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Comparative Susceptibility of Freshwater Fish Species to Metals from Ultramafic Soils

E.C. OLIVEIRA-FILHO^{1,2}, D.H.F. MUNIZ¹, I.S. FREIRE², F.G. AQUINO³ & L.R.M. ANDRADE³

¹Laboratory of Ecotoxicology, Embrapa Cerrados, Rod. BR020, Km 18, Planaltina, DF 73310-970, Brazil.

²Centro Universitário de Brasília, UniCEUB, SEPN 707/907, Asa Norte - Brasília - DF 70790-075,

³Laboratory of Plant Biology, Embrapa Cerrados, Rod. BR020, Km 18, Planaltina, DF 73310-970, Brazil.

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Abstract

Soils derived from ultramafic rocks have a mineralogy rich in metals like Co, Cr, Fe and Ni, and the process of extracting these metals from soil generates highly metallic waste. When disposed near of water bodies these residues pose a risk on the aquatic environment. Two fish species are used in ecotoxicity tests worldwide: *Danio rerio* (zebrafish), an Asian species, and *Oreochromis niloticus* (tilapia), an African one. However, *Hyphessobrycon eques* (mato grosso), a native of South America and found in Brazilian rivers, is as yet little used in ecotoxicity tests. The aims of this study were to evaluate the acute toxicity of the metals Co, Cr, Fe and Ni to the fish *D. rerio*, *O. niloticus* and *H. eques*, and compare its susceptibility. Tests were conducted following the recommendations of the Brazilian Association for Technical Standardization (ABNT). Stock solutions were prepared with the analytical reagents $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; $\text{K}_2\text{Cr}_2\text{O}_7$; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ e $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. The quantification of metals was carried out in the stock solutions by ICP-AES. The dilution water used was synthetic softwater pH 7.3 ± 0.1 , hardness 43 mg L^{-1} ; DO 95% and temperature $25 \pm 1^\circ\text{C}$. To perform static tests 10 fish from each species were exposed for 96 hours, in duplicate, to different concentrations of the metals tested. The measured 96-hr initial lethal concentrations $\text{LC}(I)_{50}$ of metals Co, Cr, Fe and Ni, calculated by the Trimmed Spearman Karber method, were 35.7 , 35.9 , 5.6 and 15.0 mg L^{-1} for *D. rerio*, 79.8 , 32.6 , 8.8 and 49.7 mg L^{-1} for *O. niloticus* and 104.1 , 23.7 , 8.5 and 34.5 mg L^{-1} for *H. eques*, respectively. The data showed that *D. rerio* has greater susceptibility to the metals tested, which justifies its increased use as a test species, except concerning Cr, for which *H. eques* was the most sensitive species.

Keywords: ecotoxicity; heavy metals; mining; cobalt; chromium; iron; nickel

Suscetibilidade Comparativa entre Espécies de Peixes de Água Doce a Metais de Solos Ultramáficos

Resumo

Os solos derivados de rochas ultramáficas tem uma mineralogia rica em metais como Co, Cr, Fe e Ni, e o processo de extração desses metais do solo gera um resíduo altamente metálico que quando disposto próximo a corpos hídricos pode apresentar risco aos ambientes aquáticos. Duas espécies de peixe exóticas são amplamente utilizadas em testes de ecotoxicidade *Danio rerio* (zebrafish) e *Oreochromis niloticus* (tilápia). Todavia, *Hyphessobrycon eques* (mato grosso), uma espécie da América do Sul e encontrada em rios brasileiros, é ainda pouco utilizada nesses ensaios. O objetivo desse estudo foi avaliar a toxicidade aguda dos metais Co, Cr, Fe e Ni para os peixes *D. rerio*, *O. niloticus* e *H. eques*, e comparar sua suscetibilidade. Os testes foram conduzidos de acordo com as normas da Associação Brasileira de Normas Técnicas. Soluções estoque foram preparadas com os reagentes analíticos $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; $\text{K}_2\text{Cr}_2\text{O}_7$; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ e $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. A quantificação dos metais foi realizada nas soluções estoque por análise em ICP-AES. A água de diluição foi a água mole pH 7.3 ± 0.1 , dureza 43 mg L^{-1} ; OD 95% e temperatura $25 \pm 1^\circ\text{C}$. Para realização dos testes estáticos 10 peixes de cada espécie foram expostos por 96 horas, em duplicata, às diferentes concentrações dos metais testados. As concentrações letais iniciais medidas $\text{CL}(I)_{50}$, em 96 horas, dos metais Co, Cr, Fe and Ni, calculadas pelo programa Trimmed Spearman Karber, foram $35,7$; $35,9$; $5,6$ e $15,0 \text{ mg L}^{-1}$ para *D. rerio*, $79,8$; $32,6$; $8,8$ and $49,7 \text{ mg L}^{-1}$ para *O. niloticus* e $104,1$; $23,7$; $8,5$ e $34,5 \text{ mg L}^{-1}$ para *H. eques*, respectivamente. *D. rerio* foi mais suscetível aos metais testados, o que justifica seu maior uso como espécie teste, exceto para o Cr, ao qual *H. eques* foi a espécie mais sensível.

Palavras-Chave: ecotoxicidade; metais; mineração; cobalto; cromo; ferro; níquel

INTRODUCTION

Metals are important contaminants of aquatic ecosystems and are widely used by humans in manufacturing processes and modern technology. The soils of the Barro Alto and Niquelândia Districts located in Goiás State (Brazil) originate in ultramafic serpentinized rocks. These rocks are primarily poor in N, K and P, and rich in metals, such as Cr, Ni, Fe and Co, which in high concentrations are potentially toxic to living species (Echevarria *et al.*, 2006; Garnier *et al.*, 2009). All regions worldwide that have this kind of soil have been explored by mining industries, and the processes that extract minerals from them generate waste products that have traces of these toxic elements in their final complex composition. This waste can release toxic compounds into ecosystems, mainly in water and soil, and it can affect species' survival and environmental quality, presenting potential risks to human health.

A number of studies show the effects of metals on aquatic invertebrates (Ravera 1977; Bellavere & Gorbi, 1981; Baral *et al.*, 2006; Deleebeeck *et al.*, 2007) and freshwater fish (Bellavere & Gorbi, 1981; Dave & Xiu, 1991; Velma *et al.*, 2009), but data showing a comparison between the acute data of chromium, cobalt, nickel and iron to freshwater fish, including exotic and Brazilian native species, are rare. It should be pointed that in Brazil, ecotoxicological assays have been performed with native or exotic species, but the exotic zebrafish *Danio rerio* predominates (Bertoletti, 2009). Considering this, the aims of this study were to provide scientific information on the acute toxicity of chromium, cobalt, nickel and iron to *Danio rerio*, *Oreochromis niloticus* and *Hyphessobrycon eques*, and to compare the susceptibility of these three species to tested metals.

MATERIAL AND METHODS

The fish species tested and their respective taxonomic families were the exotic Zebrafish (*Danio rerio*; Cyprinidae; Hamilton, 1822) and Nile tilapia (*Oreochromis niloticus*; Cichlidae; Linnaeus, 1758); and the native Mato-Grosso (*Hyphessobrycon eques*; Characidae; Steindachner, 1882). *D. rerio* and *H. eques* were purchased from a commercial supplier in Brasília, weighed 0.2 to 0.4 grams and had an average length of 2.0 to 3.0 cm. *O. niloticus* were obtained from the local municipality's fish farm in Brasília, where breeding conditions are controlled and monitored constantly, and had an average weight of 2.0 to 3.0 grams and average length of 4.0 to 6.0 cm. The fish were acclimatized to laboratory conditions for one week prior to testing.

Metal concentrations were obtained by serial dilutions of the reagents NiCl₂·6H₂O (Pro Analysis grade, purity 97% from Vetec, Brasil), FeCl₃·6H₂O (Pro Analysis grade, purity 97% from Vetec, Brasil), CoCl₂·6H₂O (Pro Analysis grade, purity 98% from Carlo Erba, Italy) and K₂Cr₂O₇ (Pro Analysis grade, purity 99% from Merck, Germany). Stock solutions of each reagent were prepared and after dilutions in test water final nominal concentrations were recalculated according to

the amount of respective metal. At this moment a sample of each concentration was taken to analyze the measured initial concentrations, determined in inductively coupled plasma optical emission spectroscopy (ICP-OES). To guarantee quality assurance and quality control of determinations, we also used blanks, duplicates, and spiked samples. Calibration coefficients were maintained at least 0.999 before proceeding with samples.

Static acute assays lasting 96 hours were carried out as standardized by the Brazilian Association for Technical Standardization (ABNT, 2004). Tests were performed in 3000 mL beakers with synthetic soft water (pH 7.3 ± 0.1, hardness 43 mg L⁻¹ as CaCO₃), maintained at 25 ± 1°C under a 16-h light/8-h dark cycle and without food. Twenty fish were exposed (10 per beaker) to each concentration and mortality was evaluated after 48 and 96 hours of exposure, and dead fish were removed daily. Negative control (synthetic soft water) beakers were included.

The Initial Lethal Concentrations to 50% of the Fish - LC(I)₅₀ values and their 95% confidence limits, after 48 and 96 hours of exposure, were determined by the Trimmed Spearman Karber method (Hamilton *et al.*, 1977) for reagents, metals' nominal concentrations and metals' measured concentrations.

RESULTS

The main objective of this study was to compare the susceptibility of different freshwater fish to metals present in ultramafic soils. As expected, values of lethal concentrations to 50% of tested fish were higher for reagents than for metals in nominal or measured concentrations.

Table 1 presents the toxicity of metallic reagents tested with fish species. Although these data do not represent the metals' toxicity, several papers publish results using these metallic compounds as reference, providing vital information on the susceptibility of fish to standard chemicals and support for inter and intra-laboratorial studies.

Table 2 shows the toxicity of metals in nominal and measured concentrations to tested fish species. It should be noted that there is a considerable difference between these two values, and this can explain the numerous discrepancies in acute toxicity data already published. However, it is important to observe that in almost all cases the obtained values to Nominal LC₅₀s are inserted in the confidence intervals of Measured LC₅₀s.

As shown in Tables 1 and 2, *Danio rerio* was the most susceptible species, except regarding chromium, where *Hyphessobrycon eques* ranks higher. On the other hand, *Oreochromis niloticus* was generally the most resistant species, except for chromium, to which *Danio rerio* was the most one.

DISCUSSION

The obtained data indicate that *H. eques*, a native species, can be appropriate for investigating toxicity of water bodies

Table 1 - Lethal concentrations 50% of metallic reagents in mg L⁻¹ to tested species after 48 and 96 hours of exposure.

Reagents	Exposure	Fish Species		
		D. rerio	H. eques	O. niloticus
CoCl ₂ ·6H ₂ O	48-hr	282.8 (211.1-378.8)	692.8 (443.7-1081.9)	1345.4 (745.7-2427.6)
	96-hr	186.6 (118.3-294.3)	544.5 (353.9-837.7)	475.7 (263.3-858.3)
K ₂ Cr ₂ O ₇	48-hr	162.4 (109.8-240.4)	123.1 (96.1-157.8)	141.4 (99.6-200.8)
	96-hr	123.1 (83.2-182.2)	93.3 (68.9-126.4)	107.2 (72.4-158.6)
FeCl ₃ ·6H ₂ O	48-hr	40.6 (31.7-52.0)	56.7 (49.5-65.0)	66.1 (60.8-71.9)
	96-hr	29.7 (16.5-53.6)	45.1 (39.4-51.7)	57.8 (49.5-67.5)
NiCl ₂ ·6H ₂ O	48-hr	107.2 (79.1-145.2)	214.3 (158.2-290.4)	649.8 (507.1-832.7)
	96-hr	81.2 (63.4-104.1)	162.4 (126.8-208.1)	237.8 (131.8-429.1)

in areas near ultramafic soils, since a recent study (Oze *et al.*, 2007) showed that, in these areas, the generation of aqueous Cr (VI) exceeds its retention in solids or its reduction to Cr (III). Furthermore, Cruz *et al.* (2008) also observed that *H. eques* was the most sensitive to potassium dichromate among three different Brazilian fish species.

For the toxicity between metals, iron was the most toxic and cobalt was the least toxic for all species. Chromium was the second most toxic, except to *D. rerio*, where nickel showed highest toxicity.

In comparison with other studies, Bertoletti (2009) obtained a nominal 96-hr LC₅₀ of hexavalent chromium to *D. rerio* of 31.8 mg L⁻¹ in softwater and Bellavere & Gorbi (1981) a nominal 96-hr LC₅₀ of 58.5 mg L⁻¹ in hardwater. These results are very similar to the LC₅₀ 96h calculated in the present study for nominal = 49.9 mg L⁻¹ and measured = 35.9 mg L⁻¹ concentrations of chromium (Table 2). As a reference chemical, the toxicity of potassium dichromate was tested with *H. eques* by Cruz *et al.* (2008). These authors found a LC(I)₅₀ of 130.8 mg L⁻¹, after 96 hours of exposure, and in the present study the 96-hr LC(I)₅₀ and 95% confidence limits of this chemical to *H. eques* was 93.3 mg L⁻¹ (68.9-126.4). In a study with the guppy *Poecilia reticulata* in hardwater Khangarot & Ray (1990) found a LC₅₀ after 96 hours of 29.28 mg Cr L⁻¹. For *O. niloticus*, Masutti *et al.* (2006) reported a median 96-hr LC₅₀ of Cr based on potassium dichromate of 133.5 mg L⁻¹, a result similar to the 107.2 mg L⁻¹ (72.4-156.6) obtained in the present study.

Regarding the toxicity of metallic chromium, the values shown in Table 2 are consistent with those obtained by Svecovicus (2006), who observed 96-hr LC50s between 28.5 and 49.3 mg Cr L⁻¹ for four European fish species. It should be noted that this European study was carried out in hardwater, low temperature and flow-through system exposure, parameters that can significantly interfere in the comparisons. With

Table 2 - Lethal concentrations 50% (nominal and measured) of metals in mg L⁻¹ to tested species after 48 and 96 hours of exposure.

		Fish Species					
Metals	Time	<i>D. rerio</i>		<i>H. eques</i>		<i>O. niloticus</i>	
		Nominal	Measured	Nominal	Measured	Nominal	Measured
Co	48-hr	71.2 (53.2-95.4)	54.1 (40.4-72.5)	174.51 (111.7-272.5)	132.5 (84.9-206.9)	333.2 (184.7-601.3)	287.7 (159.5-519.2)
	96-hr	47.0 (29.8-74.1)	35.7 (22.6-56.3)	137.1 (89.1-211.0)	104.1 (67.7-160.2)	92.4 (68.2-125.2)	79.8 (58.9-108.1)
Cr	48-hr	57.4 (38.8-85.0)	41.3 (27.9-61.1)	43.5 (34.0-55.8)	31.3 (24.4-40.1)	50.0 (35.2-71.0)	43.0 (30.3-61.0)
	96-hr	49.9 (35.1-70.9)	35.9 (25.3-51.0)	33.0 (24.3-44.7)	23.7 (17.5-32.1)	37.9 (25.6-56.1)	32.6 (22.0-48.2)
Fe	48-hr	8.4 (6.5-10.7)	7.6 (5.9-9.7)	11.7 (10.2-13.4)	10.7 (9.3-12.2)	13.7 (12.6-14.9)	10.1 (9.2-10.9)
	96-hr	6.1 (3.4-11.1)	5.6 (3.1-10.0)	9.3 (8.1-10.7)	8.5 (7.4-9.7)	12.0 (10.2-14.0)	8.8 (7.5-10.3)
Ni	48-hr	26.5 (19.5-35.9)	22.8 (16.8-30.8)	52.9 (39.1-71.7)	45.5 (39.6-61.7)	121.6 (94.9-155.8)	114.2 (89.2-146.4)
	96-hr	20.0 (15.6-25.7)	15.0 (11.2-20.1)	40.1 (31.3-51.4)	34.5 (26.9-44.2)	52.9 (39.1-71.7)	49.7 (36.7-67.4)

respect to iron, few studies have reported its acute toxicity to fish. Dalzell & MacFarlane (1999) found a 96-hr LC₅₀ of 28 mg Fe L⁻¹ to brown trout weighing 15 to 30 grams. This result is also similar to those obtained in the present research with smaller fish (Table 2). Other studies have focused on the use of FeCl₃.6H₂O to purify wastewater samples, testing water samples, effluents or sludge from treatment plants. Sotero-Santos *et al.* (2007) observed non acute effects of ferric chloride sludge on *H. eques*. It should be noted that in these samples ferric salt is added for removal of phosphate ions and other organic matter. After complexation with these compounds and FeCl₃ precipitation of larger particles occurs, modifying the metal bioavailability and protecting aquatic species from acute toxicity (Randall *et al.* 1999; Weltens *et al.* 2000; Van Anholt *et al.* 2002).

Nickel was the third most toxic of the metals tested. The 96-hr measured LC(I)₅₀ to *D. rerio* was 15.0 mg L⁻¹ (Table 1) and this is consistent with results from previous studies with fish. Although working with hardwater, Khangarot & Ray (1990) found a similar result with *Poecilia reticulata*. These authors calculated a LC₅₀ in 96 hours of 29.28 mg Ni L⁻¹. For adults of *Pimephales promelas* in softwater (48 mg L⁻¹) Hoang *et al.* (2004) found a 96 hr-LC₅₀ of 8.4 mg L⁻¹ and Pickering (1974) reported a nominal 96 hr-LC₅₀ for this species ranging from 27 to 32 mg L⁻¹ of nickel, in hardwater. Brix *et al.* (2004) observed a 96 hr-LC₅₀ of 20.8 mg L⁻¹ to rainbow trout in hardwater (hardness 91 mg L⁻¹), Nebeker *et al.* (1985) estimated a 96-hr LC₅₀ of 10.0 mg L⁻¹ in softwater (hardness 33 mg L⁻¹) and Pane *et al.* (2003) calculated a 96-hr LC₅₀ of 15.3 mg L⁻¹ at a water hardness of 140 mg L⁻¹.

About the acute effects of cobalt, its low toxicity in softwater has been described by Diamond *et al.* (1992), who found that the LC₅₀ values for fathead minnows could not be calculated due to their low susceptibility to high concentrations of cobalt. In this experiment the authors defined the lethal concentration to 50% as being higher than 5.8 mg L⁻¹. This effect was also reported by Dave & Xiu (1991), who observed no mortality of embryos and larvae of *D. rerio* exposed for 9 days at concentrations up to 15.3 mg Co L⁻¹. Both of these studies reinforce the low toxicity of cobalt to freshwater fish, as observed in the present study, when compared with the other metals investigated.

The toxic effects of metals and metallic compounds depend on the rate and extent to which metals or compounds are transformed into a bioavailable form (Muniz & Oliveira-Filho, 2006). In natural waters several factors can interfere with the toxicity of metals, including temperature, pH, dissolved salts and complexation with organic matter (Sprague, 1995). Thus, the bioavailability of metals in natural water bodies may be different from that observed in standardized assay water and so any extrapolation of data should be done with caution in environmental management policies.

The acute toxicity results from the present investigation were generally consistent with those of previous acute studies involving fish species. The higher toxicity of iron and the lower toxicity of cobalt were the main data obtained. The

comparison between the values of nominal and measured LC₅₀ showed a significant relationship, because in almost all cases the nominal values could be inserted into the 95% confidence intervals of measured values.

CONCLUSIONS

Danio rerio was the most susceptible species except to chromium, and this information reinforces data from other authors and guidelines that recommend this fish for use in Brazilian ecotoxicity assays. *Hyphessobrycon eques* has been demonstrated to be the most sensitive to chromium, and its application to research in areas with ultramafic soils could be a promising alternative. On the other hand, *Oreochromis niloticus*, an indigenous and introduced species in Brazil, was the least acutely susceptible to the metals tested, but its size is better suited to tests involving biomarkers or the investigation of physiological effects. Finally, the results of this study indicate that the presence of these metals in aquatic ecosystems can significantly interfere with the distribution of species, mainly due to their high lethality to fish.

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