Environmental Science Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Adv.*, 2025, **4**, 1501

Metals and metalloids in macrophytes and fish from an acid mine drainage-impacted river system in South Africa: aspects of bioindication and phytoremediation†

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The consequences of acid mine drainage (AMD) are apparent in water and sediment of the upper reaches of the Crocodile River (West) system, which is located in the western basin of the Witwatersrand mountain chain in South Africa. Another significant indicator for metal and metalloid pollution in aquatic systems is biota. In particular, in the case of AMD-impacted areas, which occur worldwide, biota could serve as an important bioindicator of contamination and also be useful in terms of remediation of pollutants. We investigated the content of Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb in the liver and muscle tissue of 4 fish species (*Oreochromis mossambicus*, *Labeobarbus polylepis*, *Labeobarbus marequensis*, and *Clarias gariepinus*) and in roots and stems of macrophytes *Pontederia crassipes*, *Typha* sp. and *Phragmites australis* taken from selected sites in the system. Metals and metalloids were analyzed in freeze-dried and acid-digested samples using graphite furnace atomic absorption spectroscopy, cold vapor atomic absorption spectroscopy, and total reflection X-ray fluorescence spectroscopy. We determined that the mean levels of Fe, Ni and Cu were increased in the roots of *Typha* sp. and *P. crassipes* at sites influenced by AMD. Both macrophytes could also be used for phytoremediation of Ni, Cu and Cd, with bioaccumulation factors above 1 at all study sites ranging from 1.1 (Cu) to 21 (Cd) for *Typha* sp. and 2.7 (Ni) to 25 (Cd) for *P. crassipes*. Plants were found to be better bioindicators for AMD than fish due to homeostatic regulation of Ni, Cu, and Zn in fish at chronic low-level pollution. An exception was the liver tissue of *O. mossambicus*, which accumulated high levels of Ni (2.98 ± 1.24 mg kg^{-1} dw), Cu (184 ± 124 mg kg^{-1} dw) and Ag (2.01 ± 0.51 mg kg^{-1} dw), demonstrating its bioindicative potential for these metals. Our study allowed a detailed look at an AMD influenced river system, revealing results that clearly demonstrate the consequences of chronic low-level pollution, although water melioration is in place.

Received 17th May 2025
Accepted 26th June 2025

DOI: 10.1039/d5va00136f

rsc.li/esadvances

Environmental significance

Acid mine drainage is a worldwide problem occurring after mines are abandoned and water fills the mine shafts dissolving metals. Mining influenced water can overflow and spill into the aquatic environment causing serious health problems for biota including humans. Even if waste-water-treatment plants are installed, a low-level contamination of aquatic systems may occur; effects on biota are not well understood in many cases. For this study, we analysed fish and plant tissues from an impacted river system in South Africa following a 13 month survey of water and sediments. Clearly elevated levels of metals were detected in plants demonstrating their bio-indicative potential as well as suggesting their use for phytoremediation. Fish liver also reflected the elevated metal levels in the environment.

1 Introduction

Elevated concentrations of metals and metalloids (MMs) in the environment pose a major risk to the health of living organisms. Even though low concentrations of some of those naturally occurring elements are essential for biota (*e.g.* Cu and Zn), all MMs are toxic above certain critical concentrations.[1] Prior to anthropogenic influences, the natural geological background levels typically explained their occurrence in aquatic environments. Through anthropogenic activities, however, the levels of some MMs spiked globally, posing a threat to biota due to their persistence and bioaccumulative properties.[2,3] One activity that releases high levels of MMs into the environment is mining, in particular coal, uranium or gold mining. Primarily, mining influenced water (MIW), which is chemically affected by mining

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5va00136f

or processing of minerals, is of major concern in terms of pollution in mining-impacted areas. A specific type of MIW with high mineral acidity called acid mine drainage (AMD) is especially problematic and carries the risk of discharging high amounts of MMs into the environment.[4] AMD occurs when oxygenated water comes into contact with sulfide minerals, mainly FeS_2 (pyrite). This contact produces Fe(II) hydroxide as well as sulfuric acid in a two-stage reaction, leading to a significant drop in the pH of mining-influenced water.[5] The water entering mines in operation is constantly mechanically evacuated, but once mines are abandoned they slowly start to fill with groundwater, triggering the reactions leading to AMD. In particular, abandoned gold mines pose a threat of developing AMD because they are usually rich in sulfide-bearing ores yet low in potentially neutralizing minerals (*e.g.* dolomite: $CaMg(CO_3)_2$). Therefore, MIW remains acidic and high loads of otherwise antisoluble MMs are dissolved from the mine's host rock. Once MIW starts to spill from the mine, it poses a risk for surrounding groundwater resources and for surface waters near the mines.[5,6] AMD is of major concern in almost all globally relevant mining countries, including Australia, Canada, China, Japan, the United States, the United Kingdom, and South Africa. Over 600 000 abandoned mines in these countries have either already generated AMD, or bear the risk of doing so. In the US alone, over 20 000 km of rivers and streams have been influenced and damaged by MIW. In South Africa, over 6000 mines have been abandoned/closed down.[7] One severely affected area is the western mining basin on West Rand near Johannesburg, South Africa, where highly acidic and MM-polluted MIW started to enter the upper reaches of the Crocodile River (West) system in 2002.[8] Despite the efforts made to treat MIW, our long-term study over 13 months on water and sediment in the system still found elevated contents of some MMs in both matrices.[9] Next to water and sediment, biota serves as an important indicator for the state of an aquatic system because they are exposed to the full range of physico-chemical influences of their proximate environment.[10] Monitoring and the use of bioindicators can provide important information on the magnitude or extent of pollution ("accumulation bioindicators"). Moreover, they also enable assessing the actual bioavailability of a pollutant in a system.[11] In aquatic systems, both plants and animal species are potential bioindicators for water and sediment contamination by MMs. Different fish species such as tilapia *Oreochromis mossambicus* (Peters 1852) have received attention in this regard because they provide long-term insights into the state of their habitat.[12] Most MMs have a long half-life in fish, showing potentially increased tissue content under both chronic and discontinuous exposure.[13] Plessl *et al.*, for example, reported fish to be valuable bioindicators for MM pollution, whereby the lifestyle of the respective species plays a role.[14] Different studies demonstrate that several tissues are suitable as bioindicators, including the liver (organ of detoxification) as well as the muscle tissue (consumed by predators and humans).[15] Apart from fish, macrophytes are well-established bioindicators. The focus is on macrophytes for several reasons: (i) they are sessile, reflecting local contamination, (ii) they are often widespread in a system and easy to collect, (iii) they consist of sufficient biomass for quantitative MM analysis, and (iv) they are often able to adapt to adverse conditions and are known to accumulate high amounts of MMs.[11,16] The accumulation of MMs in the macrophyte tissue can play a key role in the eco-balance of aquatic systems. Furthermore, this specific property of plants introduces an approach to removing MMs from polluted systems *via* so-called phytoremediation. Phytoremediation describes the potential of plants to effectively immobilize, remove, or detoxify MMs (or pollutants in general). Different mechanisms of phytoremediation include phytoextraction, rhizofiltration, and phytostabilization.[17,18] The efficiency or ability to remove MMs from sediments can be calculated using the bioaccumulation factor (BAF), which indicates phytoremediation potential at factors >1.[19] The free-floating water hyacinth *Pontederia crassipes* Mart. is a plant known for effectively phytoremediating a number of MMs including Cd, As, Ni, Hg, and Pb.[18] Beyond free-floating macrophytes, some submerged aquatic plants as well as emergent aquatic plants have the ability to bioaccumulate large amounts of MMs. Emergent aquatic plants usually grow in sediments covered by water. The ability to bioaccumulate MMs mainly in subterraneous parts is known for the cattail *Typha* sp. and the common reed *Phragmites australis* (Cav.) Trin. ex Steudel. This makes them promising candidates for the phytoremediation of Fe, Cd, Zn, Ni, and Cu from contaminated sediments.[18]

Extremely poor quality of water and sediment have been reported for the upper reaches of the Crocodile River (West) due to the occurrence of AMD, with high fluctuations in pH, total dissolved solids (indicated by electrical conductivity) and levels of some dissolved metals including Fe, Ni, and Zn drastically exceeding quality standards.[8,9] Although these three elements are considered essential for biota, they exhibit toxicity when present in high concentrations and may have adverse effects on plants as well as animals.[1,20] The data on MM levels from biota of this system are still very limited, and the potential use of plants and fish as bioindicators as well as plants as phytoremediation for MM pollution have not been scientifically discussed. We therefore performed a comprehensive study on the bioaccumulation of MMs in different biota. The most abundant fish species in the system, as well as the three water plant species occurring in sufficient abundance, were sampled to provide a first overview on the effects of MIW. The fish species were the Mozambique tilapia *O. mossambicus*, the yellowfish species *Labeobarbus polylepis* (Boulenger, 1907) and *Labeobarbus marequensis* (Smith, 1841), and the African sharptooth catfish *Clarias gariepinus* (Burchell, 1822). The plant species were the water hyacinth *P. crassipes* (a free-floating macrophyte) as well as the emergent macrophytes *Typha* sp. and *P. australis.* Analyzing the levels of 10 MMs (Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb) in different compartments of the biota yielded knowledge on bioindicative as well as phytoremediative aspects in the upper reaches of the Crocodile River (West) system. This information can also serve as an example for other comparable MIW-influenced sites around the world.

2 Materials and methods

2.1. Study area

The Crocodile River (West) originates in the Witwatersrand to the east of Krugersdorp, near the city of Johannesburg in South

Africa. It is the main tributary of the Hartbeespoort Dam and later runs into the Limpopo River. In the upper reaches of the river urbanization, industry, mining, and agriculture heavily influence the water quality.[21] Some smaller tributaries in the West Rand add water to the system; the two streams "Bloubankspruit" and "Tweelopiespruit" are amongst them. They are located near Krugersdorp in the Gauteng province in South Africa (Fig. 1). The Tweelopiespruit is fed with water from an abandoned gold mine in Randfontein and is a tributary to the Bloubankspruit, which then confluences with the Crocodile River (West). Shortly after, the Crocodile River (West) is dammed up to the Lake Heritage Dam by the Franz Richter wall, constructed in 2006. As a lodge is located on the lake, it is used for fly fishing, paddling, canoeing, and open-water swimming.[9]

Regarding the work of Windisch *et al.*, in which water and sediments were observed over a period of 13 months, seven sampling sites were chosen along the river system, especially considering confluences (Fig. 1).[9] Coordinates of these sites are given in Table S1.† For this study, plants and fish were collected, where available: plants from sites 1, 3, 5 and 7, fish only from site 7. For clarity, we left the numbering as in Windisch *et al.*;[9] therefore, numbers 2, 4 and 6 do not occur in this paper. Site 1 is located at Tweelopiespruit directly influenced by AMD, as its water mainly consists of treated MIW entering the system from the abandoned gold mine. Site 3 is situated along Bloubankspruit after the inflow of Tweelopiespruit. Site 5 is located along the Crocodile River (West) before the inflow of Bloubankspruit. Site 7 is the dam, Lake Heritage.[9]

2.2. Sampled plant and fish species

Common reed *P. australis* is widely distributed and is a significant part of many wetlands all over the world.[22] It has been identified to be adaptive to different environments and achieves high productivity under diverse pH and salinity conditions of sediments as well as diverse climatic conditions.[23] This species has an underground system of rhizomes and roots, making it an emergent macrophyte. It also accumulates metals from sediments, in particular Fe and Al in its roots.[24] Cattail *Typha* spp. belong to the family Typhaceae, and most species are native to North and Central Europe, with some also abundant in South Asia, Africa, and Australia.[25] They are considered semi-aquatic or aquatic, rooted in sediment and achieve fast growth rates. *Typha* spp. emerge from rhizomes, which the plant uses for vegetative growth and proliferation.[26] Amongst others, one species, namely *T. latifolia*, has been identified as a bioindicator of Mn, Zn, Cd, Pb, Ni, Cu and Co by Klink *et al.*[27] As it was not possible to obtain 100% identification down to species level in the Crocodile River system, we refer to the plants used in our study as *Typha* sp. The water hyacinth *P. crassipes* (syn. *Eichhornia crassipes*) is native to South America. It is a highly invasive species and has been spreading in subtropical and tropical regions of the world for the last 200 years. The plant is free floating and tolerates extremely contaminated environments.[28] It grows rapidly, doubling its population in 6 to 18 days. The average root length of *P. crassipes* is around 20–60 cm, but roots can extend to 300 cm and account for more than 60% of the plant's total weight.[29] Through so-called passive transfer, the submerged roots directly interact with the polluted medium, during which bioaccumulation takes place. Although due to fast growth rates and quick reproduction, the species leads to a number of environmental and socioeconomic issues, it has also been successfully used for phytoremediation of metals, *e.g.* Cr and Pb.[30]

The Mozambique tilapia *O. mossambicus* is indigenous to eastward flowing waters of Southern and Central Africa. The species mainly feeds on phytoplankton, algae, as well as zooplankton and smaller insects.[31] Of all fish species investigated, it is the species lowest in the aquatic food chain with a trophic level of 2.2 (Table S2†). This cichlid mouthbrooder tolerates a wide range of ecological conditions.[32] Several studies have investigated the use of this species as a bioindicator of As, Cd, Cr, and Cu exposure.[12] The smallscale yellowfish *L. polylepis* and the closely related largescale yellowfish *L. marequensis* belong to the family Cyprinidae and are distributed in the southern tributaries of the Limpopo River system (Skelton 2001). Both have similar feeding habits and primarily feed on algae, zoobenthos and small fish.[33] Their trophic levels are not significantly different at 3.3 and 3.2, respectively (Table S2†). Neither of

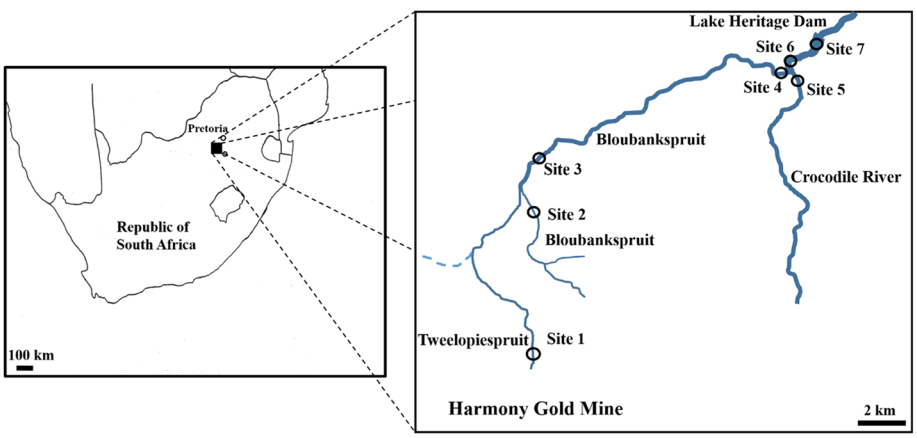

Fig. 1 Map of the Republic of South Africa, a black square indicating the sampling area and black rings specifying the locations of sampling sites 1–7.

the two species has been used for bioindication of MM pollution. The African sharptooth catfish *C. gariepinus* is a member of the family Clariidae. It is a bottom-sediment-dwelling omnivore and the highest in the food chain of all fish investigated here (3.8).[33,34] This species has been used for indicational purposes of environmental MM contamination by Farombi *et al.*, who reported high levels of Zn, Cd, As, Pb, and Cu in its liver, kidney, heart, and gill tissues as bioindicators of MM pollution.[35]

2.3. Sampling of plants and fish

Sampling of fish and water plants took place at the end of the long-term study on water and sediments in January 2020.[9] The fish were euthanized following the South African National Standard: Care and Use of Animals for Scientific Purposes (2008) and after obtaining the required ethical clearance from the University of Johannesburg (Protocol number: 2018-02-15/Gilbert). Collection permit CPE2-000129 from the Gauteng Department of Agriculture and Rural Development was used. Plant samples were taken where available, namely *P. australis* ($n = 13$), from site 1; *Typha* sp. ($n = 17$) from sites 3, 5 and 7; *P. crassipes* ($n = 16$) from sites 5 and 7. Plants were hand-picked using nitrile gloves. We collected rhizomes and stems of *P. australis* and roots and stems of *Typha* sp. and *P. crassipes*. For simplicity, all underground parts are addressed as "roots" in the following, being aware that rhizomes and roots have different anatomy and functions and are not biologically equivalent. Parts of root and stem (~5 g wet weight) of the respective plant were cut off in the field using stainless steel instruments. They were transferred into acid pre-washed 50 mL PP tubes and repeatedly, vigorously rinsed with lake water and thereafter thrice with distilled water, until the sediment was removed from the surface. The water was then discarded and samples were placed into a portable freezer, where they were kept until further processing. Thereafter, frozen samples were freeze-dried (Christ ALPHA 2-4LDplus) and sent to the University of Vienna, Institute of Inorganic Chemistry.

Adult fish in similar sizes were caught at site 7 using gill nets. This site was chosen for the sampling of fish, as it was the only site where adult fish were available. Measurements (total length and weight) of all fish are presented in Table S2.† In total, 4 specimens of *O. mossambicus*, 13 specimens of *L. polylepis*, 28 specimens of *L. marequensis* and 30 specimens of *C. gariepinus* were included. After sampling, the fish were killed by cervical dislocation. Before dissection, their total length and weight were documented. Liver and dorsal muscle tissues (app. 3 g) were sampled using a ceramic knife as well as plastic tweezers, put into 15 mL PP tubes, and frozen immediately at −18 °C. Subsequently, frozen samples were freeze-dried at the Department of Zoology at the University of Johannesburg and transported to the Institute of Inorganic Chemistry at the University of Vienna.

2.4. Sample preparation and analyses

Lyophilized plant and fish samples were homogenized using a mortar and pestle, and approximately 0.2 g of the respective tissue was digested in 4.5 mL of >68% HNO_3 (PrimarPlus, trace analysis grade, Fisher Scientific), 4.5 mL Milli-Q water and 1 mL of 30% H_2O_2 (>30%, for trace analysis, Sigma-Aldrich) using a microwave MARS XPRESS system (CEM Corporation). The digested samples were transferred into 15 mL volumetric flasks and brought to volume using Milli-Q water. The samples were filtered through 0.45 μm Nylon pre-syringe filters (VWR) and stored in 15 mL PP tubes for further analysis. Approximately 0.2 g (dry weight) of reference material fish proteins (DOLT-3 and DOLT-5) obtained from the National Research Council Canada (NRCC), as well as lichen (BCR-482) from the Institute for Reference Materials and Measurements (IRMM), were digested and diluted in the same manner as described for the samples ($n = 5$, respectively).

Sediment samples of sites 3, 5 and 7 taken by Windisch *et al.* were used for the determination of Fe.[9] On an analytical balance, approximately 0.5 g of the selected freeze-dried, homogenized sediment sample was weighed into a glass tube. Samples were leached using 4.5 mL Milli-Q water, 4.5 mL *aqua regia*, which was always freshly prepared in a molar ratio of 3 : 1 of HCl : HNO_3 (HCl: 34–37%, Trace Metal Grade, Fisher Scientific, HNO_3: >68%, PrimarPlus-trace analysis grade), and 1 mL of H_2O_2 (>30%, for trace analysis, Sigma-Aldrich). Topped with air coolers, the sediment samples were leached for 2 h at 130 °C in a heating block. The leached samples were transferred into 20 mL volumetric flasks and filled up to volume with Milli-Q water. The content was subsequently filtered through 0.45 μm PTFE syringe filters (VWR) and stored in 15 mL centrifuge tubes until analyses.

The elements Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb in plant and fish samples were measured using total X-ray reflection fluorescence spectroscopy (TXRF; S2 PicoFox, Bruker) or graphite furnace atomic absorption spectroscopy (GF-AAS; PinAAcle 900Z, PerkinElmer), depending on the element and content. Their total mercury (Hg) content was measured using cold vapor atomic absorption spectroscopy (CV-AAS; FIMS 400, PerkinElmer). In addition to the other MMs determined and discussed in Windisch *et al.*,[9] Fe in sediment leachates was determined using flame atomic absorption spectroscopy (F-AAS).

The MM content in all matrices was determined as mg kg^{-1} dry weight (dw). The recovery rates of the reference materials DOLT-3 and DOLT-5 by NRCC as well as BCR-482 by IRMM for the respective methods under the applied conditions as well as the LODs for the methods used are presented in Table S3.†

2.5. Statistical analyses

Statistical analyses were carried out using R (Version 4.2.1) with the package "FSA".[36,37] The Shapiro–Wilk test was used to check for normal distribution of values in each group. A one-way ANOVA was performed for groups that were normally distributed, and a nonparametric Kruskal–Wallis test was used when groups were not normally distributed. Following one-way ANOVA, Tukey's honest significant difference test was chosen for pairwise comparison of the means of all groups. Following the Kruskal–Wallis test, Dunn's test was chosen for pairwise comparison of the groups, adjusted for multiple testing by the

Holm–Bonferroni method. All differences were tested for significance at $p < 0.05$. For determination of the correlation between Fe and Ni contents in roots of *Typha* sp., a Spearman's rank order correlation was performed using Origin 2020b (OriginLab).

The bioaccumulation factor (BAF) was determined to estimate the efficiency of MM accumulation from the sediment and was calculated according to the following formula:

$$\mathrm{BAF}_{\left(\frac{\mathrm{root}}{\mathrm{sed}}\right)} = \frac{\text{concentration of element} \in \text{roots}}{\text{concentration of element} \in \text{sediment}}$$

Sediment data for Ni, Cu, Zn, As, Ag, Cd, Hg, and Pb were taken from Windisch *et al.*,[9] and Fe concentrations were determined for this study.

Additionally, the translocation factor was determined to evaluate the translocation of MMs from the root to stem tissue. The results are presented in Table S4.†

$$\mathrm{TLF}_{\left(\frac{\mathrm{stem}}{\mathrm{root}}\right)} = \frac{\text{concentration of element} \in \text{stems}}{\text{concentration of element} \in \text{roots}}$$

3 Results and discussion

3.1. Metals and metalloids in macrophytes

The MM contents of *P. australis, Typha* sp. and *P. crassipes* are presented in Table 1. In general, the MM content is higher in roots than in stems in all macrophytes. These findings emphasise the role of root cell wall binding during the translocation of metals in macrophytes from the root symplast to the xylem apoplast.[38] A number of processes regulate this translocation from roots to shoots, with cell wall binding being the main reason why MMs are found more abundantly in root tissues than in shoots.[39,40] MMs are described at concerning levels in water and sediment by Windisch *et al.*, calling for investigating these elements in plants.[9] The levels of Au and U were below the limit of detection in all plant samples (LODs of 0.035 mg kg^{-1} and 2 mg kg^{-1} for Au and U, respectively). In the following, the results of MMs of interest in plants are discussed.

3.1.1. Fe. Iron was found to be the most abundant element in all the analysed plant samples. The content was determined as total Fe, *i.e.*, no differentiation was made between internal Fe and potential Fe-plaque on the root surface. The mean Fe content in roots of *Typha* sp., *P. crassipes*, and *P. australis* was significantly higher than in stems, which agrees with previously published work on those macrophytes.[41–43] The mean Fe values in roots were higher in *P. crassipes* at site 7 and in *P. australis* at site 1 compared to *Typha* sp. at all sites and *P. crassipes* at site 5. The site-specific difference of *P. crassipes* and *Typha* sp. root content is depicted in Fig. 2. In particular, the roots of *P. australis* from site 1 accumulated high levels of iron (11 064 ± 7445 mg kg^{-1}). Significantly lower contents in this plant's roots were reported by Bonanno at an urban site in Italy and by van Deventer & Cho at an unpolluted site in South Africa.[41,43] A study by van Deventer & Cho in 2011 and 2012 also included samples from the same site as this study, with the iron results being well comparable.[43] The significant difference in mean iron content between polluted and unpolluted sites showed that high iron loads in the sediment at site 1 led to an excessive accumulation in *P. australis* roots not only in 2011/12, but more importantly still in 2019/2020. *P. australis* is a known bioindicator for iron and other metals in polluted sediments.[44] Considering that iron is the signature element of MIW, an ongoing impact of AMD on

Table 1 Mean (±SD) metal and metalloid content in roots and stems of *P. australis* (site 1), *Typha* sp. at sites 3, 5 and 7, as well as *P. crassipes* at sites 5 and 7. All values are given in mg kg^{-1} dw. Significant differences between mean values of root and stem samples for *P. australis* are indicated by the following letter "a" ($p < 0.05$). For *Thypha* sp. and *P. crassipes* same superscript letters indicate significant differences between the sites for the respective tissue ($p < 0.05$)

Element	Tissue	*P. australis* Site 1 ($n = 13$)	*Typha* sp. Site 3 ($n = 9$)	*Typha* sp. Site 5 ($n = 4$)	*Typha* sp. Site 7 ($n = 4$)	*P. crassipes* Site 5 ($n = 3$)	*P. crassipes* Site 7 ($n = 13$)
Fe	Root	11064^{a} ± 7444	3262^{a} ± 2929	1053^{b} ± 1169	1601^{a,b} ± 483	1225^{a} ± 969	14690^{a} ± 8550
	Stem	112^{a} ± 95	1138^{a} ± 1594	502^{b} ± 381	61.2^{a,b} ± 15.9	161 ± 46.6	265 ± 151
Ni	Root	28.9^{a} ± 29.5	32.0^{a} ± 27.1	28.9^{b} ± 8.4	160^{a,b} ± 21	47.4 ± 75.4	99.8 ± 41.7
	Stem	4.36^{a} ± 4.41	10.1^{a} ± 12.2	2.44^{a,b} ± 0.41	5.60^{b} ± 1.48	75.2 ± 60.9	17.5 ± 34.6
Cu	Root	14.7^{a} ± 10.6	11.8 ± 8.5	18.8 ± 10.7	34.4 ± 10.2	22.7 ± 27.5	37.1 ± 24.4
	Stem	4.65^{a} ± 3.87	8.86^{a} ± 4.18	2.65^{a,b} ± 0.85	20.7^{b} ± 20.7	18.3 ± 11.4	5.13 ± 4.03
Zn	Root	68.6 ± 48.1	292 ± 293	154 ± 74	51.7 ± 22.4	164 ± 217	87.2 ± 33.4
	Stem	48.8 ± 33.0	116 ± 120	27.4 ± 18.8	11.7 ± 1.3	263^{a} ± 190	21.1^{a} ± 8.0
As	Root	4.01^{a} ± 2.51	3.41 ± 3.02	4.41 ± 2.17	1.69 ± 1.23	2.32 ± 0.44	3.17 ± 1.23
	Stem	0.485^{a} ± 0.936	0.112 ± 0.062	0.142 ± 0.124	0.505 ± 0.912	<0.04	0.275 ± 0.299
Ag	Root	0.028 ± 0.015	0.031 ± 0.044	0.031 ± 0.041	0.021 ± 0.014	0.051 ± 0.043	0.061 ± 0.044
	Stem	0.011 ± 0.010	0.045^{a} ± 0.051	0.005^{a} ± 0.003	0.005^{b} ± 0.005	0.046 ± 0.043	0.017 ± 0.036
Cd	Root	0.074^{a} ± 0.051	0.191 ± 0.232	0.250 ± 0.345	0.150 ± 0.110	0.294 ± 0.252	0.129 ± 0.072
	Stem	0.029^{a} ± 0.030	0.039^{a} ± 0.037	0.013^{b} ± 0.003	0.001^{a,b} ± 0.001	0.355^{a} ± 0.225	0.037^{a} ± 0.082
Hg	Root	0.066 ± 0.063	0.062 ± 0.055	0.072 ± 0.061	0.030 ± 0.023	0.047 ± 0.045	0.112 ± 0.072
	Stem	0.030 ± 0.018	0.069 ± 0.046	0.064 ± 0.031	0.031 ± 0.013	0.057 ± 0.029	0.042 ± 0.048
Pb	Root	0.109 ± 0.124	4.15 ± 3.36	<0.01	<0.01	18.5 ± 4.9	17.7 ± 7.4
	Stem	0.322 ± 0.188	0.107 ± 0.128	<0.01	<0.01	<0.01	0.191 ± 0.186

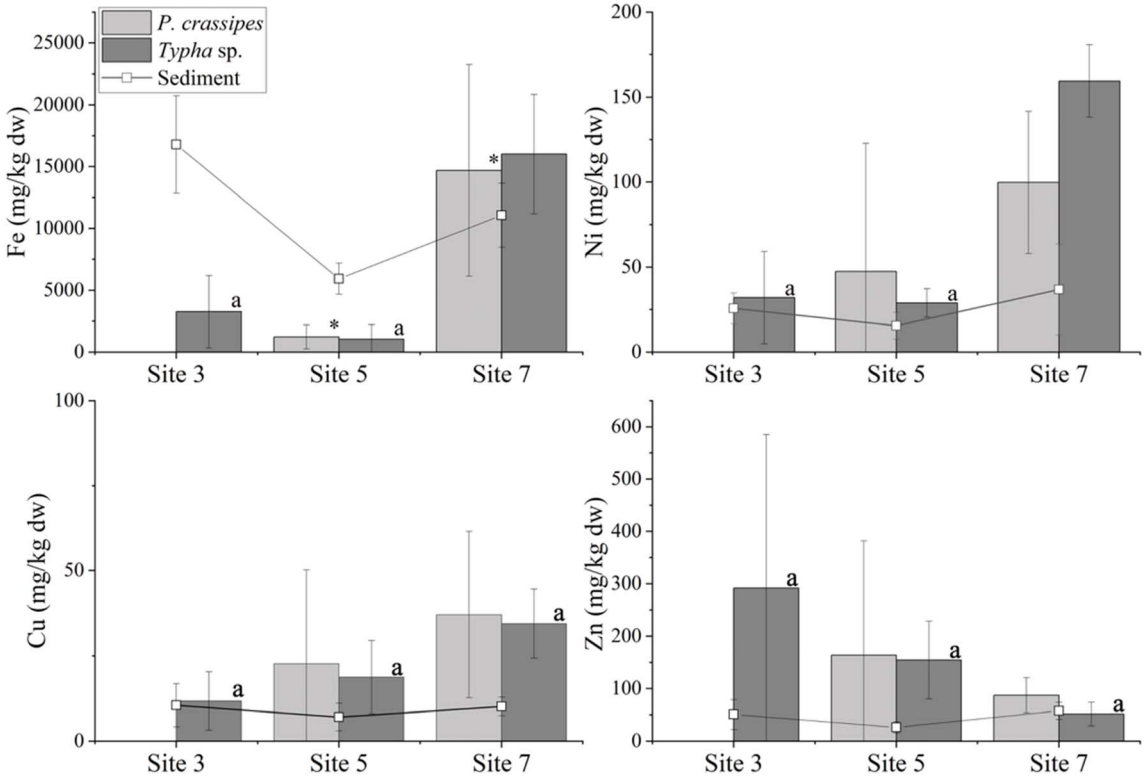

Fig. 2 Mean (±SD) content of elements Ni, Cu, Zn and Fe in *P. crassipes* and *Typha* sp. roots, as well as in sediment at different sampling sites. Values for *Typha* sp. from different sites do not differ significantly if followed by the letter "a" ($p > 0.05$). Values for *P. crassipes* differ significantly if followed by "*"($p < 0.05$).

biota at this study site was evident, even years after initiating water treatment. The influence of AMD was also apparent further downstream, where higher mean Fe contents in *Typha* sp. and *P. crassipes* roots were detected at AMD-influenced sites, correlating with higher concentrations in sediments (Fig. 2). These results point to the potential use of roots of both plant species for biomonitoring because root Fe levels quantitatively reflected the proximate environment. In particular, the roots of *P. crassipes* accumulated significantly higher loads of iron at site 7 *versus* roots at site 5. Comparison with earlier work on the plant's Fe root content in a former mining pond in Malaysia showed that our values were slightly lower at site 5 but significantly higher at site 7.[42] Interestingly, the bioaccumulation capacities for both plants investigated here were site-dependent, with $BAFs_{(root/sed)} > 1$ at site 7 for *Typha* sp. and *P. crassipes* and <1 at sites 3 and 5 (Table 2). The phytoremediation properties of *P. crassipes* and *Typha* sp. were well investigated and were mainly conducted in microcosms and batch studies in standing waters, where both plants showed potential for iron removal from sediments.[45–47] In our study, the lower bioaccumulation capacities at upstream sites were most likely linked to the flow velocity. Sites 3 and 5 are part of a lotic aquatic system, whereas site 7 is a lentic waterbody, implying sedimentation and suspension with longer contact time of macrophytes and water/sediment at the latter site.

3.1.2. Ni. The mean Ni content was higher in roots of macrophytes at site 7 compared to sites 3 and 5. The mean root Ni content of *P. australis* at site 1 was lower than that of both *Typha* sp. and *P. crassipes* at site 7, but not compared to sites 3 and 5. A literature comparison showed that the root content of *Typha* sp. agrees well with published values at sites 3 and 5, whereas the value at site 7 was higher than the previously published values.[27] A potential explanation is the slow flow velocity at site 7, as previously noted for iron above. The *P. crassipes* root content at all sampling sites agreed well with concentrations in other polluted waterbodies.[42,48,49] The mean Ni content of *P. australis* roots (28.93 ± 29.58 mg kg^{-1}) was higher compared to 1.625–3.585 mg kg^{-1} reported by Duman *et al.* in Lake Sapanca (Turkey) and 23.8 mg kg^{-1} reported by Parzych *et al.* in northern Poland.[50,51] Other results with higher Ni contents (125 mg kg^{-1}) were published by van Deventer & Cho, which is especially of interest considering that those plants were taken from the same site as for this study.[43] At first sight, this might point to a significant decrease of Ni content in the plant's roots over time at this site. However, the highest root content of an individual root sample in our study was 94.3 mg kg^{-1}, with a similar value in some other individual roots, pointing to a inhomogeneous distribution of Ni in roots of *P. australis* at sampling site 1. In terms of bioaccumulation, *P. australis* showed a lower potential than *Typha* sp. and *P. crassipes*. The BAF for *P. australis* was below 1 (0.27) (Table 2), which agrees with data of Bonanno & Vymazal from Italy.[41] In *P. crassipes*, the BAF for Ni was 3.1 and 2.7 at sites 5 and 7, respectively. Similar results were obtained for *Typha* sp., with BAFs ranging

Table 2 Calculated mean bioaccumulation factor (BAF) for macrophytes at different sites

			$BAF_{(Root/Sediment)}$								
	Site	*n*	Fe	Ni	Cu	Zn	As	Ag	Cd	Hg	Pb
P. australis	1	13	—	0.27	0.75	0.41	0.05	0.34	0.76	0.66	0.01
Typha sp	3	9	0.19	**1.2**	**1.1**	**5.8**	0.49	0.71	**9.9**	**1.2**	0.75
	5	4	0.17	**1.9**	**2.7**	**5.9**	**1.6**	**1.1**	**21**	**1.7**	—
	7	4	1.4	**4.3**	**3.4**	0.89	0.11	**1.0**	**6.2**	—	—
P. crassipes	5	3	0.21	**3.1**	**3.2**	**6.2**	0.84	**1.6**	**25**	**1.1**	**3.8**
	7	13	1.3	**2.7**	**3.6**	**1.5**	0.21	**3.0**	**5.4**	—	**2.8**

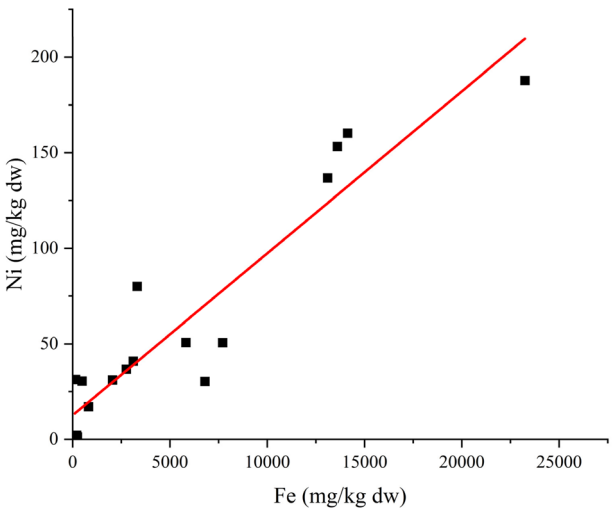

Fig. 3 Positive correlation of Fe and Ni contents in roots of *Typha* sp. from all sampling sites; $n = 15$; Pearson's $r = 0.9371$.

between 1.2 and 4.3, indicating that both macrophytes are accumulators of Ni (BAF > 1). These results support the findings of previously published work, where both aquatic plants were associated with potential Ni bioaccumulation and a possible use for phytoremediation.[40,52,53] Interestingly, a similar trend as seen for iron was evident for Ni in the roots of *P. crassipes* and *Typha* sp., with higher mean content along the course of MIW (Fig. 2). Furthermore, sediment concentrations are correspondingly related to root content in plants, pointing to a potential use of roots as bioindicators for Ni as well. There was a strong positive correlation between the *Typha* sp. root content of Ni and Fe (Fig. 3). In particular, in the case of AMD-influenced environments, co-precipitation of Fe and Ni and subsequent accumulation of the two metals might be responsible for this.[54]

3.1.3. Cu. As for Ni, a higher mean Cu content was found in roots of *Typha* sp. and *P. crassipes* at site 7, with mean concentrations of 34.4 ± 10.2 mg kg^{-1} and 37.1 ± 24.4 mg kg^{-1}, respectively. The mean copper content in the roots of both plants at other sampling sites and of *P. australis* was below 30 mg kg^{-1} (Table 1 and Fig. 2). Several publications found similar concentration ranges of Cu in underground parts of all three macrophytes.[27,50,51,55] Comparing the content of underground parts to stems, a rather high translocation of Cu occurred compared to other essential elements investigated here. Significant differences between root/stem were found solely for *P. crassipes* and *Typha* sp. at sites 5 and 7, respectively. Klink *et al.*, for example, also found relatively high translocation factors for Cu in *Typha* sp. compared to other investigated metals.[27] The high mobility of Cu within the plants can be explained by active transport due to its essentiality for plant growth and its occurrence in enzymes.[27] Besides better translocation, the trends were similar to those described for Fe and Ni. The root content of *Typha* sp. and *P. crassipes* was relatively higher at sites influenced by AMD, where the Cu sediment content was higher as well. Additionally, trends for Ni also aligned in terms of Cu bioaccumulation in roots of investigated plants. BAFs for Cu were above 1 for *Typha* sp., with values of 1.1, 2.7, and 3.4 at sites 3, 5, and 7, respectively. The corresponding values for *P. crassipes* were 3.2 and 3.6 at sites 5 and 7, respectively (Table 2). Only *P. australis* showed a BAF < 1. These results emphasize the potential use of *P. crassipes* and *Typha* sp. for the phytoremediation of Cu from polluted environments.

3.1.4. Zn. The mean Zn content ranged between 292 ± 293 (*Typha* sp.) and 51.7 ± 22.4 mg kg^{-1} (*Typha* sp.) in the roots and between 263 ± 190 (*P. crassipes*) and 11.7 ± 1.3 mg kg^{-1} (*Typha* sp.) in the stems of the analysed macrophytes, with no species-dependent differences. Comparison with previously published work showed that especially the levels in the roots were all in the same range.[27,42,55,56] The mean root and stem contents of *P. australis*, for example, differed only minimally from those published by van Deventer & Cho[43] and pointed to an unchanged Zn burden at study site 1. This was further confirmed by comparing the sediment content of Zn (114 mg kg^{-1}) in van Deventer & Cho with levels published by Windisch *et al.* from 2019 to 2020, who reported a mean sediment value of 171 ± 100 mg kg^{-1}.[9,43] There was no significant difference between root and stem contents of Zn in all investigated macrophytes. The translocation of Zn from underground to aboveground parts of *Typha* sp. has been described as poor.[57,58] Our results, however, showed a stronger translocation, but the high standard deviations made a definitive interpretation difficult and pointed rather to a location-dependent, inhomogeneous distribution of Zn in roots as well as stems. We also discovered an accumulation behavior of Zn that deviated from the metals discussed above (Fig. 2). The Zn content in *Typha* sp. roots did not show the same accumulation pattern, with BAFs of 5.8 and 5.9 at sites 3 and 5, respectively, and a value below 1 at

Table 3 Mean ± SD trace element levels in muscle and liver tissues in mg kg^{-1} dw; tissues of different species followed by the same letter do not differ significantly for the respective element ($p > 0.05$)

	Tissue	*O. mossambicus* ($n = 4$)	*L. polylepis* ($n = 13$)	*L. marequensis* ($n = 28$)	*C. gariepinus* ($n = 30$)
Fe	Liver	$1008^{a} \pm 293$	$398^{b} \pm 175$	$321^{b} \pm 138$	$3444^{a} \pm 3445$
	Muscle	$20.5^{a} \pm 6.79$	$20.6^{a} \pm 13.2$	$26.9^{a} \pm 32.0$	55.2 ± 27.2
Ni	Liver	2.98 ± 1.24	$0.277^{a,b} \pm 0.323$	$0.447^{a} \pm 0.326$	$0.284^{b} \pm 0.335$
	Muscle	$0.323^{a} \pm 0.214$	$0.214^{a} \pm 0.361$	$0.071^{a} \pm 0.104$	$0.117^{a} \pm 0.146$
Cu	Liver	$184^{a} \pm 124$	$72.7^{a} \pm 27.2$	$41.1^{b} \pm 16.8$	$35.7^{b} \pm 20.0$
	Muscle	$1.15^{a,b} \pm 0.24$	$3.74^{a} \pm 10.34$	$4.16^{a,c} \pm 8.79$	$1.37^{b,c} \pm 0.355$
Zn	Liver	$65.3^{a} \pm 42.4$	$158^{b} \pm 26$	$116^{a,b} \pm 39$	$105^{a} \pm 31$
	Muscle	$59.6^{a} \pm 45.1$	$20.1^{a} \pm 5.4$	$34.4^{a} \pm 41.8$	$38.9^{a} \pm 37.4$
Se	Liver	$11.5^{a} \pm 1.2$	$7.24^{a} \pm 7.53$	$10.1^{a} \pm 2.29$	29.4 ± 8.71
	Muscle	13.4 ± 0.9	$1.30^{a} \pm 1.08$	$1.98^{a,b} \pm 1.52$	$5.51^{b} \pm 4.16$
Ag	Liver	2.01 ± 0.51	$0.095^{a} \pm 0.042$	$0.121^{a} \pm 0.070$	0.020 ± 0.015
	Muscle	< 0.003	0.011 ± 0.004	0.073 ± 0.003	< 0.003
Cd	Liver	$0.191^{a,b} \pm 0.197$	$0.117^{a,b} \pm 0.091$	$0.040^{b} \pm 0.028$	$0.243^{a} \pm 0.199$
	Muscle	< 0.007	< 0.007	< 0.007	< 0.007
Hg	Liver	$0.245^{a} \pm 0.232$	$0.139^{a} \pm 0.103$	$0.250^{a} \pm 0.334$	$0.587^{a} \pm 0.307$
	Muscle	$0.138^{a} \pm 0.109$	$0.357^{a} \pm 0.188$	$0.253^{a} \pm 0.318$	$0.703^{a} \pm 0.459$
Pb	Liver	$0.035^{a} \pm 0.035$	$0.024^{a} \pm 0.034$	$0.013^{a} \pm 0.017$	$0.014^{a} \pm 0.022$
	Muscle	$0.031^{a} \pm 0.031$	$0.013^{a} \pm 0.017$	$0.008^{a} \pm 0.010$	$0.013^{a} \pm 0.028$

site 7 (Table 2). Hadad *et al.* found a similar variation in *Typha domingensis*, with BAFs generally above 1, but some exceptions throughout the study.[59] Explanations for these differences in Zn accumulation remain unclear, but based on our finding are not single observations. This topic requires future investigations. Similar to Fe, Ni and Cu, the BAF for *P. crassipes* was above 1, again pointing to the possible use of this plant for phytoremediation for Zn pollution.

3.1.5. Other metals and metalloids in macrophytes. In general, the content of As, Ag, Cd and Hg was similar in all three macrophyte species (Table 1). The only exception was Pb: significantly higher amounts were present in roots of *P. crassipes* compared to *Typha* sp. and *P. australis*. The levels of lead in *P. crassipes* roots were still relatively low compared to the results from other studies conducted in Egypt and Malaysia.[42,48] The lower content in our samples could be attributed to the generally low lead content in sediments and water of the system.[9] Nevertheless, the BAF of lead for *P. crassipes* was well above 1 at both sites (5 and 7). Therefore, this plant is not suitable for the biomonitoring of lead: the root content might lead to an overestimation of Pb content in an investigated system. Nonetheless, our data confirm the potential use of this species for Pb phytoremediation, supporting the conclusion based on Ehr-Chung wetlands in Taiwan.[60] Next to Pb, As was more abundant in plants than Cd, Hg and Ag. In all plants, the arsenic content was significantly higher in roots compared to stems. As previously discussed and reported for other elements, the higher abundance of MMs in roots compared to stems agrees with previously published work on *Typha* sp., *P. australis* and *P. crassipes.*[61,62] All three macrophytes showed poor capacity for sediment to root biotranslocation for the metalloid As, with most BAFs below 1.

The mean Cd content in the roots and stems of all investigated plants was comparatively low, indicating no Cd pollution in the system.[27,42,48,56] The bioaccumulation factor of this element was below 1 for *P. australis*, following the trend seen for other metals. Some studies suggested stronger bioaccumulation properties of *P. australis*, but Cicero-Fernández *et al.* also reported BAFs below 1 for Cd and Pb.[63,64] *P. crassipes* and *Typha* sp. showed extremely strong accumulation properties regarding Cd, with BAFs in the range 6.2–22 for *Typha* sp. and 5.4–25 for *P. crassipes*. These results agree well with previously published articles on Cd accumulation in the roots of both species, with BAFs well above 1.[62,65] Due to the overproportional accumulation of Cd from the sediment, the roots of *P. crassipes* would not serve as a potential biomonitor of Cd in the system. Silver and mercury were present in very low levels in the plants, matching the results for sediment, where very low concentrations (<0.1 mg kg^{-1}) of both metals were found.[9] This rather points to background levels than to any anthropogenic contamination in this river system, but also to non-hyper accumulative properties of the examined plant species for these metals. In particular, information on Ag in the three plant species examined here is still scarce, and the levels we describe could be used as background for comparison in future studies on this emerging pollutant. The content of Se in root and stem samples was below the LOD (2 mg kg^{-1}).

3.2. Metals and metalloids in fish

The mean fish length, weight, as well as the trophic level are depicted in Table S2.† The total lengths (TL) of the two closely related species *L. marequensis* and *L. polylepis* were very comparable, pointing to a similar age range.

The contents of MMs in liver and muscle samples of *C. gariepinus*, *L. marequensis*, *L. polylepis* and *O. mossambicus* are presented in Table 3. Next to macrophytes, the metal content in fish provides important insights into the degree of pollution in aquatic systems and might additionally provide bioindicative information. Therefore, the same elements as presented above

for macrophytes were analyzed and are discussed below in more detail.

3.2.1. Fe. The mean iron content in the liver of all four fish species was significantly higher than that in the muscle tissue, a well-known phenomenon.[66,67] These results emphasize that Fe is mainly accumulated in the liver tissue, where Fe storage and detoxification as well as the breakdown of hemoglobin lead to higher iron concentrations than in the muscle tissue.[68] Of the four species, *C. gariepinus* and *O. mossambicus* accumulated significantly higher amounts of Fe in the liver tissue, with mean levels in the range of those presented by Coetzee *et al.* and Avenant-Oldewage & Marx from another metal-contaminated river system in South Africa.[68,69] We found lower mean liver values in *L. polylepis* and *L. marequensis* of 398 ± 175 and 321 ± 138 mg kg^{-1}, respectively. In comparison, the liver tissue of *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913) and *Labeo umbratus* (Smith, 1841) from the Vaal Dam, South Africa, showed lower mean contents of 154 ± 331 and 225 ± 232 mg kg^{-1}, respectively.[67,70] A potential explanation for the elevated liver values in our study could be the higher iron concentrations in the sediment at the sampling site due to AMD. This would correspond to our results for the macrophytes here. Bury *et al.* described such increased Fe contents, where elevated values in sediment were associated with higher levels in fish.[71]

3.2.2. Ni. The mean Ni content was higher in the liver than in muscles for all fish species, with statistically significantly higher levels in *O. mossambicus*, *C. gariepinus*, and *L. marequensis*. Elevated Ni contents in freshwater fish have been described in several tissues, including the liver and muscle.[72] Note that, according to Pyle & Couture, mechanisms of Ni detoxification and storage in freshwater fish remain unknown, with the essentiality of Ni still under discussion.[72] Comparing the liver content in the four species, a significantly higher mean amount was found in the liver of *O. mossambicus* (2.98 ± 1.24 mg kg^{-1}). This was higher than the value reported by Outa *et al.*, namely a range between 0.166 and 0.190 mg kg^{-1} Ni in the liver tissue of the closely related *Oreochromis niloticus* (Linnaeus, 1758) from Lake Victoria (Kenya).[73] The liver content of *C. gariepinus* (mean value: 0.284 ± 0.335 mg kg^{-1}) was significantly lower than the 16.6 mg kg^{-1} found by Coetzee *et al.* in a heavily polluted waterbody.[69] Another publication on cyprinids in a rather unpolluted waterbody in South Africa listed liver and muscle contents in similar ranges as found by us in *L. marequensis*, and *L. polylepis*.[70] Moreover, the mean Ni content in muscles of *O. mossambicus* actually agreed well with the values published by Gilbert *et al.*, showing that the muscle content varied little within the investigated fish species.[70] An explanation lies in the homeostatic regulatory processes within fish, which allows them to maintain Ni levels even though they might inhabit Ni-richer environments.[74] Accordingly, the muscle tissue of all fish indicated no Ni contamination in the system, whereas macrophytes certainly did.

3.2.3. Cu. Copper levels were significantly higher in the liver *versus* muscles of all examined fish species, with a mean content up to two magnitudes higher in *O. mossambicus* liver. The hepatic copper accumulation, documented in all investigated fish, can be attributed to a very effective plasma Cu clearance by the organ. This implies a major role of the liver in the homeostasis of Cu in fish.[75] Accordingly, the liver tissue often exhibits greater concentration ranges and depends on local conditions and the investigated species.[76] The order of mean Cu content in the liver in the four species was: *O. mossambicus* > *L. polylepis* > *L. marequensis* > *C. gariepinus*. In particular, the mean value of *O. mossambicus* (184 ± 124 mg kg^{-1}) was above previously published values of 6.21–41.5 mg kg^{-1} for this species.[77,78] Chatterjee *et al.* compared *O. mossambicus* from an unpolluted and a polluted fishpond and reported a mean liver Cu content of 277 ± 50 mg kg^{-1} in the latter.[77] These ranges were similar to ours, thereby enabling determination of Cu pollution of site 7 based on the liver of this species. Using the liver of *O. mossambicus* as an indicator of potential Cu pollution was already suggested.[12] The thereby predicted Cu contamination would also agree with the macrophyte data, showing a higher content at this sampling site. This is further supported by differences in trophic levels and the alimentation pattern of the fish in our study: *O. mossambicus* is at the lowest level, and its diet mainly consists of plants and small invertebrates. Other fish species did not show a potential as Cu indicators because their levels did not mirror the elevated Cu levels in the system. This is evident when comparing the mean liver level of *O. mossambicus versus L. polylepis* and *L. marequensis* (72.7 ± 27.2 mg kg^{-1} and 41.1 ± 16.8 mg kg^{-1}, respectively). The latter values are in the same range as described by Plessl *et al.* for *L. kimberleyensis* (70.3 ± 9.0 mg kg^{-1}) from the rather unpolluted Vaal Dam in South Africa.[76] Although we recorded elevated Cu levels in plants, it is highly plausible that Cu, as an essential element, is highly regulated in uptake and excretion and that *Labeobarbus* species can therefore balance out the higher levels in their environment. The results are similar to those of *C. gariepinus*. Data from Vaal Dam published for this species (mean Cu content in liver of 40.2 ± 28.1 mg kg^{-1}) aligned well with our study (35.7 ± 20.0 mg kg^{-1}).[66] Compared to plants, fish did not reflect the rather mild pollution with Cu in the river.

3.2.4. Zn. The mean levels of Zn were generally higher in the liver compared to the muscle tissue, which agrees with the values of previously published work on freshwater fish.[76,79] The mean liver content ranged from 158 ± 26 (*L. polylepis*) to 65.3 ± 42.3 mg kg^{-1} (*O. mossambicus*), with significant differences between the two species. Other mean values did not differ significantly: 116 ± 39 mg kg^{-1} and 105 ± 31 mg kg^{-1} for *L. marequensis* and *C. gariepinus*, respectively. These ranges agreed with previously published literature on *C. gariepinus* and *L. kimberleyensis* but were lower than that reported for *O. mossambicus*.[66,67,76,78] The liver is the only fish organ with potential Zn storage capacity.[80] Considering that our levels were either similar to or lower than published values, we conclude that our study site was not contaminated with Zn. This assessment is supported by the observed levels in plants. The Zn content in muscles of all species did not differ significantly and was similar to those in the literature, indicating a tight regulation of this essential metal and a control system for maintaining Zn homeostasis in fish, as described by Hogstrand.[80]

3.2.5. Other metals and metalloids in fish. The elements Au and U were measured but were below the limit of detection in fish samples, with LODs of 0.035 mg kg^{-1} and 2 mg kg^{-1} for Au and U, respectively.

Although selenium was below the LOD in plants, it was well detectable in fish tissues. The mean selenium content was significantly higher in the liver *versus* muscle tissue of *C. gariepinus*, *L. polylepis*, and *L. marequensis*, which agrees with values reported by Plessl *et al.*[76] The exception is *O. mossambicus*, which showed no such significant difference ($p > 0.05$). Higher Se levels in the liver are expected because the synthesis and catabolism of selenoprotein take place in that organ.[81] Nevertheless, due to the occurrence of selenocysteine and selenomethionine in proteins, Se might be present in all protein-bearing tissues.[82] As this element has been the focus of research regarding detoxification and other beneficial properties in humans and animals, these findings seem noteworthy.[83] The literature on the Se level in fish tissues is still scarce, and future investigations should shed light on the distribution of this element in fish and fish as a Se source in food for humans and animals.

The mean Ag levels were significantly higher in fish liver *versus* muscle samples, ranging from 2.01 ± 0.51 mg kg^{-1} in *O. mossambicus* to 0.020 ± 0.015 mg kg^{-1} in *C. gariepinus*. In the muscle tissue, Ag could be quantified only in *L. polylepis* and *L. marequensis*, with mean contents of 0.011 ± 0.004 mg kg^{-1} and 0.073 ± 0.003 mg kg^{-1}, respectively. Higher liver values and great differences in related fish species have been described by various authors.[14,73] This can be explained by homeostatic mechanisms that regulate the Ag content in muscles, blood as well as gills and that lead to accumulation in liver and kidney tissues.[84] The liver of *O. mossambicus* contained 100 times higher Ag levels than in the sediment of the sampling site, indicating silver hyperaccumulation in the liver of this species.[9] Outa *et al.* identified the liver tissue of the closely related *O. niloticus* as a potential indicator for Ag contamination, with higher levels in fish liver than in the surface sediment of the sampling sites.[73] Whether *O. mossambicus* is very sensitive to Ag pollution or hyperaccumulates even when no contamination with Ag can be detected cannot be answered from our data but should be considered for future investigations, maybe using different age-classes of this fish species.

Cadmium was detected only in fish liver. Lower levels in muscles can be explained by fast absorption by the liver from the plasma after Cd uptake, which mainly occurs through the gills.[85] In our study, *C. gariepinus* had the highest mean liver content (0.243 ± 0.199 mg kg^{-1}) and *L. marequensis* the lowest (0.040 ± 0.028 mg kg^{-1}). Among the four fish species studied, these were the only two significant differences. The liver contents were similar to previously published work on tissues of cyprinids from a rather unpolluted waterbody.[76] We consider these values to be background levels, pointing to no Cd contamination in the investigated fish here.

The mean total Hg content was higher in muscles compared to liver tissues in *C. gariepinus, L. polylepis* and, *L. marequensis.* This emphasizes taking the muscle tissue as the leading target for Hg storage in fish.[86] The mean total Hg levels in muscle tissue ranged from 0.703 ± 0.459 mg kg^{-1} in *C. gariepinus* to 0.138 ± 0.109 mg kg^{-1} in *O. mossambicus*. Interestingly, the order of these values followed the same pattern as the trophic level of the fish, which is highest for *C. gariepinus* (3.8 ± 0.4) and lowest for *O. mossambicus* (2.2 ± 0.0).[34] These findings again underline the role of Hg biomagnification in fish.[86] The total Hg content in both tissues of *L. kimberleyensis* taken from the Vaal Dam in South Africa was well comparable to our results.[76] Those authors described the Hg content as being relatively high for a non-polluted waterbody and saw the potential source in the wet and dry deposition of Hg into the system from coal power plants near the investigated waterbody. Similarly, Windisch *et al.* showed that the sediment and water in the system we investigated can be defined as "not Hg polluted", but the metal content in fish muscles nonetheless pointed to elevated levels compared to other unpolluted systems worldwide.[9] A potential explanation for a quick turn over and bioaccumulation of Hg in fish is the high biological productivity in the system and the rather high temperatures. This would lead to a quick transformation of Hg into bioavailable species rather than to sedimentation of the metal.[87]

The lead contents of muscle and liver tissues did not differ significantly in any fish, with results ranging from 0.035 ± 0.035 in the liver of *O. mossambicus* to 0.008 ± 0.010 mg kg^{-1} in the muscle tissue of *L. marequensis*. Moreover, no significant differences were found when comparing the different species to one another. Comparing these results to studies on *O. niloticus* from Lake Victoria, as well as on *L. kimberleyensis* and *C. gariepinus* from Vaal Dam, showed that levels we detected were significantly lower in all species. Previous analysis of the sediment and water here already showed that the system was not contaminated with lead, which our study on the biota confirmed.[9]

4 Conclusion

This study identified a potential influence of acid mining drainage (AMD) on biota in the upper reaches of the Crocodile River (West) system. In particular, the Fe, Ni, and Cu contents in roots of *Typha* sp. and *P. crassipes* were higher at AMD-influenced sites. In contrast, Cd and Pb levels in those two plant species were not significantly higher at AMD-influenced sites, indicating that the system is not polluted by those MMs. Both *Typha* sp. and *P. crassipes* bioaccumulated high amounts of Ni, Cu, and Cd in the root tissue, demonstrating their potential use for phytoremediation through phytostabilization and rhizofiltration, respectively. In contrast, *P. australis* showed no such bioaccumulation properties, with BAFs < 1 for all analyzed metals. However, the contents of Fe, Cu, and Zn in this plant taken from the most AMD-influenced site were similar to those reported 10 years ago. We conclude that these elements present a continuous burden to the vegetation here, even years after implementing AMD treatment. In terms of impact on biota, we identify macrophytes as being better bioindicators for AMD and MMs than fish. Our findings show that a chronic low-level pollution with Ni, Cu, and Zn in the system generally does

not lead to excessive accumulation in tissues of *C. gariepinus*, *L. marequensis*, and *L. polylepis*. Homeostatic regulatory processes of these metals in fish explain those findings. Note that only adult fish were investigated: a potential impact of such chronic low-level pollution on early development stages was not assessed. Nonetheless, bioaccumulation was recorded in the liver tissue of *O. mossambicus* (significantly higher contents of Ni, Cu, and Ag) and *C. gariepinus*, which accumulated high levels of Fe. These findings point towards a potential use of liver tissue from both species for bioindication purposes for the respective metals. No AMD-induced contamination with Se, Cd, Hg, or Pb was detected in the examined fish species. Our long-term study allowed a detailed look on an AMD influenced river that presents a good example for such a system. Our results clearly demonstrate the consequences of chronic low-level pollution, although water melioration is in place. This system is a good example for the "eternity burden" caused by anthropogenic activities and calls for more awareness for consequences that will challenge future generations. Tight monitoring of biota in AMD-influenced waterbodies around the globe including a steady surveillance of the development of biodiversity should be the priority to be aware of anthropogenic changes caused in these ecosystems.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its ESI† files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Author contributions

Jakob Windisch: conceptualization, investigation, formal analysis, validation, visualization, writing – original draft. Andreas Gradwohl: conceptualization, investigation, formal analysis. Beric Michael Gilbert: investigation, validation, writing – review & editing. Quinton Marco Dos Santos: investigation, validation, writing – review & editing. Annemarièe Avenant-Oldewage: conceptualization, methodology, resources, writing – review & editing, project administration, funding acquisition. Franz Jirsa: supervision, conceptualization, methodology, resources, writing – review & editing, project administration, funding acquisition.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgements

This project was partly funded by the OeAD, Austria's Agency for Education and Internationalisation, with a grant within a Scientific and Technological Cooperation (S&T Cooperation) with Grant no. ZA072019. We gratefully acknowledge the South African National Research Foundation (Grant no. STGR180409318751) bilateral program funding, University of Johannesburg (UJ) URC and FRC funding and infrastructure to Annemarièe Avenant-Oldewage, UJ-GES for postdoctoral funding to Quinton Marco Dos Santos (2018–2021), and UJ Spectrum for postdoctoral funding to Beric Michael Gilbert. The Oppenheimer Memorial Trust (2022–2023) is also thanked for a postdoctoral fellowship to Quinton Marco Dos Santos. We wish to thank Jabulani Ndaba for assisting with sampling and Maximilian Eiser for helping with the statistical analysis. Michael Stachowitsch proofread this article. Open access funding provided by University of Vienna.

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