Water hardness and the effects of Cd on oxygen consumption, plasma chlorides and bioaccumulation in *Tilapia sparrmanii*

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Abstract

Closed system respirometry was performed on captive juvenile (30 ± 8 g; mean ± S.E.M) *Tilapia sparrmanii* exposed for 96 h to low (1 mg·ℓ$^{-1}$) and high (20 mg·ℓ$^{-1}$) levels of cadmium in soft and hard water. In hard water (235 mg·ℓ$^{-1}$as $CaCO_3$), cadmium (Cd) applied as $CdCl_2$, precipitates completely out but in very soft water (16.5 mg·ℓ$^{-1}$as $Ca\text{-}CO_3$), 23% of Cd is in solution 96 h after it was dissolved. Cadmium complexation is not caused by the presence of chlorides but probably depends on carbonates and sulphates present in Mooi River water. Handling stress, that lasts for at least 6 h, increased the specific oxygen consumption rate of *T. sparrmanii* (MO_2) by more than 30% compared to resting oxygen consumption levels. In hard water no change in the $\dot{M}O_2$ was found when *T. sparrmanii* was exposed to 1, 5, 10, or 20 mg of Cd·ℓ$^{-1}$ of water. In soft alkaline water all fish died when exposed for 96 h in 20 mg Cd·ℓ$^{-1}$. For 10 mg Cd·ℓ$^{-1}$, the $\dot{M}O_2$ was reduced significantly ($p< 0.05$) by 30%. The percentage cadmium dissolved in hard water was, after 96 h, below 1%, 96 h after it had been dissolved. About 2 000 µg Cd·g^{-1} accumulates per gram dried gill mass when fish are exposed to 20 mg Cd·ℓ$^{-1}$ in hard water. For soft water the Cd accumulation is about twice as much. In liver tissue more than 60 µg Cd accumulates per gram dried liver mass in hard water. In soft water the accumulation was three times as much. Blood plasma chlorides decreased from a mean of 130 mmol to 60 mmol when exposed to 20 mg Cd·ℓ$^{-1}$ in soft water. The differences were statistically significant ($P< 0.05$). No decrease in blood plasma chlorides was found in hard water when fish were exposed to 20 mg Cd·ℓ$^{-1}$ of water. Fish handling reduces the oxygen consumption rate by 35% but $\dot{M}O_2$ returns to normal resting levels 6 h after handling stress. It is concluded from the results that $\dot{M}O_2$ and blood chlorides can be used as parameters for Cd toxicity in a 96 h exposure period provided that the precipitation of Cd in the water is known and the pH of the water is monitored.

Keywords: fish, Cd, $\dot{M}O_2$, body electrolytes, hard water, soft water

Introduction

The introduction of toxic metals such as Cd into the environment by anthropogenic sources is an important challenge to toxicologists and ecological management. The concentration of the metal, its geographical transportation, exposure to a target organism and the responses of the individual organism to a specific toxic metal should be known (Truhaut, 1974; Carpené et al., 1990). The peltic sediments adjacent to the gold mine slimes dams near Carletonville, South Africa, have Cd concentrations of between 0.18 and 0.86 µg·g^{-1} with an enrichment factor of 3 compared with standard South shale. These determinations were made before 1977 (Wittmann and Förstner, 1977). Since then no physical changes of the mine drainage around the slimes dams have taken place. A tributary of the Mooi River, receiving dolomitic water (Midgley et al*.,* 1990), but no drainage from the peltic sediments, from the mines, drains into the upper reaches of the Mooi River. It has been estimated that the global input of 9 400 t of Cd into the aquatic ecosytems per year is caused by anthropogenic activities where mining, smelting and refining contributes 41.5% of the total input (Nriagu and Pacyna, 1988).

Nothing is known what the effect of Cd is on the oxygen consumption rate of South African fishes. It has been shown that exposure of cadmium to fish affects the kidney and liver functions (Friberg et al., 1979). The use of gill ventilation, respiration or metabolic rate for individual fish to measure the responses of fish to toxic metals has been poorly studied (Anderson and D' Apollina, 1978; Kelly, 1988; Rand et al., 1995). According to Depledge (1990) individual physiological variability can be used as a tool to investigate toxicity effects above traditional methods such LC_{50} values especially for aquatic organisms. In this regard he strongly advocates the use of respiratory responses to pollutants. Furthermore, Klerks and Levinton (1989) have demonstrated that selection pressure has resulted in the evolution of resistance to Cd in an aquatic oligocheate.

Gill epithelial damage by metal toxicants in fish is often characterised by excessive excretion of mucus (Mallatt, 1985). Heath (1984), Felts and Heath (1984) and Heath (1987), working on the fish *Lepomis macrochirus,* disputed the view put forward by Burton et al. (1972), that heavy metals cause only tissue hypoxia due to excessive secretion of mucus on the gill surfaces. They provided evidence that heavy metals acted on the gill physiology, resulting in a decrease in the oxygen consumption because of ion-regulatory and acid-base balance disturbances (Goss and Wood, 1988).

An increase in the oxygen consumption rates by heavy metals has been described, effected by the stressor responses in fish. (Schreck and Lorz, 1978; Wendelaar-Bonga, 1997). These stressor responses induce a dramatic increase in adrenaline, resulting in increased oxygen uptake rate, initiated by higher gill ventilation rates. Furthermore, stimulation of the branchial blood flow and branchial oxygen diffusion capacity is enhanced. Most of these actions are caused by cathechol-amines acting through adrenergic mechanisms (Wendelaar-Bonga, 1997; Pelgrom et al*.,* 1994). It is

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Received 19 November 2002; accepted in revised form 22 October 2003.

TABLE 1
Chemical composition and water quality from three Mooi River water types, Rooipoort borehole water and reconstituted water+(Rand et al. (1995). VS*, very soft water; HW*, hard water; VH*, very hard water; n a, not analysed

	Mooi River	Fish tank	Tap water	Rooipoort borehole	VS*+	HW*+	VH*+
Electrical conductivity(µS. cm^{-1})	680	840	660	110	179	569	937
pH (-log H^+ conc.)	8.19	8.14	8.70	7.21	8.23	8.69	8.76
Calcium ($mg \cdot \ell^{-1}$)	61	67	61	5	2.2	35.3	70.6
Magnesium($mg \cdot \ell^{-1}$)	41	50	42	8	3.9	63.9	127.9
Potassium ($mg \cdot \ell^{-1}$)	2	4	2	>1	0.2	4.1	8.3
Chloride ($mg \cdot \ell^{-1}$)	27	36	28	5	0.23	3.80	7.60
Sodium($mg \cdot \ell^{-1}$)	21	28	22	5	3.2	52.5	105.0
Sulphates ($mg \cdot \ell^{-1}$)	76	115	80	0.5	11.2	180	360.8
Total alkalinity as $CaCO_3$ ($mg \cdot \ell^{-1}$)	230	252	223	23	16.5	115	235
% Cadmium in solution after 96 h	n a	>1%	n a	23%	23%	1%	>1%

therefore surprising that in extensive reviews (Butler, 1978; Anderson & Conning, 1993; Rand et al., 1995) most of these researchers do not explicitly mention the use of oxygen uptake rates by fish gills as an index or biomarker in toxicity studies on metals. Because of the fish gill's large epithelial surface for gas exchange and its role in water and ion regulation, fish gills may act as a physiological index organ if damaged by toxic metals. Heath (1987) strongly propagates the use of respiratory gas exchange organs as a means to indicate the degree of toxic metal damage. This apparent neglect of using oxygen consumption rates on metal toxicology studies is difficult to explain. It is possible that $\dot{M}O_2$ measurements used in basic animal physiology (Bridges and Butler, 1989) are not well known or properly applied to experimental toxicology (Anderson and Conning, 1993).

The dolomitic nature of the area draining the Mooi River basin, results in hard water with total alkalinity of more than 230 $mg \cdot \ell^{-1}$ as $CaCO_3$ and an electrical conductivity between 557 and 680 $\mu S \cdot cm^{-1}$ (Table 1). It is known (Rand et al., 1995) that heavy metals such as cadmium and lead undergo a precipitation process in hard water where the metal ion and various ligands join chemically to form a new chemical complex (Zitko and Carson, 1976). These complexation reactions result in insoluble precipitates (Kotrly and Sucha, 1985). Nothing is known of the toxicity of high levels of cadmium complexation compounds, caused by hard water, on the biology of fish.

Tilapia sparrmanii is widely distributed south of the Congo basin, occurring both in rivers and lakes and also in temperate regions in South Africa. It is a hardy omnivorous fish that is well adapted to high (32°C) as well as low (10°C) temperatures and also to a variety of habitats (Skelton, 1993).

The aim of the present study is to make a contribution as to whether mass specific oxygen consumption rates, and body electrolyte changes, measured in *T. sparrmanii*, can be used to evaluate the short-term (96 h) biological effects of cadmium. Particular attention was given to the effects of hard and soft water on the toxicity of complexation compounds of cadmium to *T. sparrmanii*

Materials and methods

Experimental fish and the fish-keeping plant

Juvenile *Tilapia sparrmanii (*A. Smith, 1841) (30 ± 8 g) were netted at Boskop Dam (S.26. 31' 531" and E.27. 07' 348") using cast nets. Approximately 200 fish per catch were transported for 13 km to the laboratory in a 400 ℓ container filled with aerated dam water. Six kilograms of table salt were dissolved in the 400 ℓ dam water to eliminate *Saprolegnia parasitica* infection (Walsh, 1984). At the fish plant the fish were exposed in water containing 2% formaldehyde and 33 $mg \cdot \ell^{-1}$ malachite green for 10s. After treatment the batch, with a specimen density of 16 $fish \cdot \ell^{-1}$, was placed in aerated 5 000 ℓ fish holding tanks. Each holding tank was thermostatically controlled at a temperature of 20°C ± 0.5 °C and formed part of a closed circulating water system consisting of a 1 000 ℓ sedimentation tank and a biological filter tank of 1 000 ℓ capacity.

A submersible pump (Little Giant, Type 6E-CIA-SFS, USA) was placed inside the biological filter tank to maintain a water turnover rate in the system of 3 000 $\ell \cdot h^{-1}$.

The water inside the fish tank was made to circulate by pumping water at an angle into the tank. In this manner the centrally placed perforated drain pipe collects faeces and left-over food in the fish tank to be deposited in the sedimentation tank. Tanks were made of polypropylene and piping from 40 mm diameter polyvinyl chloride. Every week the biological filter tank as well as the sedimentation and holding tanks were recharged with 33% fresh tap water after it was cleaned from remaining food and faeces by suction. High-protein food (Koi-fish pellets, Rainbow, Johannesburg, RSA) was given three times per week. Under these conditions *T. sparrmanii* thrived, gained body mass and no deaths occurred after the initial 2 d recovery period. Fish were kept for at least three weeks in the holding tanks before starting the experiments.

Water analysis

Samples from Mooi River water, used in the fish tanks for exposing the fish to cadmium, were analysed for total hardness, sodium, potassium, magnesium, calcium, sulphates, electrical conductivity, and pH at 25°C. Furthermore, water samples from the fish tanks, tap water and from a borehole at the farm Rooipoort 29 (26°35'882" and 26°50'348"), were analysed by Midvaal Water Co. (accredited laboratory, number T0132) and presented in Table 1. Reconstituted freshwater (Rand et al., 1995) presenting very soft, hard and very hard water (all above pH 8.0) was made up in 2 ℓ containers. The amount of Cd precipitated in the 2 ℓ containers from 1 mg $Cd \cdot \ell^{-1}$ was determined after 96 h and after vigorous aeration.

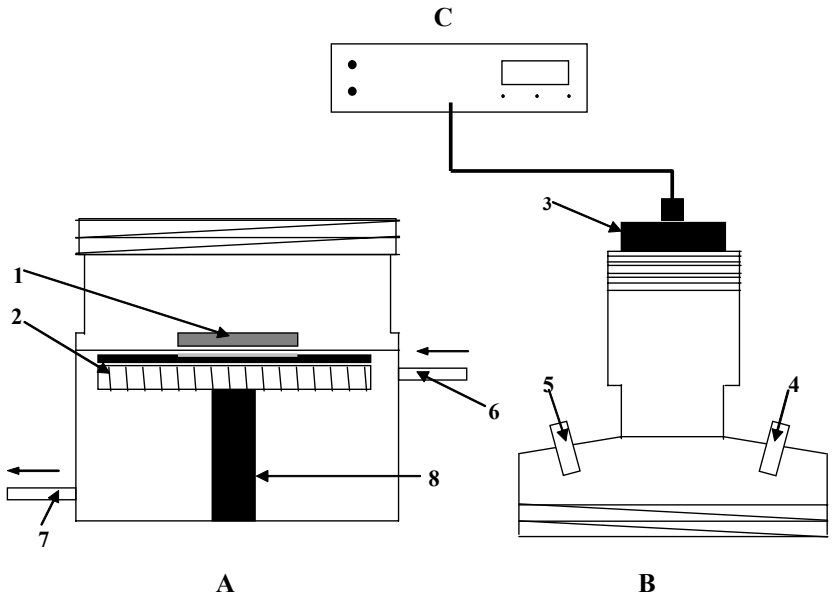

Figure 1
The PO_2 measuring cell (50 mℓ capacity), made from Perspex, to measure start and end PO_2 values. A, Perspex chamber; B, Perspex-head housing the Radiometer oxygen elektrode; C, Oxygen meter; The B part of the cell is screwed on part A. 1, magnetic stirrer; 2, water turbine; 3, Clark-polarographic oxygen electrode (Radiometer); 4, inlet for water sample; 5, outlet for water sample; 6, water inlet and, 7, water outlet for turbine; 8, axle. Scale, 1 : 1 cm.

During the exposure experiments to 1, 5, 10 and 20 mg $Cd \cdot \ell^{-1}$, the water was vigorously agitated by aeration to constant oxygen saturation levels, because decreased oxygen content in the water increases the toxicity of heavy metals to fish (Lloyd, 1961a).

Closed system respirometry

The respirometers were made of 130 mm diameter PVC-tubing provided with two water-tight screw-on lids and two removable perforated PVC lids at the open ends. At opposite sides, along the tube, two Perspex windows were installed to be able to observe the fish and count operculum frequency. The respirometers were suspended in the 1 000$\cdot \ell^{-1}$ exposure tanks. Single fish were kept inside the respirometers during the 10 h recovery period from handling stress, with the two screw-on lids removed but the two perforated lids in place.

Oxygen consumption rate ($\dot{M}O_2$) measurements in normoxic (PO_2:130 mm Hg) water

To operate the 1.180 respirometer, a 50 mℓ water sample was taken from inside the respirometer (start PO_2), followed (after a respiration period of about 20 min.) by a 50 mℓ water sample (end PO_2) when the lid was again opened. Usually the initial PO_2 was measured at 130 mm Hg and the respiration time and PO_2 were noted when the end PO_2 decreased to 80 mm Hg.

Oxygen consumption rate ($\dot{M}O_2$) measurements in progressive hypoxic (PO_2: 130 to 20 mm Hg) water

Respirometers (0.75 ℓ capacity) were used to measure the time needed by Cd-exposed banded tilapia individuals to deplete the sealed-off water from oxygen. After the 96 h Cd exposure period, fish were individually placed inside the respirometers and the lid closed. The specific oxygen consumption rate ($\dot{M}O_2$) was calculated from start (130 mm Hg) and end $P0_2$ values. The end PO_2 of the water was measured from a 50 mℓ sample after 90 min when the oxygen partial pressure was usually below 20 mm Hg.

The PO_2 measuring cell

The temperature-controlled (20°C (± 0.1°C) cell used to determine start and end PO_2 for the respirometers, is made from Perspex, with a water capacity of 45 mℓ (Fig. 1) and houses the oxygen electrode. The cell has two 3 mm diameter ports (Van Aardt and Frey, 1979). Water samples (50 mℓ) from the respirometers, to be analysed for PO_2, were gently injected into the measuring cell through one of the ports. The test water in the cell was constantly stirred by a water-driven turbine. The time taken to measure the PO_2 of a 50 mℓ test water sample from the respirometers was between 2 and 4 min. A polarographic PO_2^- electrode, provided with a 20 µm diameter platinum cathode from Radiometer, Copenhagen (Model E5046) was used. The PO_2 depletion rate of the test water by the cathode at 25°C was negligible, e.g. 0.035 mm $Hg \cdot min^{-1}$ when a Teflon membrane of 0.01 mm thickness was used (Severingshause and Bradley, 1971). The PO_2-electrode was calibrated and the membrane changed after every three days according to Hitchman (1983).

$\dot{M}O_2$ measurements in hard or in soft water at pH 8.34

Per experiment 25 fish were exposed to four different concentrations of cadmium chloride ($CdCl_2H_2O$ *pro analisi*, Merck, Germany) for 96 h in a tank with a fish density of 34 ℓ of water per fish. The tank was thermostatted at 20°C (± 0.2°C). Hard water, from dolomitic origin (Midgley et al., 1990) with a pH of 8.4 was obtained from municipal tap water pumped from the Mooi River. The tap water was treated for 2 d by vigorous aeration and by the substrate of the biological filter. Soft water with a pH of 7.51 was obtained from a borehole drilled into quartszite/shale contact (Nel, 1934) at the farm Rooipoort 29. Forty-eight hours before the fish were placed in the exposure tanks the water was aerated. Twelve hours before the fish were exposed to Cd, the appropriate amount of $CdCl_2$ was dissolved in the 1 000 ℓ exposure tanks to obtain 1, 5, 10, and 20 mg metal$\cdot \ell^{-1}$, respectively. Eighty-four hours after the fish were exposed, 12 to 15 specimens were caught and each fish placed into a respirometer with the water-tight lids removed. After 12 h, the respirometers were gently closed under water and the PO_2 values measured as described. The respirometers were suspended just below the water surfaces of the exposure tanks. The specific oxygen consumption rates of at least 10 specimens per Cd exposure were measured.

Blood plasma and bile fluid analyses

Blood samples (0.3 mℓ) were collected by cardiac puncture and centrifuged at 9 000 G for 5 min to obtain blood plasma. Bile (0.5 mℓ) was collected with a syringe from the gall bladder after

TABLE 2
The concentration of Cd in the gill-lamellae and liver tissue after 96 h exposure to different Cd concentrations in hard and soft water. For each exposure 10 fish were used. ± : Standard deviation form the mean

	Gills: control fish (µg·g^{-1} dry mass)			Exposure concentration	Gills: experimental fish (µg·g^{-1} dry mass)		
	Range	Mean	±	Cd (mg·ℓ$^{-1}$)	Range	Mean	±
Hard water	4 – 59	28	18	1	14 – 326	116	114
	4 – 15	7	3	5	132 – 964	397	264
	4 – 15	7	3	10	686 – 3762	2457	1104
	20 – 213	67	57	20	570 – 3772	1903	1128
Soft water	38 – 163	87	38	1	31 – 171	103	37
	38 – 163	87	38	5	94 – 358	212	77
	28 – 280	146	90	10	113 – 439	260	99
	28 – 280	146	90	20	945 – 6371	3955	1778
	Liver: control fish (µg·g^{-1} dry mass)			**Exposure concentration**	**Liver: experimental fish (µg·g^{-1} dry mass)**		
	Range	**Mean**	**±**	**Cd (mg·ℓ$^{-1}$)**	**Range**	**Mean**	**±**
Hard water	1 – 56	10	17	1	2 – 46	13	14
	0 – 2	1	1	5	13 – 129	48	36
	0 – 2	1	1	10	14 – 130	61	38
	0 – 3	1	1	20	10 – 133	72	50
Soft water	1 - 6	3	2	1	18 – 134	52	43
	1 – 6	3	2	5	68 – 321	179	101
	1 – 7	4	2	10	105 – 251	160	50
	1 – 7	4	2	20	65 - 724	219	210

opening of the abdominal cavity.

After the MO_2 measurements, dissolved solids in blood plasma and bile fluid were measured using a refractometer (Model T/C 10402, American Optical Company). Chlorides in the blood plasma were determined using a chloride titrator (Clinical Model 4-4495, American Instrument Company).

$\dot{M}O_2$ calculations and mass-specific oxygen consumption

To calculate $\dot{M}O_2$ the obtained ΔPO_2 values were first multiplied by the molar solubility coefficient for oxygen (Green and Carrit, 1967; Cameron, 1986) at 20°C, followed by the calculations described by Van Aardt (1990). All $\dot{M}O_2$ values for specimens ranging from 15 g to 125 g were corrected to be equivalent to the specific oxygen consumption rate of a live body mass of a 30 g *T. sparrmanii*. This was obtained by measuring the specific oxygen consumption rates of 30 specimens ranging from 13 to 131 g body-mass. The data were plotted on a graph with $\dot{M}O_2$ at the y-axis on a linear scale and the live body mass on a logarithmic scale. From the data a straight line on the graph could be calculated.

Effects of handling stress on $\dot{M}O_2$

To determine the effect of handling stress on the resting metabolic rate of the banded tilapia, ten specimens were taken from the holding tanks by netting and each placed in a respirometer suspended in the 1 000 ℓ temperature controlled tank. The $\dot{M}O_2$ was measured immediately after handling as described above. In the same manner individual $\dot{M}O_2$ was measured in other groups of ten *T. sparrmanii,* 2, 4, 8, 10 and 13 h after they had been handled (Wedemeyer, 1976).

Oxygen consumption by bacteria and other organisms

The $\dot{M}O_2$ in the holding fish tank water was measured in clean respirometers without fish for 2 h.

Cd analysis of the different hard water types

The applied Cd concentrations were determined in the exposure tanks in Fish tank hard water, Rooipoort soft water and the 3 reconstituted fresh-water types (Rand et al., 1995), 96 h after the $CdCl_2.H_2O$ was dissolved. The water samples were taken from the exposure tanks and from the three reconstituted fresh water types while the water was vigorously agitated by means of aeration stones. Cd was extracted from the water samples with tetrachlorometane using Kit No 13 from Macherey-Nagel, Germany and analysed spectrophotometrically for cadmium (accuracy 0.002 mg Cd·ℓ$^{-1}$)

Cd analysis of gill and liver tissue

After the 96 h exposure period to Cd the gill tissue on the branchial arch and also liver tissue was cut from freshly dissected fish and the wet mass per fish determined. From a previous experiment the dry mass at 60ºC was determined after 24 h drying in the oven. For *T. sparrmanii* dried gill tissue mass was 17.6 % of the wet gill tissue mass and liver tissue 29.9%. To analyse the wet tissue it was first digested with 4 mℓ HNO3 (pro-analisi, Merck, Germany 0.000001 % Cd) at 70ºC for 12 h. The digested samples were diluted to a volume of 10 mℓ with de-ionised water. One hundred µℓ of sample was diluted 20 times and used to determine Cd with a Varian Spectra 250 Plus and a GTA95 electrothermal oven (Varian, Australia). Palladium and Triton X-100, as modifiers, were used. Accuracy better than 5 µg Cd·ℓ$^{-1}$ was achieved. However, a straight regression line through the standards could not be achieved. The analysis was expressed per gram of dry tissue. The certified reference material used was obtained as dogfish reference material (National Research Council, Canada) and used to determine analytical efficiency.

Statistical analysis

A statistical package was used to compare control with experimental data using one-way analyses of variance [ANOVA and Tukey's

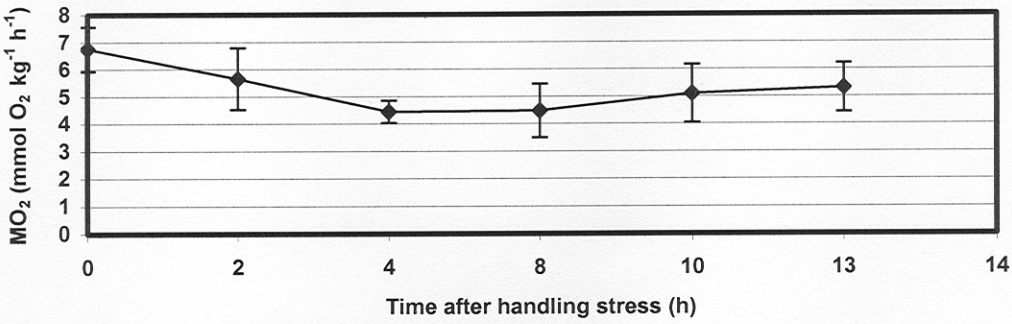

Figure 2
The effect of handling stress on the specific oxygen consumption rate ($\dot{M}O_2$) of Tilapia sparrmanii. *Each point on the graph represent ten fish. The vertical bars represents the standard deviation from the mean.*

post hoc test for multiple comparisons of significant different means ($P<0.05$)]

Data are presented as means (± = standard deviation from the mean).

Results

Complexation of Cd in different hard water types

The concentrations of cadmium dissolved in Mooi River water, reconstituted freshwater, Rooipoort soft water, precipitate from solution after 96 h (Table 1). For hard water less than 1% of cadmium could be analysed from water samples taken during vigorous aeration. It was observed that within minutes of the application of $CdCl_2$ in the water, precipitation occurred, presumably as cadmium sulphate (Fridberg et al., 1979). According to Bodek et al. (1988) the solubility product constants for Cd are such that total precipitation in hard and soft water is possible.

Cd accumulation in gill lamelae and liver tissue

Fish exposed to 1 and 5 mg·ℓ$^{-1}$ cadmium, for both hard and soft water, accumulate about ten times less in their gill tissue compared to 10 and 20 mg·ℓ$^{-1}$ cadmium exposure (Table 2). The Cd accumulation in the liver is nearly twenty times less compared with gill tissue and is found in the liver when fish are exposed to both hard and soft water. The differences, however, are not significant.

Oxygen consumption rate of the water medium

Metabolic activity in the respirometer water, probably caused by bacterial or algal consumption of oxygen, does not change the PO_2 by more than 3 mm Hg during the 2 h measurement period for both the respirometers in hard water. In soft water no changes in the partial pressure of oxygen were found.

Oxygen consumption rate and fish size

For the 30 fish measured the $\dot{M}O_2$ values fell on a regression line represented by the equation, $Y = -3.3523\ X + 10.06$. The slope (-3.3523) was used to derive the correction factor for $\dot{M}O_2$ of fish ranging between 15 g and 140 g to a 30 g standard banded tilapia.

$\dot{M}O_2$ and handling stress

Handling stress resulted in a 37% increase of the specific $\dot{M}O_2$ to 6.7 mmol O_2·kg^{-1} fish·h^{-1} (Fig.2). The differences are statistically significant ($P<0.05$). The value of 4.5 mmol O_2·kg^{-1} fish·h^{-1}, 4 h after handling, was not significantly different 8, 10, or 13 h after the fish had been handled. From these findings it was decided that experimental fish would be kept in the open respirometers submerged in the exposure tanks for at least 10 h before resting specific $\dot{M}O_2$ measurements were made.

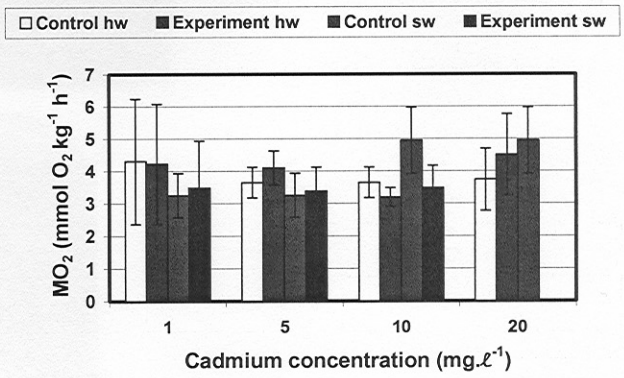

Figure 3
The effect of different Cd concentrations on the specific oxygen consumption rate ($\dot{M}O_2$) of Tilapia sparrmanii *measured in hard water (hw) and soft water (sw). All fish died before 96 h exposure to 20 mg Cd·ℓ$^{-1}$ in soft water. The vertical bars represent the standard deviation from the mean.*

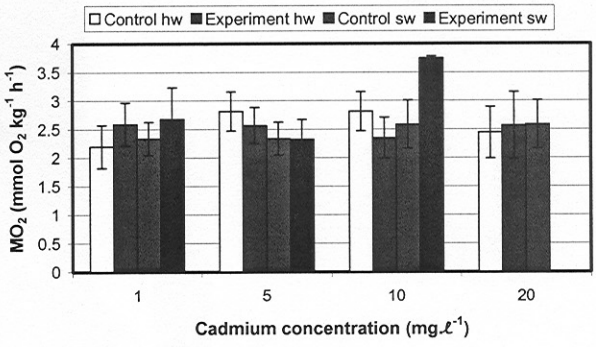

Figure 4
The effect of different Cd concentrations on the specific oxygen consumption rate ($\dot{M}O_2$) of Tilapia sparrmanii measured in hard (hw) and soft water (sw) exposed for 96 h to Cd. The $\dot{M}O_2$ was measured at 94 h with fish kept at progressively hypoxic conditions (130 mm Hg to 10 mm Hg) for 2 h. All fish died at 20 mg Cd·ℓ$^{-1}$ exposure to soft water. The vertical bars represent the standard deviation from the mean.

Effects of hard water on the $\dot{M}O_2$ for Cd exposed fish

No effects of 1, 5, 10 and 20 mg Cd·ℓ$^{-1}$ hard water on the $\dot{M}O_2$ were found when compared with values from control fish (3.2 and 4.5 mmol O_2·ℓ$^{-1}$, Fig. 3). The mean oxygen consumption rates were between 3.55 and 4.37 mmol O_2·kg^{-1}·h^{-1}.

TABLE 3
Blood chloride concentration and dissolved solids in bile and blood plasma after 96 h exposure to different Cd concentrations in hard and soft water. For each exposure 10 fish were used. ± : Standard deviation.

	Blood plasma chlorides: control fish (mmol NaCl)			Exposure concentr.	Blood plasma chlorides: exp. fish (mmol NaCl)		
	Range	Mean	±	Cd (mg·ℓ$^{-1}$)	Range	Mean	±
Hard water	138 – 152	144	4	1	133 – 147	141	4
	139 – 164	149	8	5	134 – 157	143	8
	139 – 164	149	8	10	139 – 155	147	5
	112 – 136	128	7	20	121 – 150	133	8
Soft water	122 – 147	137	8	1	134 – 158	150	8
	122 – 147	137	8	5	106 – 129	114	17
	98 – 134	122	10	10	85 – 137	108	16
	98 – 134	122	10	20	32 – 90	58	17
	Dissolved solids in plasma: control fish (g·100 mℓ$^{-1}$)			**Exposure concentr.**	**Dissolved solids in plasma: experimental fish(g·100 mℓ$^{-1}$)**		
	Range	**Mean**	**±**	**Cd (mg·ℓ$^{-1}$)**	**Range**	**Mean**	**±**
Hard water	5 – 6	5	0.4	1	5 – 6	5	0.4
	4 – 6	5	0.7	5	5 – 7	6	0.5
	4 – 6	5	0.7	10	4 – 6	5	0.6
	5 – 6	5	0.4	20	5 – 8	6	1
Soft water	5 – 7	6	0.5	1	4 – 7	6	1
	5 – 7	6	0.5	5	6 – 7	7	2
	5 – 6	5	0.3	10	6 – 8	6	0.7
	5 – 6	5	0.3	20	6 – 9	7	1
	Dissolved solids in bile: control fish (g·100 mℓ$^{-1}$)			**Exposure concentr.**	**Dissolved solids in bile: experimental fish(g·100 mℓ$^{-1}$)**		
	Range	**Mean**	**±**	**Cd (mg·ℓ$^{-1}$)**	**Range**	**Mean**	**±**
Hard water	15 – 17	16	0.5	1	15 – 16	16	0.3
	15 – 16	16	0.3	5	15 – 17	16	0.5
	15 – 16	16	0.3	10	16 – 17	16	0.2
	14 – 16	16	0.5	20	15 – 17	15	0.6
Soft water	14 – 16	16	0.7	1	14 – 16	16	0.5
	14 – 16	16	0.7	5	14 – 16	16	0.2
	14 – 15	15	0.5	10	12 – 14	13	0.6
	14 – 15	15	0.5	20	8 – 12	11	1.3

Effects of soft water (pH 8.4) on the $\dot{M}O_2$ for cadmium exposed fish

During the 96 h exposure period to Cd in soft water the oxygen consumption rate for control and experimental fish stayed the same except for the 20 mg Cd concentration, where all the experimental fish died before the 96 h exposure period expired (Fig.3).

Effects of hypoxic water on the $\dot{M}O_2$ for Cd exposed fish

a) Hard water

The $\dot{M}O_2$ was between 2.41 and 2.80 mmol $O_2 \cdot kg^{-1}$ fish$\cdot h^{-1}$ and is not significant between control and experimental fish in hard water. This value is 34% less compared with $\dot{M}O_2$ of fish in normoxic hard water (Fig.4).

b) Soft water

For experimental fish exposed to 10 mg Cd, the $\dot{M}O_2$ increased to 3.7 mmol $O_2 \cdot kg^{-1}$ fish h^{-1}. This is significantly above the 10 mg·ℓ$^{-1}$ normoxic Cd exposure control values of between 2.3 and 2.6 mmol $O_2 \cdot kg \cdot h^{-1}$. All fish died at 20 mg·ℓ$^{-1}$ Cd, both in normoxic and progressive hypoxic water conditions. The oxygen consumption rates for hypoxic fish are 35% less compared with $\dot{M}O_2$ of fish in normoxic (130 mm Hg) soft water (Fig. 4) for all other exposure concentrations.

Blood plasma chlorides decrease significantly from 122 mmol NaCl to 108 mmol for 10 mg Cd·ℓ$^{-1}$ and to 58 mmol NaCl for 20 mg Cd·ℓ$^{-1}$ exposed fish compared with a value of 122 mmol NaCl for the controls. Dissolved solids in bile for the 10 and 20 mg·ℓ$^{-1}$ Cd concentrations also decreases significantly compared with the control values. However, dissolved solids in blood plasma stays the same for both experimental and control fish for all Cd exposed concentrations.

Discussion

In seawater and estuarine conditions, Cd complexation is caused by chloride as ligand (Sunda et al., 1978) but in Mooi River hard water the carbonate and phosphate ions are probably the major complexation ligands and not chlorides. This is so because, as shown in results from Table 1, in reconstituted hard water with low chloride concentration, Cd complexation still takes place.

From these experiments it is not clear whether *T. sparrmanii* are resistant to Cd toxicity. It appears that the differences in toxic responses are probably related to the bioavailable fraction of the administered dose being very low at hard water conditions. At exposure levels in soft water, with 23% of Cd in solution (after speciation) and bioavailable, the metal concentration is about ten times higher compared to Cd exposed to the grass shrimp and other aquatic animals (Sunda et al., 1978). At these concentrations no deaths were experienced for *T. sparrmanii*.

Compared to other heavy metals such as Pb, Zn and Cu, Cd toxicity is much more influenced by water hardness. The 48 h LC_{50} for rainbow trout is below 0.1 mg Cd·ℓ$^{-1}$ for water hardness of 20 mg·ℓ$^{-1}$ $CaCO_3$, but toxicity (using 5 mg Cd·ℓ$^{-1}$) decreases about 50-fold in hard water with a hardness of 250 mg·ℓ$^{-1}$ as $CaCO_3$

(Brown, 1968). However, banded tilapia are more resistant to Cd toxicity compared to trout, because at 10 mg Cd all specimens were still alive after 96 h exposure at the same hardness level.

The toxicity tests done with Cd on the $\dot{M}O_2$ reveal an unanticipated variation of the $\dot{M}O_2$ values. Only a small part of this variation could be ascribed to variation in the individual specimens tested. This variation phenomenon was also discussed by Rand et al., (1995) who mention that for LC_{50} values it is the "result of unrecognised changes in the organisms or in the test conditions". The same set of parameters in the same laboratory for a particular experiment done on hard water using trout, show remarkably different LC_{50} results. This was also found for inter-laboratory experiments using the same experimental conditions (Rand et al., 1995; Sprague, 1995; Mount, 1966).

In these experiments we suggest that the variation in $\dot{M}O_2$ found in all the exposure experiments can be the result of the physical nature of the precipitated Cd complexes. The Cd complex is mechanically brought into suspension by vigorous aeration. In this manner varied Cd deposits on the gill surfaces could be the result, especially for fish that stay and swim deeper in the exposure tanks. The deposits, in turn, cause damage to the gill epithelium that differs from fish to fish, resulting in variations in the $\dot{M}O_2$ values. Even though Cd precipitates as a metal complex on the external gill surface there is a likelihood that the lower pH at the gill micro-environment could result in uptake of Cd thereby increasing bio-availability. In this regard a mechanism how positive metal compounds react with low pH gill epithelial constituents was put forward by Reid and Macdonald (1991). This mechanism could be substantiated by the fact that bioaccumulation of Cd in or on the gill tissue, with large variation in concentration, was demonstrated in our experiments.

Analysis of cadmium in hard water, 96 h after it was dissolved showed that this metal completely precipitates out when dissolved in hard water. This is also true for relatively soft water from the Rooipoort farm. Precipitation of Cd is in accord with the very low solubility characteristics described for Cd (Rand et al., 1995). Despite the low solubility of the Cd-complexation compound, biological effects of its toxicity can be detected, starting at 10 mg $Cd{\cdot}\ell^{-1}$ soft alkaline water. This finding should be evaluated with the knowledge that the precipitate is deposited on the gill epithelium, damaging the gill structure that leads to changes in its respiratory function.

The fact that bile-dissolved solids and chloride plasma levels in *T. sparrmanii* are decreased and blood plasma solids increased during Cd exposure, suggests that the effects of Cd on the gill epithelium are not only the result of gill epithelium damage but that the metal toxicity mechanism, via gill uptake, has its effects on the whole physiology of the fish, including a rise in the $\dot{M}O_2$. This is substantiated by the finding that a sharp increase in the accumulation of Cd, both in gill and liver tissue was established in our experiments. Cd bioaccumulation would increase in the softer water due to the greater bioavailibility. However, since the bioavailability of Cd is <1% in the hard water the mechanism of toxicity has to be associated with the precipitation of Cd complexes on the gill surface rather than uptake-based toxicity. This view is not shared by Playle et al., (1993) who showed that Cd strongly binds to fat head minnow gills. They reasoned that the uptake of Cd takes place through high affinity Ca channels, in addition to surface binding. The decreased plasma Cl observed in our experiments could be an indication of altered acid-base balance with more CO_2 being buffered in the red blood cells, due to the increased diffusion distance, resulting in the production of HCO_3. This would result in Cl ions being shifted from the plasma into the erytrocytes to maintain electroneutrality. Increase of plasma solids could in turn be related to compensatory mechanisms initiated to maintain plasma osmolality (Wilson and Taylor, 1993).

It is known that the highest Cd accumulation in vertebrates' organs occurs in kidneys. Unfortunately in *T. sparrmanii,* the collection of tissue from the small and elongated kidney for analysis was unpractical and prone to cross-contamination with blood. Undoubtedly the liver is involved in the collection and detoxification of Cd by Cd-metallothionein formation, as was proven for kidneys by Van der Malie and Garvey (1979).

The decrease in the $\dot{M}O_2$ during progressive hypoxia in closed-off respirometers indicates that when these measurements are done in closed-off respirometers, PO_2 readings should be done before the critical PO_2 tension of the water is reached (Herreid, 1980). In practice, for fish, the oxygen tension in the water should not decrease more than 20% compared to the starting PO_2. The high MO_2 found for hypoxic treated tilapia is reflected by an increase in the gill frequency, a physiological parameter not measured in these experiments but was observed in both the large mouth bass *(Micropteris salmoides)* and the mud fish *(Labeo capensis)* during progressive hypoxia (personal observations).

It is recommended that before the toxic effect of a heavy metal is tested, its solubility and complexation properties at different pH values, together with its toxicity in hard and soft water, should be studied and evaluated.

It can be stated that Cd, released through mining activities (Wittman and Förstner, 1977), in the Mooi River catchment area can be precipitated by hard water from dolomitic origin. These insoluble metal complexes can, after precipitation, be stored in the river sediments of the Mooi River and the river's three dams. Any future change in the solubility of the precipitated metals, such as a dramatic and sudden decrease in the pH of the water and physical disturbance of the sediment, could potentially release these toxic metals in a soluble form into the water. Therefore, future measurements of metal accumulation in the sediments of the Mooi River are important to assess the potential dangers of toxic metals in this area as a whole.

Acknowledgements

We wish to thank the Research Focus Area: Environmental Sciences and Management, for financial assistance and the School of Environmental Sciences and Development, Potchefstroom University for CHE, Potchefstroom, South Africa, for providing the research facilities.

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