The effect of short-term Cu exposure on the oxygen consumption and Cu accumulation of mudfish (Labeo capensis) and the largemouth bass (Micropteris salmoides) in hard water

WJ van Aardt* and M Hough
School of Environmental Sciences and Development, N.W.U, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2025, South Africa

Abstract

Sediment samples from 3 dams near the gold-mining area in the Mooi River catchment, South Africa, and fish tissue from the mudfish (Labeo capensis) and largemouth bass (Micropteris salmoides) were analysed for Cu to assess environmental pollution. Copper concentrations of sediment samples in 50 mm deep profiles at Klerkskraal Dam (22.2 mg Cu·kg⁻¹) Boskop Dam (14.1 mg Cu·kg⁻¹) and Potchefstroom Dam (21.7 mg Cu·kg⁻¹) and profiles 100 mm and 150 mm deep were above the risk assessment values for Cu, as implemented by the US EPA. Lowest Cu concentrations were found in gonads and blood samples in fish from both species in the 3 dams, but accumulated 3 to 5 times more, to 110.1±17.8 mg Cu·kg⁻¹ dry mass in the liver. After 120 min Cu exposure at 20ºC to 10 mg Cu·ℓ⁻¹ (157.3 mmol Cu·ℓ⁻¹) and a 96 h Cu exposure to 1 mg Cu·ℓ⁻¹ (15.73 mmol Cu·ℓ⁻¹) Cu accumulated mainly in liver tissue and gills. For the mudfish, upon exposure to 10 mg Cu·ℓ⁻¹ (157.3 mmol Cu·ℓ⁻¹), the opercular frequency increased significantly from 80 (± 5.7) cycles·min⁻¹ to above 100 (± 5.8) cycles·min⁻¹ after 90 min, but thereafter decreased to zero cycles·min⁻¹. For largemouth bass the same increase in opercular frequency was found during 10 mg Cu·ℓ⁻¹ exposure, but this Cu level did not stop opercular frequency. For L. capensis the oxygen consumption rate MO₂ for the two hour exposure period at 10 mg Cu·ℓ⁻¹ decrease significantly from 5.17 (± 0.32) mmol O₂·ℓ⁻¹·h⁻¹ for the controls to 4.5 (± 0.37) mmol O₂·ℓ⁻¹·h⁻¹ and for experimental M. salmoides from 4.91 (± 0.45) mmol O₂·ℓ⁻¹·h⁻¹ to 3.13 (± 0.74) mmol O₂·ℓ⁻¹·h⁻¹. For the exposure period of 96 h at 1 mg Cu·ℓ⁻¹, MO₂ for both fish species, decreased to 2.9 (± 0.3) mmol O₂·ℓ⁻¹·h⁻¹. It is concluded that:

- The imported M. salmoides from the USA is biologically more tolerant to acute Cu exposure compared to the endemic mudfish, Labeo capensis
- For the 2 fish species Cu accumulates mainly in the liver, followed by the gills and kidney
- [Cu] above 20 mg Cu·kg⁻¹ dry sediment may be released in the water column if the pH value decreases below 5 and, together with the physical disturbance of the sediment layer, acute Cu pollution will be the result
- Copper is about 10 times more toxic for the 2 fish species studied compared to Pb and Cd in hard water as found in previous studies.

Keywords: Cu, sediment, fish, MO₂, opercular frequency, hard water

Introduction

Effluents from eroded or disused slimes dams at the Anglo-Gold Mine at Carletonville, previously known as the West Wits Goldfields, contain 84.97 µmol Cu·ℓ⁻¹ (5.4 mg·ℓ⁻¹) and 3.97 mmol Zn·ℓ⁻¹ (26.0 mg Zn·ℓ⁻¹) while the dry pelitic sediments contain 484 mg Cu·kg⁻¹ (7616.2 µmol Cu·kg⁻¹) and 6440 mg Zn·kg⁻¹ (98501 µmol Zn·kg⁻¹). The slimes dams receive discharges of high acidity (pH 1.7) containing large amounts of soluble ions (Wittmann and Förstner, 1977). About 139 Mℓ·d⁻¹ (Winde, 2007) of underground water from these mines, mainly dolomitic in origin (Midgley et al., 1990), are pumped into the Wonderfonteinspruit, part of the upper Mooi River system. Since that study the polluted status of the slimes dams and the mining activities has not changed appreciably (Wade et al., 2002; Coetzee et al., 2006). Van Aardt and Erdman (2004) found a mean value of 35 (± 7.5) mg Cu·kg⁻¹ and 60 (±10.4) mg Zn·kg⁻¹ in the dried clay fraction of sediment samples from the Boskop Dam about 38 km downstream from the slimes dams. The [Cu] in the water of the Wonderfonteinspruit is between 2 and 6 µg Cu·ℓ⁻¹ but in sediment from a dam located in the Wonderfonteinspruit 305 mg Cu·kg⁻¹ was found (Coetzee et al., 2006). The average [Cu] in soils world-wide is 20 mg·kg⁻¹ (Nriagu, 1979). In Labeo capensis, a mudfish from the Mooi River Dams, Van Aardt and Erdmann (2004) found 150 (±17.8) mg Cu·kg⁻¹ and 130 (± 23.1) mg Zn·kg⁻¹ in dried liver. Copper concentrations for relatively unpolluted water include a mean of 5 µg Cu·ℓ⁻¹ for ‘average river water’ while the Amazon and its tributaries range from 0.3 µg·Cu·ℓ⁻¹ to 2.3 µg·Cu·ℓ⁻¹ (Boyle, 1976; Kelly, 1980). According to the South African Water Quality Guidelines (DWAF, 1996) for aquatic ecosystems the acute effect value for Cu is 12 µg Cu·ℓ⁻¹ in very hard water.

Generally for aquatic animals such as tubificids, amphipods, crayfish, mud snails and mussels, (Hodson et al., 1979) and in tilapia fish (Van Aardt and Hough, 2006) Cu decreases the oxygen consumption rates. Water hardness affects acute Cu toxicity because Cu-complexes, especially from carbonates such as CuCO₃²⁻ and Cu (CO₃)₂⁻, are less toxic than cupric or...
Cu hydroxyl ions (Andrew et al., 1977). In hard water systems Cu tends to accumulate in sediments but, if dissolved in water, can still be as high as 25 µg Cu·ℓ⁻¹ (Smith et al., 1996). Toxic Cu levels found in water systems originate mainly from mining processes, the leaching of Cu from minerals due to acid mine drainage and, thirdly, effluents from heavily urbanised and industrialised areas. Generally Cu in aquatic systems is 10 times more toxic compared to Pb, Ni and Zn (Kelly, 1988).

Rainbow trout, Oncorhynchus mykiss, at a 96 h exposure to 311 µg Cu·ℓ⁻¹ in pH 7.9 water with only dissolved calcium, undergoes severe ionic-regulatory failure combined with a progressive systemic hypoxia and massive haemoglobin concentration (Wilson and Taylor, 1993). This is usually accompanied by excessive production of mucus by the mucus cells on the gill epithelium. The mucus largely stays on the gill surface probably as a protective layer (Tkatcheva et al., 2004). The 96 h LC₅₀Cu concentrations for fish start from 10 µg Cu·ℓ⁻¹ and end at 10.0 mg Cu·ℓ⁻¹ (McKim et al., 1978). Water hardness, high pH values, or water alkalinity reduce the lethality of Cu to fish (Zitko and Carson, 1976; Howarth and Sprague, 1978; Erickson et al., 1996) but at a pH of lower than 6.5 toxic Cu hydrides are formed (Stouthart et al., 1996). Compared to blue gill and fathead minnow, LC₅₀ for Cu is about 10 times lower in the salmon family (Hodson et al., 1979).

A change in respiration rate (ΜΟ₂) is one of the common physiological responses to metal toxicants (Connell et al., 1999) and is easily detectable through changes in the oxygen consumption rates. Generally the ΜΟ₂ in fish is reduced when exposed to sub-lethal levels of Cu (Beaumont et al., 2003). However, at lethal Cu concentrations for blue gill (O’Hara, 1971) and O. mykiss (Wilson and Taylor, 1993) ΜΟ₂ first increases above normal values before it declines before death. At a water pH of lower than 4.0, standard oxygen consumption in fish is reduced and the values of critical oxygen tension (Herried, 1980) increase, with the result that fish are less able to handle environmental hypoxia at low pH values (Ultsch et al., 1978; Van Dijk et al., 1993). This paper reports the acute effects of Cu on the ΜΟ₂ and gill frequency in hard water of an indigenous mudfish, L. capensis and the largemouth bass, Micropterus salmoides, imported from the USA. Measurements were also made of the Cu concentrations in the water and sediments of the dams in the Mooi River that harbours these fish and also receives water from the Carletonville mining area. Copper levels in the fish tissue for the 2 fish species, before and after exposure to Cu in the laboratory, were also analysed.

Material and methods

Collection and preparation of sediment samples

Six core samples were collected during the summer and winter season of 1998 from each of the 3 dams (Klerksraal Dam: 26.3.745/27.09.063; Boskop Dam: 26.31.331/27.07.348; Potchefstroom Dam: 16.39.714/27.05.328) in the Mooi River catchment. Sampling localities were situated in the water near the dam inflow and were between 50 and 100 m apart. The core samples were collected using a specially made stainless steel core sampler with a mechanical valve (to be able to salvage the sediment sample), a cutting face and an integral hammer device to be able to drive the core sampler deeply into the dam sediment. The sampler was operated from a boat and took sediment samples from about 2 m below the water surface. Each sample, with an average mass of 900 g, was taken from the core sampler, enclosed in its polyvinyl jacket, and air dried in a vertical position for 24 h (Van Aardt and Erdmann, 2004).

The top 50 mm sample layer was removed and dried for 12 h at 80°C. With the aid of a Wolfram-ring swing mill (Sieltotechnik, Mulheim, Germany) the samples were pulverised for 15 s. One gram from each sediment sample was weighed, 1 ml de-ionised water, 2 ml HNO₃ (70% pro analysi) and 1 ml HClO₄ (65% pro analysi) from Merck were added and digested at 80°C for 12 h. The digested samples were filled up with de-ionised water to a total volume of 10 ml.

Copper analysis of the sediment and Mooi River water was performed by flame atomic absorption spectrometry at 324.8 nm using standard reference materials (MESS-2) from The National Research Council, Canada, in quality assurance protocols. The calibration graph, with an accuracy of 10 µg Cu·ℓ⁻¹ was linear between 0.01 mg Cu·ℓ⁻¹ to 8.88 mg Cu·ℓ⁻¹. One gram samples from the pulverised cores were also used for energy dispersive X-ray spectrometry analysis (EDAX), using a Philips EDAX-analyser (EDAX, CDU LEAP Detector) and an electron microscope (XL 30 Phillips DZi). The sample buttons for each sample were prepared using ‘sticking film’. The sample was slightly pressed onto the film and covered with a layer of carbon (Emscope TB 500).

Fish collection and the fish-keeping plant

Labeo capensis were collected by gill-netting from the 3 dams in the Mooi River during the summer and winter of 1998. During the same period Micropterus salmoides were caught by line fishing. Klerksraal Dam was used as a control dam compared to Boskop and Potchefstroom Dams (Fig. 1a). The latter 2 dams receive water from mining effluents via the Mooi River (Wade et al., 2002). The fish were transported in a 400 ℓ container filled with aerated dam water and spiked with 12 g NaCl·ℓ⁻¹ water (Walsh, 1984). At the fish plant the fish were treated with 2% formaldehyde and 33 mg malachite green·ℓ⁻¹ water for 10 s and transferred to the 5 000 ℓ fish-holding tanks (Van Aardt and Booysen, 2004). M. salmoides were fed earthworms or fish fry (Tilapia sparrmanii) from Boskop Dam. All fish were kept for at least 3 weeks in the fish-holding tanks at 20°C ±0.5°C before experiments were undertaken at this temperature range.

Chemical analysis of Mooi River water

Water samples were analysed for hardness and macro-elements (Midvaal Water Co., accredited laboratory number T0132).

Copper levels in fish before experimental Cu exposure

From 20 fish caught per dam a 1 ml blood sample was immediately taken by heart puncture whereupon the fish were transported to the laboratory. One gram fish tissue (gonads, the jejunal part of the intestines, gills, liver, kidney and muscle) was dissected out. In a glass vial 2 mℓ HNO₃ (70% pro analysi) and 1 ml HClO₄ (65% pro analysi) from Merck were added and each type of tissue was processed separately and digested as described above for sediment samples. The analysis protocols for Cu were the same as for the sediments except that the standard reference material used was (Dorm-2) from the National Research Council, Canada. To express the metal concentrations obtained from L. capensis per gram dried tissue, the percentage of water for each of the seven tissue types was determined as described by van Aardt and Erdmann (2004).
Because of the low numbers of *S. micropteris* caught in Boskop and Klerkskraal Dams, only the tissues from *L. capensis* were analysed for Cu (Fig. 5).

Copper levels in fish after copper exposure

After the Cu exposure experiments, and MO₂ determinations for both *S. micropteris* and *L. capensis* to different Cu concentrations, the 7 types of fish tissue were also harvested from the experimental fish and digested and analysed as described above for control fish.

Oxygen consumption rate measurements (MO₂)

Measurements were done on individual fish after exposure for 120 min to 10 mg Cu·ℓ⁻¹ (157.3 µmol Cu·ℓ⁻¹) as Cu(NO₃)₂·3H₂O. Individual fish were also exposed for 96 h to 1 mg Cu·ℓ⁻¹ (15.7 µmol Cu·ℓ⁻¹) as Cu(NO₃)₂·3H₂O. Control fish, without Cu exposure, were measured for MO₂ using the same batch caught during the same season as for the experimental fish. All MO₂ measurements for Cu exposed fish were made 2 h after handling of the fish and 24 h after feeding (Jobling and Davies, 1980; Jobling, 1981). To determine the effects of handling on the MO₂ oxygen consumption measurements were also made 0, 2, 4, 6, and 12 h after handling of the fish (Fig. 1a). The mean live fish mass for both control and experimental fish was 864.1 (± 85.1) g for *S. micropteris* and 992 (±112.0) g for *L. capensis*.

MO₂ and opercular frequency was determined after 10 mg Cu·ℓ⁻¹ exposure using individual fish in a large 23.03 ℓ Perspex respirometer (Fig. 2) set up as a closed system respirometer (Bridges and Butler, 1989) with continuous PO₂ monitoring of the water. MO₂ calculations and MO₂ corrections for the different fish masses were done according to Van Aardt (1990) and Van Aardt and Booysen (2004). The PO₂ values to calculate MO₂ for each fish were taken at a starting PO₂ value of 120 mm Hg and, after about a 20 min measuring time, again at a PO₂ of 80 mm Hg. In another experiment the progressive decrease of the PO₂ in the respiration water was determined for both fish species at 10 min intervals for 120 min (Fig. 3, a, b). At the same 10 min intervals the opercular frequency was also visually counted (Fig. 4, a, b). The MO₂ determined in 10 control fish (no Cu) and 10 experimental fish exposed to 1 mg Cu·ℓ⁻¹ for 96 h at 20°C (±0.5°C), was done in a smaller volume (5.15 ℓ) respirometer without PO₂ and opercular monitoring. This was done by exposing the free swimming fish for 84 h in a 1 000 ℓ exposure tank. The fish were then caught and placed individually in open respirometers (Van Aardt and Booysen, 2004) in the same exposure tank for a further 12 h in order to eliminate handling stress on MO₂. After this period the water-tight lids were screwed on for 20 min. The ΔPO₂ was then determined to enable the calculation of MO₂ (Van Aardt, 1990; Van Aardt and Booysen, 2004) (Fig. 8a, b).
The water in the 3 dams of the Mooi River can be classified as very hard (Cooney, 1995) with total alkalinity (as CaCO₃) of 252 mg l⁻¹; Ca: 67 mg l⁻¹; Cl: 36 mg l⁻¹; Mg: 50 mg l⁻¹; K: 4 mg l⁻¹; Na: 28 mg l⁻¹; SO₄: 115 mg l⁻¹; pH 8.2. Copper levels in dam sediments (Fig. 3) were 21.1 ± 6.1 µg Cu·g⁻¹ dry tissue. This was less than half the value found for liver tissue in Boskop Dam (110.1±17.8 mg Cu·kg⁻¹ dry tissue). This value represents the percentage occurrence of Cu on the surface of the scanned film compared to the other metal elements.

For the 3 dams Cu in mudfish accumulates 3 to 5 times more in the liver compared to other tissues (Fig. 6). Lowest Cu concentrations were found in gonads and blood samples. The Cu concentration in mudfish liver in the Klerkskraal Dam was 42.10 ±9.8 mg Cu·kg⁻¹ dry tissue. This was less than half the value found for liver tissue in Boskop Dam (110.1±17.8 mg Cu·kg⁻¹) or Potchefstroom Dam (90.5 ±12.2 mg Cu·kg⁻¹) dried liver.

Copious secretion and production of mucus on the gill lamellae were observed during the 10 mg Cu·ℓ⁻¹ exposure for both L. capensis and M. salmoides but not for the 1 mg Cu·ℓ⁻¹ exposure experiment. In both fish species the cough reflex was observed when exposed to 10 mg Cu·ℓ⁻¹. Presumably the inside surface of the oral and opercular cavity was irritated or blocked with mucus (Heath, 1995). For both fish species, Cu accumulated mainly in the liver during the exposure of 1 mg Cu·ℓ⁻¹ for 96 h or 10 mg Cu·ℓ⁻¹ for 2 h (Figs. 6 and 7). Copper concentrations in fish tissue, after a 96 h exposure to 1 mg Cu·ℓ⁻¹ or after 2 h to 10 mg Cu·ℓ⁻¹, increased more than twofold in liver, kidney and gills for both species. The data were almost the same obtained for both L. capensis and M. salmoides. The mean Cu concentration (µg Cu·g⁻¹) in 1 g dried tissue from L. capensis collected from the three dams during 1998. Twenty fish per dam were analysed. Vertical bars denote the standard deviation from the mean.

The progressive decrease of the mean PO₂ in the water measured for (a) L. capensis and (b) M. salmoides in the large respirometer. Each fish was monitored for 2 h, exposed to 10 mg Cu·ℓ⁻¹ (n=10). Vertical bars denote the standard deviation from the mean.

Results

The water in the 3 dams of the Mooi River can be classified as very hard (Cooney, 1995) with total alkalinity (as CaCO₃) of 252 mg l⁻¹; Ca: 67 mg l⁻¹; Cl: 36 mg l⁻¹; Mg: 50 mg l⁻¹; K: 4 mg l⁻¹; Na: 28 mg l⁻¹; SO₄: 115 mg l⁻¹; pH 8.2. Copper levels in dam sediments (Fig. 3) were 21.1 ± 6.1 µg Cu·g⁻¹, 14.0 ± 3.3 µg Cu·g⁻¹ and 21.2 ± 4.7 µg Cu·g⁻¹ respectively for Klerkskraal-, Boskop- and Potchefstroom Dam. The percentage Cu in liver samples from Boskop Dam, analysed by energy dispersive X-ray analysis, (EDAX) was 0.55 ± 0.06%. For Potchefstroom Dam it was 0.54 ± 0.05% and for Klerkskraal Dam it was 0.51± 0.1%. This value represents the percentage occurrence of Cu on the surface of the scanned film compared to the other metal elements.

For the 3 dams Cu in mudfish accumulates 3 to 5 times more in the liver compared to other tissues (Fig. 6). Lowest Cu concentrations were found in gonads and blood samples. The Cu concentration in mudfish liver in the Klerkskraal Dam was 42.10 ±9.8 mg Cu·kg⁻¹ dry tissue. This was less than half the value found for liver tissue in Boskop Dam (110.1±17.8 mg Cu·kg⁻¹) or Potchefstroom Dam (90.5 ±12.2 mg Cu·kg⁻¹) dried liver.

Copious secretion and production of mucus on the gill lamellae were observed during the 10 mg Cu·ℓ⁻¹ exposure for both L. capensis and M. salmoides but not for the 1 mg Cu·ℓ⁻¹ exposure experiment. In both fish species the cough reflex was observed when exposed to 10 mg Cu·ℓ⁻¹. Presumably the inside surface of the oral and opercular cavity was irritated or blocked with mucus (Heath, 1995). For both fish species, Cu accumulated mainly in the liver during the exposure of 1 mg Cu·ℓ⁻¹ for 96 h or 10 mg Cu·ℓ⁻¹ for 2 h (Figs. 6 and 7). Copper concentrations in fish tissue, after a 96 h exposure to 1 mg Cu·ℓ⁻¹ or after 2 h to 10 mg Cu·ℓ⁻¹, increased more than twofold in liver, kidney and gills for both species. The data were almost the same obtained for M. salmoides exposed to 1.0 mg Cu·ℓ⁻¹ or 10 mg Cu·ℓ⁻¹ (Fig. 6).

The PO₂ depletion capacity by both M. salmoides and L. capensis of the water medium was significantly reduced when
fish were exposed to 10 mg Cu·ℓ⁻¹ compared to the controls (Fig. 3a, b). However, when the opercular frequencies between the two fish species were compared, *L. capensis* showed an increase in both control and experimental fish before a traumatic collapse in the opercular frequency for experimental mudfish occurred (Fig. 4a, b). This happened at PO₂ values of the water below 30 mm Hg and after about 80 min exposure to Cu. All *L. capensis* specimens died before the end of the 120 min exposure time to 10 mg Cu·ℓ⁻¹. For the largemouth bass an initial increase in opercular frequency was found in acute Cu exposure, but it levelled off after about 60 min exposure time (Fig. 4). No deaths or collapse of the opercular frequency were found for largemouth bass. During progressive hypoxia (Fig. 3) it was visually observed that the opercular stroke volume for bass was increased by lowering the lower jaw and the floor of the oral cavity during each opercular opening. This reaction of the gill ventilation to Cu was not observed for mudfish. The MO₂ values obtained for *M. salmoides* using the closed respirometry system compares favourably with the open system operated by Beamish (1970) and Beamish (1974) on the same fish species.

The MO₂ of largemouth bass and mudfish decreased nearly twofold from 5.9 mmol O₂·kg⁻¹·h⁻¹ to 2.12 mmol O₂·kg⁻¹·h⁻¹, 6 h after handling (Fig. 1). After this period the oxygen consumption rate stabilised, most probably as resting (standard) MO₂. Beamish (1970) found for *M. salmoides* at 20°C a standard MO₂ of 3.43 mmol O₂·kg⁻¹·h⁻¹ for a 150 g fish. In our experiments a statistically significant decrease in the MO₂ between control fish and fish exposed for 2 h to 10 mg Cu·ℓ⁻¹ was found. For the 96 h exposure period to 1 mg Cu·ℓ⁻¹ for both the mudfish and largemouth bass the MO₂ for experimental fish was significantly lower compared with the controls.

**Discussion**

The hard water in the Mooi River system probably played an important role in the precipitation of Cu compounds before the water from the mines reached the Potchefstrom and Boskop dams. The Cu concentration in the water decreased by more than half, 2h after the pH of the water was increased from a value of below 7 to above 8, in agreement with the findings reported by Shaw and Brown (1974). The result of this pH effect is that a relatively low Cu level is found in Mooi River water. Furthermore the high pH values of the water in the 3 dams increased the adsorption of Cu onto the small clay particles (Kishk and Hassan, 1973; Farrah and Pickering 1977; Daoust et al. 2006).
functions which enables it to operate its opercular muscles during acute Cu exposure, than the relatively sedentary mud fish.

Generally the oxygen consumption rate in fish exposed to Cu decreases. However, an initial stimulation of \( \text{MO}_2 \) was found for blue gill (O’Hara, 1971), although this was not found by De Boeck et al. (1995) who found an immediate \( \text{MO}_2 \) decrease in carp. Higher than normal \( \text{MO}_2 \) values were also noted in our experiments but it could be ascribed to irradiation of the gill membranes as well as the epithelium of the oral cavity causing the fish to move around more frequently in the large volume (23.03 l) respirometer compared to the small volume (5.15 l) respirometers. The standard \( \text{MO}_2 \) of 1.8 mmol \( \text{O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) in acidified water at 15°C was found for 1.960 kg carp, \( \text{Cyprinus carpio} \), (Ultsch et al., 1980) while De Boeck et al. (1995) measured standard \( \text{MO}_2 \) as 5.3 mmol \( \text{O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) in their small 25 g carp. However, for 25 g carp exposed to 53.37 µg Cu·ℓ⁻¹ (0.84 µmol Cu·ℓ⁻¹) the \( \text{MO}_2 \) decreased substantially in hard water at 20°C to well below 3 mmol \( \text{O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) (De Boeck et al., 1995).

In general, the ability of fish, exposed to Cu, to deplete the oxygen content in the water is impaired, compared with controls. This points to a disruption of the branchial structure in fish, probably due to apoptotic death (Wendelaar-Bonga and Lock, 1992; Booysen et al., 2000) and not due to damage of the oxygen receptors in the gill arteries that govern gill frequency and ventilation volume (Hughes and Shelton, 1958; Hughes, 1966). It is concluded that:  

It is known that acute as well as prolonged Cu exposure to Cu is physiologically more tolerant to Cu exposure compared to Cd and Pb. These metals are known for their high toxicity to fish in soft water but in high alkalinity hard water, Cd and Pb completely precipitate from the water column compared to Cu.

Conclusions

It is concluded that:

- The imported \( \text{M. salmoides} \) is physiologically more tolerant to acute Cu exposure compared to the endemic mudfish.
The high [Cu] found in the sediments in the Mooi River catchment, if released in the water through pH and physical changes would result in acute Cu environmental pollution.

Copper is about 10 times more toxic for the 2 fish species studied compared to Pb and Cd in hard water.

The PO₄ depletion capacity for *M. salmoides* exposed to Cu decreases significantly after 15 min compared with the controls. For *L. capensis* PO₄ depletion capacity decreases significantly after 45 min.

Copper toxicity decreases in hard water but is still toxic if tested at between 1 mg and 10 mg Cu·ℓ⁻¹.

Copper as toxicant accumulates mainly in fish liver, gills and kidney. This metal can be evaluated, in short-term exposure regimes, using oxygen consumption rate and opercular frequency as physiological parameters.

Experimental data from the literature (Wendelaar Bonga and Lock, 1992; Tkatcheva et al., 2004) advocate that electrolyte concentrations of Cl, Na and K and the osmotic value of blood plasma should also be measured to evaluate Cu uptake during Cu exposure studies.

Acknowledgements

We wish to thank the Research Focus Area: Environmental Sciences and Management; the National Research Foundation, for financial assistance and the School of Environmental Sciences and Development, NWU, Potchefstroom Campus, Potchefstroom, South Africa, for providing the research facilities.

References

ANDERSON DP, DIKSON OW, BODAMMER JE and LIZZIO EF (1989) Suppression of antibody-producing cells in rainbow trout spleen sections exposed to copper in vitro. *J. Aquat. Anim. Health* 1 57-61.

ANDREW BW, BIESINGER KE and GLASS GE (1977) Effects of inorganic complexing on the toxicity of copper to Daphnia magna. *Water Res.* 11 309-315.

BAKER JTP (1969) Histological and electron microscopic observations on copper poisoning in the winter flounder, *Pseudopleuronectes americanus*. *J. Fish. Res. Bd. Can.* 26 2785-2790.

BEAMISH FWH (1970) Oxygen consumption of largemouth bass, *Micropterus salmoides* in relation to swimming speed and temperature. *Can. J. Zool.* 48 1221-1228.

BEAMISH FWH (1974) Apparent specific dynamic action of large-mouth bass *Micropterus salmoides*. *J. Fish. Res. Board Can.* 31 1763-1769.

BEAUMONT MW, BUTLER PJ and TAYLOR EW (2003) Exposure of brown trout to a sublethal concentration of copper in soft acidic water: effects upon gas exchange and ammonia accumulation. *J. Exp. Biol.* 206 153-162.

BRIDGES CR and BUTLER PJ (1989) Techniques in Comparative Respiratory Physiology. An Experimental Approach. Cambridge University Press, Cambridge.

BOYLE EA (1976) Copper in natural waters. In: Niriagiu JO (ed.) Copper in the Environment. Part I: Ecological Cycling. John Wiley and Sons, New York, 77-88.

BURLY NR, WALKER PA and GLOVER CN (2003) Nutritive muscle uptake in teleost fish. *J. Exp. Biol.* 206 1-23.

COETZEE H, WINDE F and WADE PW (2006) An Assessment of Sources, Pathways, Mechanisms and Risks of Current and Potential Future Pollution of Water and Sediments in Gold Mining Areas of the Wonderfonteinspruit Catchment. WRC Report No. 1214/1/06. Water Research Commission, Pretoria, South Africa. 266 pp.

COLLIVIN L (1985) The effect of copper on growth, food consumption and food conversion of perch *Perca fluviatilis* L. offered maximal food rations. *Aquat. Toxicol.* 6 105-109.

CONNELL D, LAM P, RICHARDSON B. and WUR (1999) Introduction to Ecotoxicology. Blackwell Science, Australia.

COONEY JD (1995) Fresh water tests. In: Rand GM (ed.) Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. Taylor and Francis, Washington, DC. 71-102.

COURTOIS LA and MEYERHOFF RD (1975) Effects of copper exposure on water balance. *Bull. Environ. Contam. Toxicol.* 14 211-219.

CUTHBERT AW and MAETZ M (1972) The effects of calcium and magnesium on sodium fluxes through the gills of *Carassius auratus*. *L. J. Physiol.* 221 633-643.

DANG ZC, LOCK RAC, FLIK G and WENDELAAR-BONGA SE (1999) The metallothionein response in gills of *Oreochromis mossambicus* exposed to copper in fresh water. *Am. J. Physiol.* 277 R320-R331.

DANG Z, LOCK R AC, FLIK G and WENDELAAR-BONGA SE (2000) Na⁺/K⁺-ATP-ase immunoreactivity in branchial chloride cells of *Oreochromis mossambicus* chronically exposed to copper. *J. Exp. Biol.* 203 379-387.

DAoust CM, BASTIEN and DESCHENES L (2006) Influence of soil properties and aging on the toxicity of copper on compost worm and barley. *J. Environ. Qual.* 35 558-561.

DE JF, SolyBE and COPPER VA (1976) Studies on the toxicology of copper sulphate to Stone Loach *Noemacheilus barbatulus* (L) in hard water. *Water Res.* 19 523-527.

DE BOECK G, SMET H and BLUST R (1995) The effect of sublethal levels of copper on oxygen consumption and ammonia excretion in the common carp, *Cyprinus carpio*. *Aquat. Toxicol.* 32 27-141.

DWAF (Department of Water Affairs and Forestry)(1996) *South African Water Quality Guidelines*. Vol.7: Aquatic Ecosystems. Pretoria.

ERIKSON RJ, BENOIT DA, MATTSON VR, NELSON HP and LEONARD NL (1996) The effects of water chemistry on the toxicity of copper to fathead minnows. *Environ. Toxicol. Chem.* 15 181-183.

FARRAH, H. and PICKERING, W.F. (1977). Influence of clay-solute interactions on aqueous heavy metal ion levels. *Water Air Soil Pollut.* 8 189-197.

FELTS PA and HEATH AG (1984) Interactions of temperature and sublethal environmental copper exposure on the energy metabolism of bluegill, *Lepomis macrochirus* Rafinesque. *J. Fish. Biol.* 25 445-453.

HEATH AG (1984) Changes in tissue adenylates and water content of bluegill, *Lepomis macrochirus* exposed to copper. *J. Fish. Biol.* 24 299-309.

HEATH AG (1995) *Water Pollution and Fish Physiology*. CRC Press, Boca Raton, Florida, USA.

HERRIED CF (1980) Hypoxia in invertebrates. *Comp. Biochem. Physiol.* 67A 311-320.

HODSON V, BORGMAN U and SHEAR H (1979) Toxicity of copper to aquatic biota. In: Niriagiu JO (ed.) Copper in the Environment Part II Environmental and Occupational Exposure to Copper. John Wiley & Sons, New York. 307-372.

HOWARTH RS and SPRAGUE JB (1978) Copper lethality to rainbow trout in water of various hardness and pH. *Water Res.* 12 455-462.

HUGHES GM (1966) The dimensions of fish gills in relation to their function. *J. Exp. Biol.* 45 177-195.

HUGHES GM and PERRY SF (1976) A morphometric study of trout gills: a light-microscopic method for the evaluation of pollutant action. *J. Exp. Biol.* 64 447-460.

HUGHES GM and SHELTON G (1958) The mechanism of gill ventilation in three freshwater teleosts. *J. Exp. Biol.* 35 807-823.

HUGHES GM and UMEZAWA S-I (1968) On respiration in the dragonfly *Callimimus lyra*. *J. Exp. Biol.* 49 565-571.

JOBLING M (1981) The influences of feeding on the metabolic rate of fishes: a short review. *J. Fish. Biol.* 18 385-400.

JOBLING M and DAVIES PS (1980) Effects of feeding on metabolic rate, and the specific dynamic action in Plaice, *Pleuronectes platessa* L. *J. Fish. Biol.* 16 629-638.

KISHK FM and HASSAN MN (1973) Sorption and desorption of copper by and from clay minerals. *Plant Soil* 39 497-505.

KELLY M (1988) *Mining and the Freshwater Environment*. Elsevier, Applied Science, London.

LAMBO RP, SLINGER SJ and HILTON JW (1985) Maximum tolerable and toxicity levels of dietary copper in rainbow-tout (*Salmo gairdneri* Richardson). *Aquacult.* 49 257-268.
