Lethal copper concentration levels for *Clarias gariepinus* (Burchell, 1822) — a preliminary study

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Van der Merwe, Marinda, J.H.J. van Vuren and H.H. du Preez. 1993. Lethal copper concentration levels for *Clarias gariepinus* (Burchell, 1822) — a preliminary study. *Koedoe* 36(2): 77-86. Pretoria. ISSN 0075-6458.

Lethal copper concentrations were determined for both adult and juvenile *Clarias gariepinus* at representative mean summer and winter temperatures. Fish were exposed to copper for 96 hours in an experimental system and mortalities monitored. Toxicity curves of percentage mortality versus actual copper concentration were drawn, and the LC50 calculated for winter and summer temperatures. The lethal copper concentrations, expressed as LC50, found in laboratory exposures, ranged for adults from 1,29 mg/l during summer to 1,38 mg/l in winter. These values are considerably higher than the levels of copper in the water of the Olifants River in the Kruger National Park during summer (0,055 ± 0,016 mg/l) and winter (0,085 ± 0,032 mg/l). The derived LC50 values predict the level of copper which should be prevented at all cost. The fish in the Olifants River are already exposed to sublethal concentrations (40% of LC50) of copper. The results can be used as an indication of what the safe concentrations of copper should be.

Keywords: copper, lethal concentration level, *Clarias gariepinus*, Kruger National Park.

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Introduction

The growing need to determine critical concentrations for toxicants in rivers and other water bodies have become inevitable in order to maintain the diversity of species. Stricter legislation has already been enforced on mining, industry, and agriculture in Canada, Europe, and the United States of America to protect natural waterbodies. In South Africa, inadequate legislation of the past and the negligence of executing the existing laws have partly led to the current problem of polluted rivers, the Olifants River being an example. Acute toxicity tests may provide meaningful answers to problems arising due to the pollution of rivers, by comparing the lethality of toxicants between organisms and test conditions or toxicants (Buikema *et al.* 1982). Toxicity tests can reliably monitor changes in the lethality of metals to an organism, but it is essential to consider that the acute toxicity test, by itself, cannot adequately predict a concentration of toxicant that will be unlikely to harm a population or ecosystem at all times (APHA [American Public Health Association] 1989).

Many factors can affect or modify the degree of sensitivity shown by an organism to different toxicants. Some examples are the specific diet, season of the year and water quality variables such as temperature, pH and hardness (Falk & Dunston 1977). The calcium content of water acts as a modifying factor with an increase in metal toxicity in water with a low calcium content (Doudorff & Katz 1953; Davies *et al.* 1976; Gregory & Macfarlane 1981). An increase in water temperature will increase the rate of chemical reactions in fish as predicted by the Arrhenius theory. According to this theory the toxicity of a toxicant also increases with a rise in temperature. This was reported during exposures of *Lepomis macrochirus* (Burton *et al.* 1972), *Tilapia zilli* and *Clarias lazera* (Hilmy *et al.* 1987). The toxicity of pollutants may be increased by a reduction in dissolved oxygen, while the hydrogen ion concentration can affect ionisation as well as the solubility of

the toxicant (Heath 1987; Everall *et al.* 1989). The importance of modifying factors cannot be overestimated, for many accounts of their influence on the variation in toxicity can be found in the available literature. It is therefore important to determine the specific LC50 value (lethal concentration for 50 percent of the individuals) for a given combination of abiotic conditions as found in a specific river. Lethal threshold concentrations may often be similar for similar species, but it is risky to transfer predictions of the effects of modifying factors from one species to another.

The study reported here was conducted to determine the lethal threshold concentration or incipient LC50 values for copper at the two selected temperatures (21 ± 1°C and 28 ± 1°C) for adult as well as juvenile *C. gariepinus*. The lethal threshold concentration is usually defined as that concentration which causes death to half the test organisms within a specified period of time e.g. 96 hours (Heath 1987). The range of copper concentrations found in the Olifants River was compared to the LC50 values found during the experimental tests, in order to predict acceptable guidelines for copper concentrations in the Olifants River. *Clarias gariepinus* was chosen, because, although it is a species of commercial importance in South Africa, copper toxicity data for it are nonexistent, and it is risky to extrapolate toxicological findings from other air-breathing species to this species (Hellawell 1988). *Clarias gariepinus* is also abundant in the Olifants River and occupies a high level in the trophic hierarchy, being an omnivorous scavenger.

Material and Methods

Clarias gariepinus specimens were obtained from Blyderiver Aquaculture, a commercial catfish farm near Hoedspruit in the eastern Transvaal. Fish were collected by means of a seine net and transported in an aluminium tank filled with aerated borehole water. Mortalities during transport did not exceed 0,3%.

Laboratory conditions

In the laboratory fish were placed in two 1 000-litre plastic tanks filled with well-aerated borehole water. The physico-chemical conditions of the water were tested once a month for three months and results are shown in Table 1. Fish were not fed for 72 hours after arrival to reduce the likelihood of lethality due to feeding during a stress condition (Carmichael *et al.* 1984).

The tanks were provided with a central, vertical pipe through which uneaten food and faeces were drawn off. Water from both tanks was collected in a plastic 100-litre cylindrical reservoir tank, fitted with a float switch, which regulated a swimming pool pump. The water was pumped from the reservoir tank via a sand filter to a biological filter, from where it was pumped back into the 1 000-litre plastic tanks. No copper or brass fittings were used due to the toxicity that these fittings might have (Sprague 1973).

Fish were acclimatised in the laboratory for a minimum period of four weeks at a temperature of 28 ± 1°C and 21 ± 1°C. The physico-chemical conditions of the dilution water are important factors that might affect the form and toxicity of any metal salt added to the water (Sprague 1973). It was thus important to regulate the levels of specific water quality variables

Table 1
Water quality variables measured for borehole water used in laboratory experiments

Water quality variable	21°C	28°C
pH	7,40 ± 0,10	7,60 ± 0,15
Total hardness ($CaCO_3$ mg/l)	61,00 ± 4,70	64,00 ± 5,01
Dissolved oxygen (O_2 mg/l)	7,90 ± 0,18	7,00 ± 0,32
Conductivity (mS/m)	22,00 ± 3,20	21,00 ± 4,00
Ammonia (NH_3 mg/l)	0,14 ± 0,02	0,15 ± 0,02

Table 2
Concentrations of copper in mg/l added as $CuCl_2$, Cu^{2+} and the actual copper concentration found in the test water used for experiments with adult and juvenile C. gariepinus *at 21°C and 28°C, respectively*

21°C	Adult *C. gariepinus*						Juvenile *C. gariepinus*					
$CuCl_2$	2,41	2,68	2,95	4,02	4,70	6,17	1,48	2,15	2,68	3,54	4,02	5,10
Cu^{2+}	0,90	1,00	1,10	1,50	1,75	2,30	0,55	0,80	1,00	1,32	1,50	1,90
Actual $[Cu^{2+}]$	0,88	0,95	1,21	1,46	1,74	2,22	0,51	0,77	0,97	1,30	1,43	1,63
28°C	Adult *C. gariepinus*						Juvenile *C. gariepinus*					
$CuCl_2$	1,73	2,65	2,95	3,45	4,87	6,17	2,15	2,95	3,44	3,83	4,83	9,09
Cu^{2+}	0,64	0,98	1,10	1,28	1,81	2,30	0,80	1,10	1,29	1,43	1,80	3,39
Actual $[Cu^{2+}]$	0,62	0,93	1,07	1,26	1,77	2,26	0,79	0,99	1,17	1,40	1,81	3,33

to prevent additional physiological stress on the fish that could not be attributed to the addition of a particular metal. The temperature, disolved oxygen and pH of the water were monitored daily, and a maximum deviation of 1,0°C in temperature, 0,32 mg/l disolved oxygen, and 0,3 pH units occured (Table 1). Water hardness was also monitored once a month and a local photoperiod sequence was regulated with an electric timer to simulate a 12 h:12 h day:night cycle.

After the initial acclimatisation period ten fish were transferred to the experimental system. This continuous-flow test system consisted of eight 100-litre glass tanks, fitted with a 50 mm diameter vertical PVC pipe

Table 3
Mean weight and length of C. gariepinus *used in toxicity tests*

21°C	Adult $n = 60$		Juvenile $n = 60$	
	Total length (cm)	Weight (g)	Total length (cm)	Weight (g)
$\bar{x}$	19,43	54,87	7,08	13,97
SD	4,57	10,91	1,98	4,07
r	7,90	23,80	3,72	4,51
28°C	Adult $n = 60$		Juvenile $n = 60$	
	Total length (cm)	Weight (g)	Total length (cm)	Weight (g)
$\bar{x}$	22,97	60,88	5,08	15,07
SD	5,83	11,90	2,97	3,08
r	6,95	25,94	2,75	1,68

r = regression coefficient percentage

of specific length beneath each tank. This pipe drained excess water and regulated the volume of water within the glass tanks. A 200-litre reservoir tank was fitted with a float switch which regulated a swimming pool pump. The water in the glass experimental tanks was constantly aerated with compressed air (Van der Merwe 1992).

A continuous-flow test is an appropriate test to estimate the toxicity of a pollutant to riverine organisms. The concentration of the toxicant and duration of exposure can be regulated. Short-term (acute) and long-term exposures can be performed to monitor behavioural and physiological changes. Furthermore, constant oxygen levels can be maintained (Buikema *et al.* 1982).

Continuous-flow systems do have disadvantages such as the high construction and operation costs, electric power requirements, large space requirements, as well as the large volumes of water and quantities of toxicant that are required (Buikema *et al.* 1982).

A toxicity curve which shows the correlation between the exposure concentration of the toxicant and the percentage mortality of the organism was drawn to provide an overall assessment of the test progress. A completely standardised method (APHA 1989) with a reference toxicant theoretically maximises comparability, repeatability and reliability and is thus essential in answering questions of relative toxicity and sensitivity or of the repeatability of tests.

Fish were kept in the glass tanks for 48 hours prior to the addition of the toxicant. The toxicant was mixed with the dilution water in a separate 200-litre cylindrical plastic tank, which was fitted with a "Little Giant" submersible pump. A separate set of taps was connected to the submersible pump and was set at a rate which replaced 90% of the water in each of the 100-litre glass tanks every twenty four hours.

Exposure of test organisms

Adult and juvenile *C. gariepinus* were exposed to various copper concentrations at a temperature of 21 ±1°C and 28 ± 1°C respectively. These selected temperatures represent the mean winter and summer temperatures of the Olifants River, as determined from field monitoring results between February 1990 to February 1991. The copper concentrations used for the acute toxicity tests, were determined by taking the prevailing concentrations in the Olifants River as a sublethal concentration with a 0% mortality rate and increasing the concentration to a 100% mortality rate. A duplicate toxicity test was conducted for copper concentrations that represented a mortality rate between 20% and 80%. Table 2 displays the concentration of copper chloride added as well as the theoretical divalent copper-ion concentration in the water determined by atomic absorbtion spectrophotometry.

Ten fish were used for each exposure test. The numbers of fish that survived each exposure were determined at intervals of 2, 4, 6, 8, 24, 48, 72, and 96 hours, respectively. Dead fish were immediately removed from the exposure tanks and their length and weight determined. The water temperature, pH, and water hardness were monitored at each interval.

Calculation of the LC50 and 95% confidence limits

The LC50 value can be determined graphically from a line drawn through the data points or by a variety of computational methods described by Litchfield & Wilcoxon (1949). A regression line ("line of best fit") was drawn of Y (% mortality) on X (percentage concentration per volume, e.g. mg/100 ml). The predicted concentration of copper, where 50% mortality will occur was determined and the 95% confidence limits of this concentration estimate were calculated. Differences in the LC50 values of the respective toxicity tests were determined by means of anova one-sample analysis using the *Statgraphics* software package. A toxicity curve was drawn by plotting the exposure time in hours (y-axis) on the median concentration of copper in percentage per volume (x-axis). Graphs were prepared with the aid of the *Statgraphics* software package.

Results

The mean mass and total length of fish used in the toxicity tests are summarised in Table 3. No mortalities were found during the control tests. On the basis of 24, 48, 72 and 96-h exposure periods, the LC50 values were estimated for *C. gariepinus* seasonally (Tables 4 -7). A regression coefficient percentage was calculated for all the LC50 values.

The 24, 48, 72 and 96-h LC50 value for juvenile *C. gariepinus* at 21 ± 1°C are significantly higher ($P < 0,05$) than the 24, 48, 72 and 96-h LC50 value for adult *C. gariepinus*. No statistically significant difference was found between the 96-h LC50 values for adult and juvenile *C. gariepinus* at 28 ± 1°C. However, there was a significant difference ($P < 0,05$) between the LC50 values at 48 and 72 hours. The toxicity curves of *C. gariepinus* to various concentrations of copper at the selected 21 ± 1°C and 28 ± 1°C are presented in Fig. 1. The curve for juveniles at 21 ± 1°C is almost linear, while it is distinctly curvilinear for the remaining bioassays.

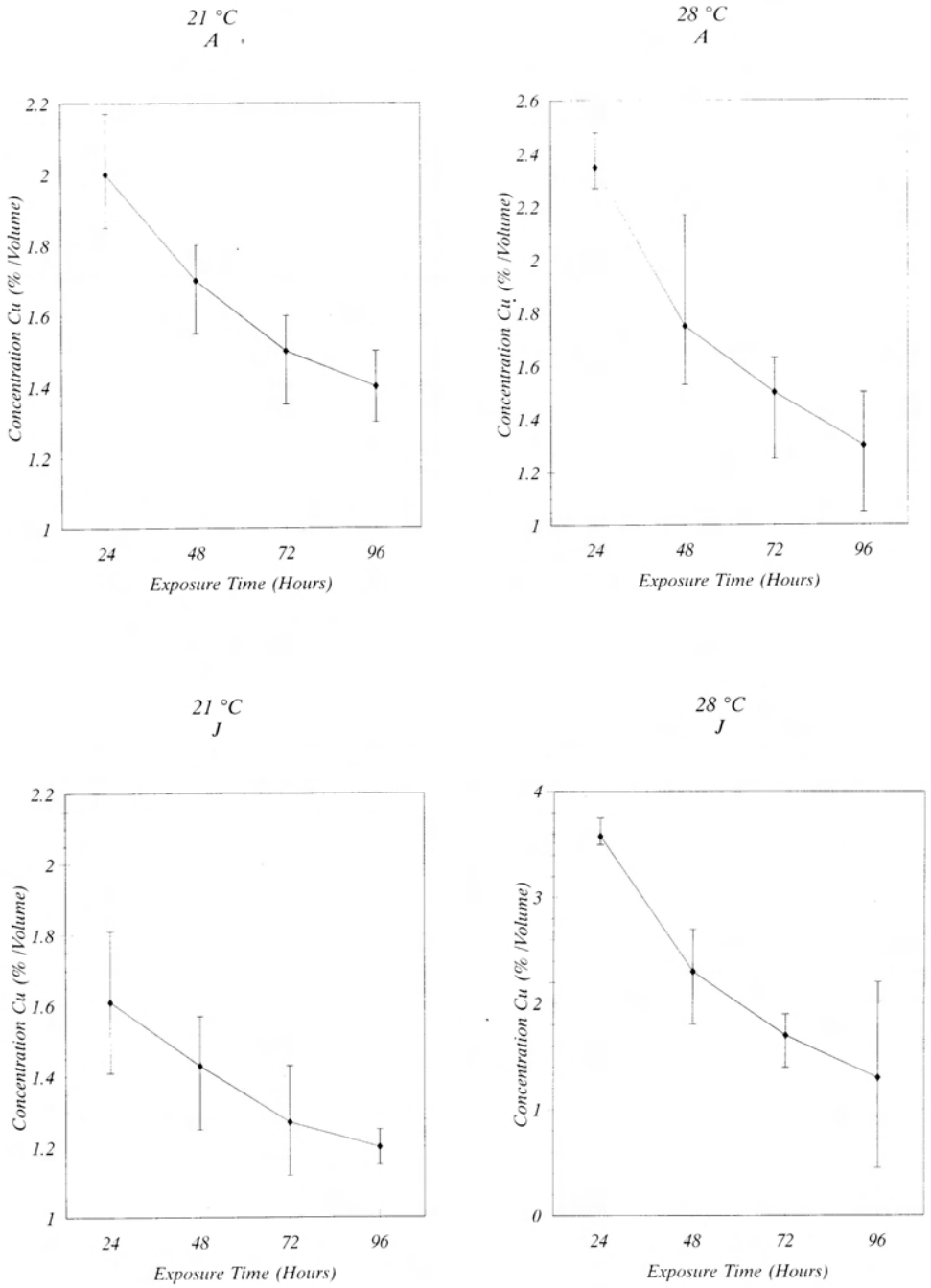

Fig. 1. Toxicity curves for *C. gariepinus* after copper exposure. A: Adults. J: Juveniles.

Table 4

Experimental data obtained during a toxicity test on adult C. gariepinus *and copper LC50 values at 21 ± 1°C after different time intervals*

Concentration of copper % Cu^{2+} / volume	Number of test organisms	Number of test organisms dead after:							
		2 h	4h	6h	8h	24h	48h	72h	96h
2,220	10	0	0	0	2	6	8	9	10
1,744	10	0	0	0	1	4	5	7	7
1,463	10	0	0	0	0	3	4	6	6
1,211	10	0	0	0	0	2	3	3	4
0,949	10	0	0	0	0	1	1	2	3
0,877	10	0	0	0	0	0	0	1	1
0,543	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		–	–	–	–	2,00	1,68	1,48	1,38
95% Confidence limits for Cu^{2+} concentration		–	–	–	–	2,17	1,80	1,59	1,46
		–	–	–	–	1,83	1,56	1,35	1,30
Regression coefficient (%)		–	–	–	–	97,10	96,42	96,98	97,04
Slope of probit line		–	–	–	–	38,26	50,68	58,13	60,79

Table 5

Experimental data obtained during a toxicity test on juvenile C. gariepinus *and copper LC50 values at 21 ± 1°C after different time intervals*

Concentration of copper % Cu^{2+} /volume	Number of test organisms	Number of test organisms dead after:							
		2 h	4h	6h	8h	24h	48h	72h	96h
1,629	10	0	0	0	3	6	6	9	10
1,517	10	0	0	0	2	6	5	6	7
1,334	10	0	0	0	0	3	4	5	6
0,968	10	0	0	0	0	1	1	2	2
0,767	10	0	0	0	0	0	0	1	1
0,514	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		–	–	–	–	1,61	1,42	1,28	1,20
95% Confidence limits for Cu^{2+} concentration		–	–	–	–	1,81	1,57	1,43	1,24
		–	–	–	–	1,31	1,27	1,13	1,16
Regression coefficient (%)		–	–	–	–	90,61	93,47	93,73	94,62
Slope of probit line		–	–	–	–	60,38	67,75	75,11	86,52

A veil-like film, which appeared to be excessive coagulated mucus covered the dead fish. A similar veil-like thickened film of mucous was found within the operculum on the gills. Other symptoms of toxicosis such as behavioural changes were observed, which indicated that copper causes sublethal effects on *C. gariepinus*. Behavioral changes recorded in *C. gariepinus* were in order of their appearance, agitated swimming rates, increased rates of operculum movement, lethargy, loss of rheotaxis. These reactions to copper were more pronounced in tanks containing the higher levels of copper, and less conspicuous at lower concentrations.

Discussion

The primary goal in the design and use of toxicity tests in biomonitoring is to predict, in combination with other environmental factors, with known accuracy, a concentration of a specific toxicant (metal) that will not harm an entire system, and to make this prediction in a responsible and cost effective manner. In any bioassay, it is important to consider the water quality used in the experimental system. Modifying factors, such as water hardness, pH, alkalinity, and temperature, can affect the copper speciation, and thus alter the copper toxicity. The critical factor regarding copper toxicity to fish is not as much the toxic action following copper speciation, but to a larger extent the controlling roles of water hardness, alkalinity, and pH (Chakoumakos *et al.* 1979).

It is widely known that metal toxicity varies with the test organism as well as the chemical water quality of the test water (Lewis & Lewis 1971). Thus, the reported data on 96-h copper LC50 values to various fish species in different waters have varied over a wide range of values from 0,1 mg/l to 20 mg/l (Wong *et al.* 1977). The LC50 value for copper at hardness of 100 mg/l $CaCO_3$ and pH 7,8, was 0,14 mg/l (Howarth & Sprague 1978), while the 96-h LC50 and 95% confidence limits observed for the common guppy at 28°C were 0,986 (0,73-1,32) mg/l (Khangarot & Ray 1990). The 96-h LC50 value obtained at 28°C for adult *C. gariepinus* falls within these 95% confidence limits. The 21 ± 1°C, 96-h LC50 value differed from results reported on the bluegill *Lepomis macrochirus*, where a LC50 value and 95% confidence limits of 0,9 (0,7-1,2) mg/l Cu^{2+} were found at 19 ± 2,5°C (Thompson *et al.* 1980). Although the LC50 values of a specific metal obtained for two different species at the same water temperature and hardness are within the same confidence limits, the variability of values indicated towards species specificity. It is therefore of utmost importance that LC50 values should be as far as possible determined for individual species.

Results indicated that water temperature may alter the lethality of copper to *C. gariepinus*, therefore copper toxicity appears to be enhanced by temperature as a stressor. Several studies have shown that as water temperature rises, waterborne toxicants become lethal to fish at lower concentrations. Heat stress decreased the LC50 values for bluegills exposed to zinc (Burton *et al.* 1972) and choline exposure on bluegills and rainbow trout (Bass *et al.* 1977). The difference in LC50 values due to changes in seasonal water temperature may occur due to a transient change in the metabolic rate of the fish. *Clarias gariepinus* possibly accumulated copper mainly through the gills as suspected in other Teleosts (Heath 1987). The volume of water passing through the gills could control the uptake rate of copper by the test organism. A water temperature of 21 ± 1°C will lead to a decrease in metabolic rate, consequently inducing longer survival by reducing ventilation rate and copper uptake. Similarly, metabolic acceleration due to heat may have shortened survival by accelerating copper accumulation. It has, however, been reported that these changes are not likely to have a large practical importance, for interspecies differences are much greater (Smith & Heath 1979).

Clarias gariepinus stayed at the bottom of the tank during stress caused by the toxicant, which forced it to use only the gills for oxy-

Table 6

Experimental data obtained during a toxicity test on adult C. gariepinus *and copper LC50 values at 28 ± 1 °C after different time intervals*

Concentration of copper % Cu^{2+} / volume	Number of test organisms	Number of test organisms dead after:							
		2 h	4h	6h	8h	24h	48h	72h	96h
2,263	10	0	0	0	2	4	7	9	10
1,772	10	0	0	0	1	4	5	7	8
1,263	10	0	0	0	0	3	5	6	6
1,071	10	0	0	0	0	2	3	5	5
0,930	10	0	0	0	0	1	1	1	2
0,617	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		–	–	–	–	2,38	1,87	1,48	1,29
95% Confidence limits for Cu^{2+} concentration		–	–	–	–	–	3,18	1,66	1,54
		–	–	–	–	–	1,56	1,20	1,06
Regression coefficient (%)		–	–	–	–	85,27	86,68	84,86	92,14
Slope of probit line		–	–	–	–	25,10	41,29	53,70	59,27

Table 7

Experimental data obtained during a toxicity test on juvenile C. gariepinus *and copper LC50 values at 28 ± 1 °C after different time intervals*

Concentration of copper % Cu^{2+} /volume	Number of test organisms	Number of test organisms dead after:							
		2 h	4h	6h	8h	24h	48h	72h	96h
3,326	10	0	0	0	0	4	7	7	10
1,810	10	0	0	0	0	3	5	7	8
1,398	10	0	0	0	0	3	4	6	6
1,167	10	0	0	0	0	2	2	4	4
0,991	10	0	0	0	0	0	1	1	2
0,790	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		–	–	–	–	3,62	2,28	1,68	1,30
95% Confidence limits for Cu^{2+} concentration		–	–	–	–	3,72	2,88	1,89	2,20
		–	–	–	–	3,52	1,88	1,21	0,40
Regression coefficient (%)		–	–	–	–	93,01	84,56	90,07	78,90
Slope of probit line		–	–	–	–	31,53	26,25	73,56	35,94

gen uptake. The veil-like mucous film, formed on the body and gills of dead fish after copper exposure, confirms the reason for death argued above. Various authors reported the mucous formation after metal exposure (Skidmore & Tovell 1972). Rapid mouth and operculum movements were also reported for the common guppy *P. reticulata*, for nickel and chromium exposure (Doudorff & Katz 1953). These findings suggest that the toxic mode of action of copper may be dependent on the toxic effect on the respiratory apparatus of the fish. Many pollutants, irrespective of type, may firstly cause damage to the gills, and this might have an effect on osmoregulation as well as respiration (Wong *et al.* 1977).

The 96-h LC50 values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specified conditions. Although the mean copper concentrations found in the water of the Olifants River in summer (0,055 ± 0,016 mg/l) and winter (0,085 ± 0,032 mg/l) are significantly lower than the predicted LC50 values, it still may exert a physiological affect on *C. gariepinus* at both temperatures, and can manifest itself in a change in blood chemistry. Adaptation to these concentrations does not necessarily reflect normality.

Despite increased sophistication in toxicity testing, increased awareness on the variety of processes operating in a natural system and the integration of information from a system of tests in various hazard evaluation protocols, the ultimate question of what is an acceptable concentration of a chemical with a known degree of accuracy is still unanswered. In the Olifants River, copper is one of several metals which pose a threat to the conservation status of the river. The LC50 values found during this study, can be used as an indication of the levels at which copper becomes lethal for this specific species. In a larger management-control program like the Kruger National Park, these results must only be seen as the limits within which the concentration of copper can be regarded as lethal for *C. gariepinus* in the Olifants River. Future research should focus on the development and validation of acceptable prediction strategies. In general, toxicants occur in mixtures in natural waters, therefore the interaction of toxicity is an important factor, which must be taken into account when assessing the hazard of an environmental pollutant to aquatic life and for setting valid water quality standards for diverse use. Additional studies on the effects of toxicants, singly and in mixtures, on biochemical and physiological processes are needed to gain more knowledge of their interactions and toxic effects.

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