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Comparative study on the susceptibility of freshwater species to copper-based pesticides

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Abstract

Copper compounds have been intentionally introduced into water bodies as aquatic plant herbicides, algicides and molluscicides. Copper-based fertilizers and fungicides have been widely used in agriculture as well. Despite the fact that copper is an essential element for all biota, elevated concentrations of this metal have been shown to affect a variety of aquatic organisms. Nonetheless, comparative studies on the susceptibility of different freshwater species to copper compounds have seldom been performed. This study was conducted to compare toxicity of copper-based pesticides (copper oxychloride, cuprous oxide and copper sulfate) to different freshwater target (*Raphidocelis subcapitata*, a planktonic alga and *Biomphalaria glabrata*, a snail) and non-target (*Daphnia similis*, a planktonic crustacean and *Danio rerio*, a fish) organisms. Test water parameters were as follows: pH = 7.4 ± 0.1; hardness 44 ± 1 mg/l as $CaCO_3$; DO 8–9 mg/l at the beginning and >4 mg/l at the end; temperature, fish and snails 25 ± 1 °C, *Daphnia* 20 ± 2 °C, algae 24 ± 1 °C. *D. similis* (immobilization), 48-h EC_{50}s (95% CLs) ranging from 0.013 (0.011–0.016) to 0.043 (0.033–0.057) mg Cu/l, and *R. subcapitata* (growth inhibition), 96-h IC50s from 0.071 (0.045–0.099) to 0.137 (0.090–0.174) mg Cu/l, were the most susceptible species. *B. glabrata* (lethality), 48-h LC_{50}s from 0.179 (0.102–0.270) to 0.854 (0.553–1.457) mg Cu/l, and *D. rerio* (lethality), 48-h LC_{50}s 0.063 (0.045–0.089), 0.192 (0.133–0.272) and 0.714 (0.494–1.016) mg Cu/l, were less susceptible than *Daphnia* to copper-based pesticides. Findings from the present study therefore suggest that increased levels of copper in water bodies is likely to adversely affect a variety of aquatic species.
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1. Introduction

Copper is an essential trace element for all biota. Essentiality of copper arises from its specific incorporation into a variety of enzymes which play important roles in physiological processes (e.g. enzymes involved in cellular respiration, free radical defense, neurotransmitter function, connective tissue biosynthesis and other functions), as well as into some structural proteins (Flemming and Trevors, 1989; WHO, 1998). Nonetheless, when organisms are excessively exposed, homeostatic control mechanisms become overwhelmed, and toxicity arises owing to copper adverse effects on the structure and function of macromolecules such as DNA and proteins (WHO, 1998).

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Natural sources of copper are manifold and this element seems to be relatively abundant (approximately 60 mg/kg) in the Earth's crust as copper oxide, copper sulfite and other ores (Flemming and Trevors, 1989; WHO, 1998). Anthropogenic sources of environmental contamination by copper include mining, smelting, foundries, municipal waste incinerators, burning of coal for power generation, and a variety of copper-based products used in building and construction, in electrical/electronic equipment and in other industries (Nor, 1987; Flemming and Trevors, 1989; WHO, 1998). Contamination of waters, sediments and soils by copper can also arise from the widespread use of copper-based products in agriculture as fertilizers and fungicides. It is of note that copper salts are also intentionally introduced into water bodies as aquatic plant herbicides, algicides and molluscicides. Copper sulfate, for instance, was employed as molluscicide in schistosomiasis control programs in Egypt and other countries (WHO, 1993).

Since copper from anthropogenic sources eventually contaminates water bodies, toxicity of this metal to aquatic organisms has been intensely studied over the past two decades (Nor, 1987; Flemming and Trevors, 1989; WHO, 1998). Comparative toxicity to different freshwater species, however, has been much less studied so far. For comparative purposes it is desirable to conduct toxicity assays with different species in parallel and at the same laboratory, because toxicity indices such as LC_{50} and EC_{50} are known to be subjected to a wide interlaboratory variability. Comparative toxicity studies are thus important to establish more precise margins of safety (magnitude of differences between toxic concentrations to target and to non-target species) when copper products are intended to be used as algicides, aquatic herbicides or molluscicides.

The present study was undertaken to compare the susceptibility of different freshwater target (planktonic green alga and a planorbidae snail) and non-target (a cladoceran and a fish) organisms to copper-based pesticides.

2. Material and methods

Copper sulfate ($CuSO_4$) (*Pro Analysis* grade, purity 99%) or 'blue copper' was obtained from Merck, Darmstadt. Copper oxychloride or 'green copper' (Cuprogarb 500®, 860 g/kg; 500 g of copper per kg), and cuprous oxide or 'brown copper' (Cobre Sandoz BR®, 560 g/kg, 500 g of copper per kg) were both purchased from a commercial supplier of agricultural chemicals.

Toxicity of copper-based pesticides to freshwater organisms was evaluated in assays with the planktonic freshwater green alga *Raphidocelis subcapitata* Korshikov (formerly known as *Selenastrum capricornutum* Printz), the microcrustacean *Daphnia similis*, the planorbidae snail *Biomphalaria glabrata* and the teleost fish *Danio* (*Brachydanio*) *rerio*.

2.1. Algal growth inhibition assay

A 96-h static growth inhibition test was conducted with *R. subcapitata* as described in a standardized protocol (ABNT, 1992). The algal growth medium (L.C. Oligo) contains a number of oligoelements—such as Fe, Zn, Mg, Ca, Mo, Cu, Mn and B—and its composition is described in the Brazilian (ABNT, 1992) as well as in the French standardized protocol (AFNOR, 1980). Briefly, assays were carried out in Erlenmeyer flasks (250 ml) containing algal assay medium (100 ml, pH 7.0 ± 0.1) inoculated with algae in log-phase growth (1.9×10^6 cells/ml). After being inoculated, culture flasks were kept in a shaking tray at 24 °C under fluorescent lamps (5000 lux). Copper-based pesticides were dissolved directly in the algal assay medium and tested concentrations (active principle) were as follows: 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 mg/l. Culture flasks were sampled at 0, 48 and 96 h and cell number was measured at each sampling time using a Neubauer chamber.

2.2. Toxicity test with microcrustaceans

A 48-h static toxicity assay was performed with *D. similis* as described in a standardized protocol (ABNT, 1993a). Copper-based pesticides were dissolved in the assay water (synthetic soft water, pH 7.2 ± 0.1, water hardness 40–48 mg/l as $CaCO_3$, dissolved oxygen 98%). All tests were carried out within an incubator at 20 °C and under a constant (16-h light/8-h dark) photoperiod. Fifteen daphnids (>6, <24 h old) per concentration (5 per 20 ml beaker containing 10 ml of assay water) were exposed to different concentrations (0, 0.01, 0.025, 0.5, 0.1, 0.25, 0.5 and 1.0 mg/l) of copper-based pesticides in the absence of food. The number of immobilized daphnids in each beaker was counted at 24 and 48 h of exposure and respective EC_{50} values were calculated.

2.3. Toxicity assay with snails

Forty eight-hours static acute tests with the snail *B. glabrata* (Mollusca; Gastropoda) were carried out in 2000 ml beakers containing synthetic softwater (pH 7.0 ± 0.1, water hardness 40–48 mg/l as $CaCO_3$). Twenty adult snails (shell diameter = 15–20 mm) per concentration, 10 per beaker, were exposed to the copper pesticides (0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/l) in the absence of food. Room temperature (25 ± 1 °C) and light/dark cycle (light on for 16 h) were kept constant. Snail lethality was evaluated at 24 and 48 h of exposure and respective LC_{50} values were determined.

2.4. Toxicity test with fish

Ninety six-hours static-renewal acute assays with zebrafish (*D. rerio*) were conducted as reported in details in a standardized protocol (ABNT, 1993b). Tests were performed in 3000 ml beakers containing synthetic softwater (pH 7.5 ± 0.1, water hardness 40–48 mg/l as $CaCO_3$), maintained at 25 ± 1 °C under a 16-h light/8-h dark cycle. Twenty fish were exposed (10 per beaker) to each concentration (0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 mg/l) of the tested substances. Testing solutions were replaced every 24 h, and fish lethality was recorded, and LC_{50} values calculated, at 24, 48, 72 and 96 h of exposure.

2.5. Statistical calculations

The LC_{50}/LC_{10} (lethality) and EC_{50}/EC_{10} (immobilization of *D. similis*) values and their 95% confidence limits were determined by probit analysis (Finney, 1971) using a computer software (USEPA Probit Analysis Program used for calculating EC values, version 1.5). IC_{50} (inhibition of algal growth) values were obtained by a linear interpolation method (Walsh et al., 1987, USEPA, 1994).

3. Results and discussion

Toxicity of copper to aquatic species depends, on one side, on the organism sensitivity and, on the other side, on the concentration of copper and its bioavailability. In natural settings, copper bioavailability is known to vary among water bodies depending on a variety of factors such as adsorption to particles, complexation by organic matter (e.g. humic and fulvic acids), presence of other cations and pH (Nor, 1987; WHO, 1998). Owing to this fact, copper bioavailability in a certain natural water body may be quite different from that in the standardized assay water and thus laboratory bioassay data should be used with caution by risk assessors.

The main objective of this study, nevertheless, was to compare the susceptibility of different freshwater organisms to copper-induced toxicity. It should be borne in mind that different toxicity indexes (LC_{50}; EC_{50}, IC_{50}) may result not only from susceptibilities of test species

Table 1
Toxicity of copper-based pesticides to freshwater planktonic green alga (*R. subcapitata*), water flea (*D. similis*), snail (*B. glabrata*) and zebrafish (*D. rerio*)

Species	Endpoint	Concentration (mg/l)		
		Copper oxychloride	Cuprous oxide	Copper sulfate
R. subcapitata	*Growth inhibition*			
	48-h IC_{50}	0.153 (0.116–0.169)	0.216 (0.122–0.270)	0.297 (0.216–0.384)
	48-h IC_{10}	0.090 (0.053–0.101)	0.037 (0.006–0.091)	0.046 (0.026–0.119)
	96-h IC_{50}	0.123 (0.093–0.159)	0.079 (0.053–0.110)	0.344 (0.207–0.439)
	96-h IC_{10}	0.015 (0.008–0.052)	0.010 (0.006–0.020)	0.046 (0.026–0.169)
D. similis	*Immobilization*			
	24-h EC_{50}	0.145 (0.101–0.207)	0.096 (0.033–0.238)	0.035 (0.030–0.042)
	24-h EC_{10}	0.038 (0.018–0.059)	0.028 (0.001–0.061)	0.026 (0.019–0.031)
	48-h EC_{50}	0.065 (0.050–0.088)	0.045 (0.030–0.064)	0.032 (0.026–0.039)
	48-h EC_{10}	0.030 (0.017–0.041)	0.012 (0.005–0.020)	0.022 (0.014–0.027)
B. glabrata	*Lethality*			
	24-h LC_{50}	2.862 (1.765–7.459)	1.389 (1.004–1.936)	1.868 (1.196–3.068)
	24-h LC_{10}	0.736 (0.196–1.237)	0.462 (0.237–0.680)	0.269 (0.091–0.488)
	48-h LC_{50}	1.433 (0.923–2.461)	0.201 (0.115–0.302)	0.477 (0.297–0.706)
	48-h LC_{10}	0.395 (0.140–0.651)	0.039 (0.010–0.077)	0.100 (0.032–0.181)
D. rerio	*Lethality*			
	24-h LC_{50}	4.741 (3.594–5.788)	1.881 (1.283–2.717)	0.349 (0.245–0.478)
	24-h LC_{50}	2.922 (1.411–3.782)	0.799 (0.318–1.193)	0.185 (0.074–0.259)
	48-h LC_{50}	1.198 (0.827–1.706)	0.213 (0.148–0.303)	0.158 (0.113–0.221)
	48-h LC_{10}	0.499 (0.240–0.738)	0.094 (0.040–0.138)	0.086 (0.041–0.119)
	72-h LC_{50}	0.257 (0.180–0.342)	0.106 (0.075–0.148)	0.134 (0.097–0.189)
	72-h LC_{10}	0.159 (0.056–0.212)	0.054 (0.025–0.076)	0.075 (0.037–0.102)
	96-h LC_{50}	0.152 (0.105–0.216)	0.083 (0.056–0.120)	0.094 (0.069–0.137)
	96-h LC_{10}	0.072 (0.033–0.105)	0.034 (0.014–0.052)	0.053 (0.022–0.072)

Data are presented as IC_{50}, EC_{50} or LC_{50} (mg of active ingredient/l) with, in brackets, their respective 95% confidence limits.

to copper, but also from different bioavailabilities of this metal due to variations of assay water composition. In the present study, however, except for the algal culture medium, compositions of all other assay waters were very similar. Under those circumstances it seems fair to assume that, in toxicity tests performed with *D. similis*, *B. glabrata* and *D. rerio*, copper bioavailabilities were similar as well. On the other hand, care should be taken in comparing *R. subcapitata* susceptibility to copper, expressed in terms of IC_{50}s, with that of the other three freshwater species, indicated by their respective $L(E)C_{50}$s (Tables 1 and 2). The algal culture medium (L.C. Oligo medium) contains metals such as iron, manganese and other that may have reduced copper bioavailability thereby increasing copper IC_{50}s or, in other words, decreasing its toxicity.

As shown in Tables 1 and 2, the cladoceran *D. similis* (48-h EC_{50}s ranging from 0.013 to 0.043 mg Cu/l) proved to be, among the four organisms tested, the most susceptible one to copper-induced toxicity. The second most susceptible species was the planktonic alga *R. subcapitata*. The growth of *R. subcapitata* was inhibited at concentrations (IC_{50}s) ranging from 0.091 to 0.195 mg Cu/l, and 0.071 to 0.137 mg Cu/l, after 48 and 96 h of exposure, respectively (Table 2). If copper is in fact less bioavailable in algal culture medium than in the assay soft water, this ranking of susceptibilities to copper would be somewhat different. In any case, *Daphnia* 48-h EC_{50} and planktonic green alga 48 and 96-h IC_{50} are very low and close to each other so that zooplankton crustaceae are likely to be drastically affected by algicidal concentrations of copper-based pesticides.

The planorbidae snail *B. glabrata* (48-h LC_{50}s from 0.179 to 0.854 mg Cu/l) was less sensitive to copper-produced toxicity than the first two species (Tables 1 and 2). The fish *D. rerio* (48-h LC_{50}s 0.063, 0.192 and 0.714 mg Cu/l), on the other hand, was somewhat more susceptible to copper-induced lethality than the mollusk *B. glabrata* at 48 h of exposure (Table 2). At longer exposure periods (96 h), however, copper-based pesticides proved to be almost as toxic to zebrafish (*D. rerio*: 96-h LC_{50}s 0.095, 0.075 and 0.038 mg Cu/l) as to green alga (*R. subcapitata*). It has been reported that bioavailability of copper from some copper-based herbicides/algicides (e.g. Clearigate and Cutrine-Plus) decreases over time following initial application into water so that, at very long periods (>7 days), toxic effects from exposures limited to the last 48 h are markedly less pronounced

Table 2
Toxicity of copper from copper-based pesticides to some freshwater species

Species	Endpoint	Concentration (mg Cu/l)		
		Copper oxychloride	Cuprous oxide	Copper sulfate
R. subcapitata	*Growth inhibition*			
	48-h IC_{50}	0.091 (0.072–0.102)	0.195 (0.110–0.230)	0.119 (0.082–0.167)
	48-h IC_{10}	0.054 (0.032–0.061)	0.033 (0.004–0.083)	0.018 (0.012–0.052)
	96-h IC_{50}	0.073 (0.049–0.086)	0.071 (0.045–0.099)	0.137 (0.090–0.174)
	96-h IC_{10}	0.009 (0.005–0.031)	0.009 (0.007–0.017)	0.018 (0.011–0.071)
D. similis	*Immobilization*			
	24-h EC_{50}	0.091 (0.064–0.128)	0.087 (0.033–0.205)	0.014 (0.012–0.017)
	24-h EC_{10}	0.025 (0.012–0.039)	0.026 (0.002–0.056)	0.011 (0.008–0.012)
	48-h EC_{50}	0.043 (0.033–0.057)	0.042 (0.028–0.059)	0.013 (0.011–0.016)
	48-h EC_{10}	0.021 (0.010–0.028)	0.012 (0.005–0.019)	0.009 (0.006–0.011)
B. glabrata	*Lethality*			
	24-h LC_{50}	1.703 (1.052–4.393)	1.241 (0.897–1.729)	0.747 (0.478–1.227)
	24-h LC_{10}	0.440 (0.121–0.737)	0.413 (0.213–0.608)	0.108 (0.036–0.195)
	48-h LC_{50}	0.854 (0.553–1.457)	0.179 (0.102–0.270)	0.191 (0.119–0.282)
	48-h LC_{10}	0.239 (0.087–0.391)	0.035 (0.009–0.068)	0.040 (0.013–0.072)
D. rerio	*Lethality*			
	24-h LC_{50}	2.822 (2.139–3.445)	1.679 (1.146–2.426)	0.140 (0.098–0.191)
	24-h LC_{10}	1.739 (0.840–2.251)	0.713 (0.285–1.065)	0.074 (0.030–0.103)
	48-h LC_{50}	0.714 (0.494–1.016)	0.192 (0.133–0.272)	0.063 (0.045–0.089)
	48-h LC_{10}	0.298 (0.145–0.440)	0.086 (0.035–0.126)	0.034 (0.017–0.047)
	72-h LC_{50}	0.157 (0.111–0.207)	0.095 (0.067–0.133)	0.053 (0.039–0.076)
	72-h LC_{10}	0.100 (0.034–0.131)	0.047 (0.023–0.066)	0.030 (0.015–0.041)
	96-h LC_{50}	0.095 (0.067–0.133)	0.075 (0.051–0.109)	0.038 (0.028–0.055)
	96-h LC_{10}	0.047 (0.023–0.066)	0.031 (0.013–0.047)	0.021 (0.009–0.029)

Data are presented as IC_{50}, EC_{50} or LC_{50} (mg of copper/l) with, in brackets, their respective 95% confidence limits.

than those that result from exposures limited to the first 48 h (Mastin and Rodgers, 2000). We do not know whether bioavailability of copper from the tested compounds was reduced—under our assay conditions (soft water)—over a 96-h exposure period. If so, toxicity indices at longer exposures (e.g. 96-h LC/EC_{50}s) could not be compared because the static/renewal exposure system, used in the fish assay, would enhance copper toxicity as compared to the static exposure system employed in assays with the green microalga.

The high vulnerability of daphnids to copper has been confirmed by different studies (Nor, 1987; WHO, 1998). In a recent summary of copper toxicity data on freshwater invertebrates, for instance, 48 h-E(L)C_{50}s (static/$CuSO_4$) as low as 0.007 mg/l (*Daphnia magna*) and 0.0134 mg/l (*Ceriodaphnia dubia*) can be found for daphnids (Oris et al., 1991; Oikari et al., 1992). We have found no literature data on copper toxicity to *D. similis*. It should also be pointed out, however, that copper sulfate 48-h LC_{50} reported by Ingersoll and Winner (1982) for *Daphnia pulex* (0.027 mg/l) is very close to that 48-h EC_{50} determined for *D. similis* (0.030 mg/l) in the present study (Table 1).

Growth inhibition of different species of freshwater algae have been noted at comparable concentrations of copper or copper salts in several studies. Copper sulfate, for instance, has been reported to inhibit (72-h IC_{50}, static) *S. capricornutum* (*R. subcapitata*) growth at concentrations as low as 0.047 mg/l, *Chlamydomonas reinhardii* at 0.079 mg/l and *Scenedesmus subspicatus* at 0.120 mg/l (WHO, 1998).

It should be pointed out that the extent to which the algal medium affected Cu bioavailability may have been different for the three copper-based pesticides tested. For *D. similis*, *B. glabrata* and *D. rerio*, in most instances, when median lethal or effective concentrations of copper-based pesticides were expressed in terms of copper content (Table 2), $CuSO_4$ yielded the lowest LC/EC_{50}s. Moreover, this difference of toxic potency between copper sulfate and the two other copper salts was apparently larger at shorter (24 h) than at longer (96 h) exposure periods (Table 2).

4. Conclusions

Toxicity data provided by the present study thus suggest that copper sulfate is more readily bioavailable in the assay soft water than the other two copper-based pesticides. In the alga growth inhibition test, on the other hand, 96-h IC_{50}—expressed in terms of Cu—for copper sulfate was slightly higher than those calculated for copper oxychloride and cuprous oxide (Table 2). Contrasting with the assay soft water, in the algal assay medium, bioavailability of copper from copper sulfate may have been lower than those of Cu derived from the two other copper-based pesticides.

In conclusion, toxicity data provided by this paper confirm that planktonic crustaceae and algae are extremely susceptible to increases in free copper levels in water bodies. Since phyto- and zooplanktonic organisms form the basis of aquatic food webs, increased levels of bioavailable copper is likely to dramatically affect freshwater ecosystems. Furthermore, our results also indicate that the use of copper compounds, either as algicides and aquatic plant herbicides, or as molluscicides, is likely to adversely affect a variety of non-target aquatic species.

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