables this species to breath air when very active or under very dry conditions. Omnivorous (prey & scavenger) |
| <i>Marcusenius macrolepidotus</i> | Bulldog                        | Invertebrates, e.g. midge and mayfly larvae and pupae.   | Prefers well-vegetated, muddy bottomed marginal habitats of rivers and floodplains. A shoaling species which moves inshore after dark. Predator/carnivorous.   |
| <i>Labeo rosae</i>                | Rednose labeo                  | Detritus, algae and small invertebrates  | Occurs in the warmer reaches of rivers, particularly in sandy stretches An active fish, leaps at barriers when migrating upstream in swollen rivers to breed in summer. Omnivorous.  |
| <i>Labeo cylindricus</i>          | Redeye labeo                   | Algae, aufwuchs, tree trunks.  | Shoaling species. Occurs in both sediment-free and sediment-rich rocky biotopes; favors clear, running waters in rocky habitats of small and large rivers, also found in lakes and dams over rocky areas. Herbivore.   |
| <i>Labeobarbus marequensis</i>    | Lowveld largescale yellowfish  | Algae, insect larvae, small fishes, snails, mussels, drifting organisms such as beetles and ants.                        | Prefers flowing waters of perennial rivers. Uncommon in dams. Omnivorous (opportunistic).  |
| <i>Micropterus salmoides</i>      | Largemouth bass                | Invertebrates and fish   | Inhabit lakes, ponds, swamps, and backwaters and pools of creeks, and small to large rivers. Usually found over mud or sand. They prefer quiet, clear water and over-grown banks. Predator.  |
| <i>Synodontis zambezensis</i>     | Brown Squeaker                 | Bottom feeder on detritus, seeds, insects, snails.   | Occurs in pools and slow flowing reaches of perennial and seasonal rivers. Active at night. Omnivorous.  |

## Results and Discussion

### Trace Metals and Metalloids in Water and Sediments

All the metals tested in the water were below the detection limit, except Zn. The concentrations of Zn were above the DWAF environmental standard of 0.002 mg/l [26] at Sites 1, 2 and 4. However, the metals in the sediment were recorded in considerable levels (Table 2). The highest concentrations of Cd, Cu, Pb and Zn were recorded at Site 2 and the highest concentrations of As and Cr were recorded at Site 1. The mean concentrations of Cr and Cu were above the recommended sediment guideline values.

### Metals and Metalloids in Fish Muscle Tissue

The concentrations of metals and metalloids in the fish muscle tissue are shown in Table 3. Metals that showed a significant difference between sites were Cd, Pb and Zn ( $p < 0.05$ ). The highest mean concentrations of Cd, Cr, and Pb were at Site 2, the highest mean concentration of As was at Site 1, the highest mean concentration of Cu was at Site 4 and the highest mean concentration of Zn was at Site 3. Similar to the sediment, the highest concentrations of most of the metals were at Site 2.

The levels of the trace metals varied significantly among fish species ( $p < 0.05$ ). The highest concentrations of Cr and Pb were recorded in *S. zambezensis*, the highest As and Zn concentrations were recorded in *L. marequensis*, the highest concentration of Cd was recorded in *C. gariepinus*, while the highest

Table 2. Mean trace metal and metalloid concentrations (mg/kg dw) in the sediments of the Groot Letaba River (Bold values exceeded the guideline values).

|                  | Site 1         | Site 2        | Site 3       | Site 4       | Guideline                           |
|------------------|----------------|---------------|--------------|--------------|-------------------------------------|
| Elements (n = 4) | Mean±SD        | Mean±SD       | Mean±SD      | Mean±SD      | values                              |
| As               | 2.7±2.1        | 2.5±1.6       | 0.9±0.5      | 0.6±0.4      | 19 <sup>a</sup> , 9.79 <sup>b</sup> |
| Cd               | n.d            | 0.009±0.02    | n.d          | n.d          | 1 <sup>a</sup> , 0.99 <sup>b</sup>  |
| Cr               | <b>219±207</b> | <b>155±30</b> | <b>71±38</b> | <b>86±51</b> | 62 <sup>a</sup> , 43.4 <sup>b</sup> |
| Cu               | <b>47±21</b>   | <b>75±14</b>  | <b>32±24</b> | <b>40±29</b> | 20 <sup>a</sup> , 31.6 <sup>b</sup> |
| Pb               | 19.4±13.9      | 28±8.7        | 11.8±9.3     | 10.6±7.8     | 40 <sup>a</sup> , 35.8 <sup>b</sup> |
| Zn               | 75±8.0         | 92±29         | 39±23        | 45±25        | 147 <sup>a</sup> , 121 <sup>b</sup> |

dw – dry weight

n.d – not detected (0.001 µg/l)

a – Flemish guidelines (De Deckere et al., 2011) [50]

b – Sediment guidelines on the consensus based on Threshold Effect Concentrations (TEC) [51] (Macdonald et al., 2000).

Table 3. Mean trace metal and metalloid concentrations and standard deviation (mg/kg) in fish muscle tissue at different sites of the Groot Letaba River and maximum permissible guideline values.

| Site                 | Fish                     | N  | As        | Cd        | Cr        | Cu        | Pb        | Zn    |
|----------------------|--------------------------|----|-----------|-----------|-----------|-----------|-----------|-------|
| Site 1               | <i>L. marequensis</i>    | 4  | 2.32±3.35 | 0.02±0.05 | n.d       | 2.0±0.42  | 0.05±0.06 | 36±6  |
|                      | <i>O. mossambicus</i>    | 2  | 2.2±3.11  | n.d       | n.d       | 1.55±0.21 | n.d       | 44±6  |
|                      | <i>S. intermedius</i>    | 7  | 0.1±0.08  | 0.01±0.04 | 0.06±0.15 | 1.31±0.24 | 0.06±0.05 | 23±11 |
| Site 2               | <i>O. mossambicus</i>    | 9  | 0.01±0.02 | n.d       | n.d       | 1.02±0.38 | 0.01±0.02 | 24±8  |
|                      | <i>S. intermedius</i>    | 4  | 0.32±0.28 | 0.01±0.01 | 0.04±0.06 | 2.52±3.13 | 0.02±0.02 | 28±8  |
|                      | <i>C. gariepinus</i>     | 6  | 0.53±1.43 | 0.54±1.31 | 0.47±1.12 | 1.14±1.28 | 0.46±1.14 | 22±6  |
|                      | <i>M. macrolepidotus</i> | 23 | 0.6±0.99  | 0.2±0.61  | 0.24±0.53 | 1.79±0.68 | 0.19±0.54 | 62±16 |
|                      | <i>M. salmoides</i>      | 2  | 0.17±0.20 | n.d       | 0.01±0.02 | 0.3±0.42  | n.d       | 23±3  |
| Site 3               | <i>L. marequensis</i>    | 4  | 0.44±0.29 | n.d       | 0.04±0.03 | 0.53±0.50 | n.d       | 22±5  |
|                      | <i>O. mossambicus</i>    | 7  | 0.4±0.87  | 0.29±0.76 | 0.35±0.60 | 0.84±0.64 | 0.28±0.65 | 27±3  |
|                      | <i>C. gariepinus</i>     | 4  | 0.01±0.02 | n.d       | 0.11±0.09 | 1.15±0.50 | n.d       | 29±4  |
|                      | <i>M. macrolepidotus</i> | 2  | 0.18±0.26 | n.d       | n.d       | 1.42±0.02 | 0.01±0.01 | 76±9  |
|                      | <i>L. rosae</i>          | 14 | 0.42±0.82 | 0.26±0.83 | 0.26±0.80 | 1.43±1.03 | 0.27±0.82 | 23±8  |
|                      | <i>L. cylindricus</i>    | 10 | 0.26±0.15 | 0.02±0.04 | 0.01±0.02 | 2.77±1.08 | 0.06±0.07 | 38±6  |
| Site 4               | <i>O. mossambicus</i>    | 9  | 0.31±0.38 | 0.02±0.02 | 0.15±0.12 | 1.54±0.43 | 0.05±0.06 | 34±9  |
|                      | <i>S. intermedius</i>    | 4  | 0.25±0.29 | 0.02±0.04 | 0.17±0.11 | 1.25±0.35 | 0.05±0.03 | 38±7  |
|                      | <i>M. macrolepidotus</i> | 1  | n.d       | n.d       | n.d       | 2.15±0.00 | n.d       | 35±11 |
|                      | <i>L. rosae</i>          | 5  | 0.6±0.41  | 0.01±0.01 | 0.04±0.03 | 1.8±0.56  | n.d       | 43±17 |
|                      | <i>M. salmoides</i>      | 1  | n.d       | n.d       | 0.02±0.00 | 0.83±0.00 | n.d       | 20±4  |
|                      | <i>S. zambezensis</i>    | 15 | 0.41±0.95 | 0.21±0.77 | 0.32±0.83 | 2.5±0.88  | 0.32±0.85 | 34±7  |
| FAO guideline values |                          |    | 1.0       | 0.05      | 1.0       | 30.0      | 0.5       | 30    |

n.d - not detected

concentration of Cu was in *L. cylindricus* (Fig. 2). The concentration of As exceeded the FAO limit of 1.0 mg/kg in *L. marequensis* and *O. mossambicus* at Site 1. The concentration of Cd exceeded the FAO limit of 0.05 mg/kg in *C. gariepinus*, *M. macrolepidotus* at Site 2, *O. mossambicus* and *L. rosae* at Site 3, and *S. zambezensis* at Site 4. While Zn concentration exceeded the FAO limit of 30 mg/kg in *L. marequensis* and *O. mossambicus* at Site 1, *M. macrolepidotus* at Site 2, *M. macrolepidotus* and *L. cylindricus* at Site 3, and *O. mossambicus*, *S. intermedius*, *M. macrolepidotus*, *L. rosae* and *S. zambezensis* at Site 4.

Arsenic

The As concentration ranged from no detection to 2.32±3.35 in the muscle tissue of *L. marequensis* at Site 1.

Cadmium

The highest mean concentration was 0.54±1.31 mg/kg in the muscle tissue of *C. gariepinus* at Site 2. The Cd concentration exceeded the maximum

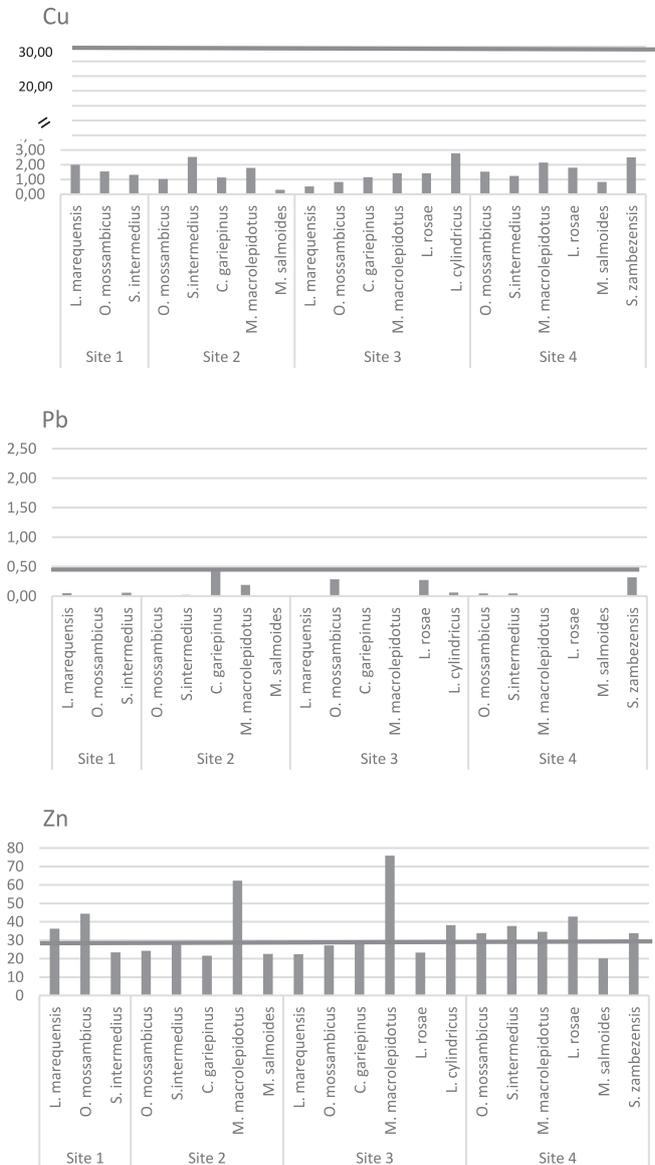
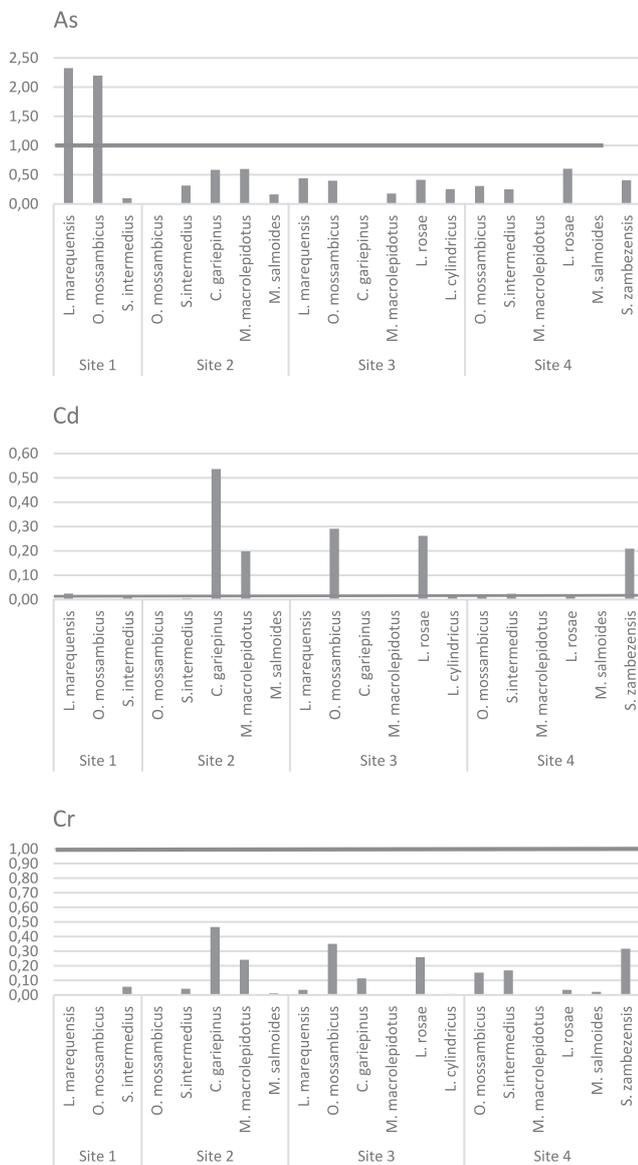


Fig. 2. Comparison of trace metal and metalloids concentrations (mg/kg) in the muscle tissue of the fish species from the Groot Letaba River (red lines represent the maximum permissible limits).

permissible limit of 0.05 mg/kg [27] in *C. gariepinus* and *M. macrolepidotus* at Site 2, *O. mossambicus* and *L. rosae* at Site 3, and *S. zambezensis* at Site 4. Industrial processes such as smelting or electroplating and fertilizers can contribute to the environmental concentration of Cd [30], in this area the possible Cd pollution could be from fertilizers. It is a highly toxic element, which can be transported in the air. It can cause kidney failure and softening of bones following long-term or high dose exposure [31] and high levels of Cd can cause prostate cancer [32].

#### Chromium

The highest mean concentration was  $0.47 \pm 1.12$  mg/kg in the muscle tissue of *C. gariepinus* at Site 2. The Cd concentration was below the maximum permissible limit of 1.0 mg/kg [27] in all the species. The highest Cr concentration at Site 2 might be coming from the citrus farms near the river. Some fungicides, pesticides and fertilizers, are known to contain Cr [30]. Several studies have shown that chromium (VI) compounds can increase the risk of lung cancer [33]. Fish health can also be affected by Cr exposure. The presence of Cr together with other metals are known to increase the glycogen level in different organs causing stress due to the metal exposure [34].

#### Copper

The mean concentration of Cu ranged from  $0.3 \pm 0.42$  mg/kg in *M. salmoides* muscle tissue at Site 2 to  $2.77 \pm 1.08$  mg/kg in *L. cylindricus* at Site 3. The mean Cu concentration was below the maximum permissible limit of 30 mg/kg [27]. The Cu concentration recorded in this study were similar to the records from the Olifants River [20, 24]. Copper is a micronutrient for plants and it plays a pivotal role in many enzymes and proteins, also with increasing crop yield or improving nutritional quality of food [35]. Fish health effects associated with Cu include, damage to target tissues from the disruption of ionic and osmotic regulations, oxidative stress and enzyme inhibition [36, 37]. Consequently, fish such as *L. cylindricus* which feed by scraping on diatoms and small algae that adhere to surfaces may be exposed to Cu, as it is either absorbed by the diatoms or settled on the surfaces. The possible source of Cu in the Groot Letaba River could be from fertilizer and pesticides used in the farms.

#### Lead

The highest mean Pb concentration of  $0.46 \pm 1.14$  mg/kg was in the muscle tissue of *C. gariepinus* at Site 2. The Pb concentration recorded in all the species was below the maximum permissible limit of 2.5 mg/kg [27] and 1.5 mg/kg [38]. Lead is a ubiquitous pollutant which could find its way into rivers through discharge of industrial effluents from various industries

and other human activities. In fish, excess Pb level may decrease in survival, growth, development, behaviour and metabolism, in addition to an increase in the formation of mucus [39]. Excess Pb concentration can cause renal, liver muscular, cardiovascular and reproductive disorders in humans [40] and chronic exposure may cause cognitive and learning deficits in children.

#### Zinc

The mean concentration of Zn ranged from  $20.0 \pm 4.0$  mg/kg at Site 4 to  $76.0 \pm 9.0$  mg/kg in *M. macrolepidotus* at Site 3. Zn concentration was the highest among all the elements in the fish species. The concentration of Zn exceeded the recommended permissible limit of 30.0 mg/kg [27] in *C. gariepinus* and *M. macrolepidotus* at Site 2, *O. mossambicus* and *L. rosae* at Site 3, and *S. zambezensis* at Site 4. The recorded levels of Zn in various fish muscle tissues in this study are similar to other records from other South African rivers, such as the Sand River, Olifants River and Nyl River [41, 42]. However, higher concentrations than the present study have been reported in *O. mossambicus* muscle tissues collected from Nyl River in the Limpopo Province [41]. The main sources of Zn could be from fertilizers [43] and sewage [44]. Bioaccumulation of Zn is generally not considered to have acute effects because it is essential for aquatic organisms, though it can be toxic at high concentrations [45-47].

Generally, the highest metal concentrations were found in the omnivorous and predators. Herbivorous fish, such as *L. cylindricus* which are primary consumers, eat aquatic macrophytes, submerged land plants and filamentous algae. Being at a lower trophic level, they do not have the variety of food intake as in omnivores and carnivores, hence biomagnification in herbivorous fish is not as large as for those in higher trophic levels [48]. The high accumulation of metals in omnivorous fish in this study may be due to the fact that most omnivorous fish caught were bottom dwellers; thus, they may obtain contaminants from both the sediments and food [48].

#### Correlation Analysis of Trace Metal in Fish Tissue

The results of the correlation analysis are shown in Table 4. There were significant correlations between As and Cd ( $r = 0.50$ ,  $p < 0.05$ ), Cd and Cr ( $r = 0.62$ ,  $p < 0.05$ ), Cd and Pb ( $r = 0.91$ ,  $p < 0.05$ ), Cr and Pb ( $r = 0.69$ ,  $p < 0.05$ ) and Cu and Zn ( $r = 0.75$ ,  $p < 0.05$ ). The relationship between As and Cd is probably due to the high concentrations of these two elements in *O. mossambicus*. The relationships between Cd and Cr, Cd and Pb, and Cr and Pb could be due to the high concentrations of these trace metals in *C. gariepinus*. The relationship between Cu and Zn could be due

Table 4. Spearman correlation of the trace metal and metalloid concentrations in the muscle tissue of the fish species from The Groot Letaba River.

|    | As    | Cd           | Cr           | Cu     | Pb           | Zn           |
|----|-------|--------------|--------------|--------|--------------|--------------|
| As | 1.000 | <b>0.504</b> | 0.240        | 0.297  | 0.324        | 0.291        |
| Cd |       | 1.000        | <b>0.767</b> | 0.259  | <b>0.917</b> | 0.005        |
| Cr |       |              | 1.000        | -0.127 | <b>0.686</b> | -0.276       |
| Cu |       |              |              | 1.000  | 0.219        | <b>0.618</b> |
| Pb |       |              |              |        | 1.000        | -0.044       |
| Zn |       |              |              |        |              | 1.000        |

to the high concentrations of these two elements in *M. macrolepidotus*. The relationship between pairs of elements could be probably due to the fact that they are originating from similar sources and being accumulated in the various fish species. For the present study, the main sources of metal(loid) pollution are agricultural and sewage discharges, for example, As, Cd, Cr, Cu, Pb and Zn are found in fertilizers, pesticides and fungicides [43], and municipal wastes contain most of these elements, if not all [44].

### Conclusion

The concentrations of six trace metals and metalloid in the muscle tissue of nine commonly consumed fish species from the Groot Letaba River were analysed. Fish species from Site 2 had higher concentrations of the metals than those from the other sites. Among the fish species, omnivorous fish species had higher levels of trace metals compared with herbivorous fish species. The following fish species, *L. marequensis*, *O. mossambicus*, *C. gariépinus*, *L. rosae*, *M. macrolepidotus* were found to contain high metal(loid) concentrations especially at Site 1 and Site 4, and therefore their consumption should be minimized. Correlation analysis showed strong positive correlations between As and Cd, Cd and Cr, Cd and Pb, Cr and Pb, and Cu and Zn indicating that they had the same source. Intensive agricultural activities in the catchment of the river might be the sources of high concentration of these metal (loids), as all the elements are found in fertilizers, pesticides and fungicides. Although the concentrations of Cr, Cu and Pb found in the fish muscle tissue may not be harmful, it is important to take into account the possible consequences of the long-term effect of consuming fish from the river and there is also a possibility that the concentrations of those elements may increase to levels that may be toxic as the activities in the catchment continues unabated and their discharges end up in the river. The river needs to be monitored regularly to prevent further trace metal(loid) pollution and to sustain a healthy ecosystem.

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### Conflicts of Interest

The authors declare no conflict of interest.

### References

1. MAHANTY A., GANGULY S., VERMA A., SAHOO S., MITRA P., PARIJA P., SHARMA A.P., SINGH B.K., MOHANTY B.P. Nutrient profile of small indigenous fish *Puntius sophore*: proximate composition, amino acid, fatty acid and micronutrient profiles. *Natl. Acad. Sci. Lett.* **37** (1), 39, **2014**.
2. BHAT R.A., SHAFIQ-UR-REHMAN M.M.A., DERVASH M.A., MUSHTAQ N., BHAT J.I.A., DAR, G.H. Current status of nutrient load in Dal Lake of Kashmir Himalaya. *J. Pharmacog. Phytochem.* **6** (6), 165, **2017**.
3. ADDO-BEDIAKO A., RASIFUDI L. Spatial distribution of heavy metals in the Ga-Selati River of the Olifants River System, South Africa. *Chem. Ecol.* **37** (5), 450, **2021**.

4. ALI H., KHAN E., ILAHI I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *J. Chem.* **2019** (4), 1, **2019**.
5. ADDO-BEDIAKO A., MARR S.M., JOOSTE A., LUUS-POWELL W.J. Human health risk assessment for silver catfish *Schilbe intermedius* Rüppell, 1832, from two impoundments in the Olifants River, Limpopo, South Africa. *Water SA* **40**, 607, **2014**.
6. RENIERI E.A., SAFENKOVA IV., ALEGAKIS A.K., SLUTSKAYA E.S., KOKARAKI V., KENTOURI M., DZANTIEV B.B., TSATSAKIS A.M. Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: risk evaluation for consumers. *Food. Chem. Toxicol.* **124**, 439, **2019**.
7. ZHAO S., FENG C., QUAN W., CHEN X., NIU J., SHEN Z. Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. *Mar. Pollut. Bull.* **64**, 1163, **2012**.
8. NAIGAGA I., KAISER H., MULLER W.J., OJOK L., MBABAZI D., MAGEZI G., MUHUMUZA E. Fish as bioindicators in aquatic environmental pollution assessment: A case study in Lake Victoria wetlands, Uganda. *Phys. Chem. Earth.* **36**, 918, **2011**.
9. VOEGBORLO R.B., ATTA A., AGORKU E.S. Total mercury distribution in different tissues of six species of freshwater fish from the Kpong hydroelectric reservoir in Ghana. *Environ. Model. Assess.* **184**, 3259, **2012**.
10. AHMED A.S.S., RAHMAN M., SULTANA S., BABU S.M.O.F., SARKER M.S.I. Bioaccumulation and heavy metal concentration in tissues of some commercial fishes from the Meghna River Estuary in Bangladesh and human health implications. *Mar. Pollut. Bull.* **145**, 436, **2019**.
11. SHEEHY J.E., REBETZKE G.J., SADRAS V.O. Facets of the maximum crop yield problem. *Field Crops Res.* **182**, 1, **2015**.
12. WORSFOLD P., POOLE C., TOWNSHEND A., MIRÓ M., BARKER A.V. Fertilizers. In *Encyclopedia of Analytical Science*, 3<sup>rd</sup> edition, 134, **2019**.
13. GAGLIARDI B., PETTIGROVE V. Removal of intensive agriculture from the landscape improves aquatic ecosystem health. *Agric. Ecosyst. Environ.* **176**, 1, **2013**.
14. KROFLIĆ A., GERM M., GOLOB A., STIBILJ V. Does extensive agriculture influence the concentration of trace elements in the aquatic plant *Veronica anagallis-aquatica*? *Ecotoxicol. Environ. Saf.* **150**, 123, **2018**.
15. DALLAS H., DAY J. The Effect of Water Quality Variables on Aquatic Ecosystems: A review. Water Research Commission. Cape Town, South Africa. 222, **2004**.
16. ANSARA-ROSS T., WEPENER V., VAN DEN BRINK P., ROSS M. Pesticides in South African fresh waters. *Afri. J. Aquat. Sci.* **37**, 1, **2012**.
17. MAURYA P.K., MALIK D.S., YADAV K.K., KUMAR A., KUMAR S., KAMYAB H. Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicol. Rep.* **6**, 472, **2019**.
18. ADDO-BEDIAKO A., MARR S.M., JOOSTE A., LUUS-POWELL W.J. Are metals in the muscle tissue of Mozambique tilapia a threat to human health? A case study of two impoundments in the Olifants River, Limpopo province, South Africa. *Ann. Limnol-Int. J. Lim.* **50**, 201, **2014**.
19. JOOSTE A., MARR S.M., ADDO-BEDIAKO A., LUUS-POWELL W.J. Metal bioaccumulation in the fish of the Olifants River, Limpopo province, South Africa, and the associated human health risk: a case study of rednose laboe *Labeo rosae* from two impoundments. *Afr. J. Aquat. Sci.* **39**, 271, **2014**.
20. MARR S.M., JOOSTE A., ADDO-BEDIAKO A., LUUS-POWELL W.J. Are catfish from metal-polluted impoundments in the Olifants River, South Africa, safe for human consumption? *Inland Waters* **5**, 215, **2015**.
21. Department of Water Affairs and forestry (DWAF) (2006). National Water Resources Strategy. Annual Report 2006, Pretoria, South Africa. **2006**.
22. STATE OF RIVERS REPORT (SOR). Letaba and Luvuvhu river systems. Water Research Commission, Pretoria. **44**, **2001**.
23. BERVOETS L., BLUST R. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. *Environ. Pollut.* **26**, 9, **2003**.
24. JOOSTE A., MARR S.M., ADDO-BEDIAKO A., LUUS-POWELL W.J. Sharptooth catfish shows its metal: A case study of metal contamination at two impoundments in the Olifants River, Limpopo river system, South Africa. *Ecotoxicol. Environ. Saf.* **112**, 96, **2015**.
25. BUAH-KWOFIE A., HUMPHRIES M.S., PILLAY L. Bioaccumulation and risk assessment of organochlorine pesticides in fish from a global biodiversity hotspot: iSimangaliso Wetland Park, South Africa. *Sci. Total Environ.* **621**, 273, **2018**.
26. DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). South African Water Quality Guidelines Volume 7: Aquatic Ecosystems. Department of Water Affairs and Forestry, Pretoria, South Africa. 1996, 145, **1996**.
27. FOOD AND AGRICULTURE ORGANIZATION (FAO). Compilation of legal limits for hazardous substances in fish and fishery production, FAO Fishery Circular, **464**, 5, **1983**.
28. CANADIAN COUNCIL OF MINISTRIES OF THE ENVIRONMENT (CCME). Canadian Water Quality Guidelines for the Protection of Aquatic Life: Arsenic. Ottawa, Ontario. 2001, 4, **2001**.
29. FLORA S.J.S., PACHAURI V., SAXENA G. Arsenic, cadmium and lead. In Gupta, R. C. (ed.). *Reproductive and Developmental Toxicology*. Academic Press. 415, **2011**.
30. WHO (World Health Organisation). (2004). Guidelines for Drinking Water Quality (3RD edn.) Vol. **1**. Recommendations. WHO, Geneva, Switzerland. 515, **2011**.
31. VANNOORT R.W., THOMSON B.M. New Zealand total diet survey: agricultural compound residue, selected contaminants and nutrients. New Zealand Food Safety Authority, 144, **2006**.
32. GRAY M.A., HARRINS A., CENTENO J.A. The role of cadmium, zinc, and selenium in prostate disease. In: Moore TA, Black A, Centeno JA, Harding JS, Trumm DA (eds). *Metal contaminants in New Zealand: sources, treatments, and effects on ecology and human health*. Resolutionz Press, Christchurch, 393, **2005**.
33. ISHIKAWA Y., NAGAKAWA K., SATOH Y., KITAGAWA T., SUGANO H., HIRANO T., TSUCHIYA E. Characteristics of chromate workers' cancers, chromium lung deposition and precancerous bronchial lesions: an autopsy study. *Br. J. Cancer.* **70**, 60, **1994**.
34. JAVED M., USMANI N. Accumulation of heavy metals in fishes: a human health concern. *Int J. Env. Sci.* **2** (2), 659, **2011**.
35. YRUELA I. Copper in plants. *Braz. J. Plant Physiol.* **17**, 145, **2005**.

36. CHEN H.R., YANG H.C., HSIEH D.J.Y., LIU Z., TSAI K. J. Zebrafish *sod1* and *spl* expression are modulated by the copper ATPase gene in response to intracellular copper status. *Chem. Biol. Interact.* **189**, 192, **2011**.
37. ZEBRAL Y.D., ANNI I.S.A., AFONSO S.B., ABRIL S.I.M., KLEIN R.D., BIANCHINI A. Effects of life-time exposure to waterborne copper on the somatotrophic axis of the viviparous fish *Poecilia vivipara*. *Chemosphere* **203**, 410, **2018**.
38. European Community (EC). Commission Regulation No 78/2005 (pp. L16/43eL16/45). Official J Eur Union. **2005**.
39. EISLER R. Lead hazards to fish, wildlife, and invertebrates: A synoptic review. US Fish and Wildlife Service Report. **85**, 1, **1998**.
40. ULLAH A.K.M.A., MAKSUD M.A., KHAN S.R., LUTFA L.N., QURAIISHI S.B. Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. *Toxicol Rep.* **4**, 574, **2017**.
41. MUSA R., GERBER R., GREENFIELD R. A Multivariate Analysis of Metal Concentrations in Two Fish Species of the Nyl River System, Limpopo Province, South Africa. *Bull. Environ. Contam. Toxicol.* **98**, 817, **2017**.
42. MOYO N.A.G., RAPATSA M.M. Trace Metal Contamination and Risk Assessment of an Urban River in Limpopo Province, South Africa. *Bull. Environ. Contam. Toxicol.* **102**, 492, **2019**.
43. REZA R., SINGH G. Heavy metal contamination and its indexing approach for river water. *Int J Reza R, Singh G. Environ. Sci. Technol.* **7**, 785, **2010**.
44. SOJKA M., JASKUTA J., SIEPAK M. Heavy Metals in Bottom Sediments of Reservoirs in the Lowland Area of Western Poland: Concentrations, Distribution, Sources and Ecological Risk. *Water* **11**, 56, **2019**.
45. ZHANG C., SONG N., ZENG G.M., JIANG M., ZHANG J.C., HU, X.J., CHEN A.W., ZHEN J.M. Bioaccumulation of zinc, lead, copper, and cadmium from contaminated sediments by native plant species and *Acrida cinerea* in South China. *Environ. Monit. Assess.* **186**, 1735, **2014**.
46. LORO V.L., NOGUEIRA L., NADELLA S.R., WOOD C.M. Zinc bioaccumulation and ionoregulatory impacts in *Fundulus heteroclitus* exposed to sublethal waterborne zinc at different salinities. *Comp Biochem Physiol C Toxicol Pharmacol.* **166**, 96, **2014**.
47. FENG J., LIN Y, YANG Y., SHEN Q., HUANG J., WANG S., ZHU X., LI, Z. Tolerance and bioaccumulation of combined copper, zinc, and cadmium in *Sesuvium portulacastrum*. *Mar. Pollut. Bull.* **131**, 416, **2018**.
48. HASHIM R., SONG T.H., MUSLIM, N.Z. M., YEN, T.P. Determination of Heavy Metal Levels in Fishes from the Lower Reach of the Kelantan River, Kelantan, Malaysia. *Trop. Life Sci. Res.* **25** (2), 21, **2014**.
49. SKELTON P. A Complete Guide to the Freshwater Fishes of Southern Africa. (2<sup>nd</sup> edition). Struik Publishers, Cape Town, South Africa. 397, **2001**.
50. DE DECKERE E., DE COOMAN W., LELOUP V., MEIRE P., SCHMITT C., VON DER OHE P.C. Development of sediment quality guidelines for freshwater ecosystems. *J. Soil Sediment* **11**, 504, **2011**.
51. MACDONALD D.D., INGERSOLL C.G., BERGER T.A. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* **39**, 20, **2000**.