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Solubility, toxicity, and accumulation of metals from biotite schist rock in *Danio rerio* fish

Abstract – The objective of this work was to determine the solubility of biotite schist rock powder by different methods, as well as the potential toxicity/accumulation of its metals in *Danio rerio* freshwater fish. Solubility was tested in ultrapure water and in $CaCl_2$ solution (10 mmol L^{-1}). Metal contents were analyzed by inductively coupled plasma optical emission spectroscopy. Fish were separated into different groups and then exposed to biotite schist powder. The solubility of toxic elements in water is not lethal, and, 30 days after the end of exposure, there is no accumulation of metals in the fish.

Index terms: bioaccumulation, bioavailability, ecotoxicology, fertilizers.

Solubilidade, toxicidade e acumulação de metais da rocha biotita xisto no peixe *Danio rerio*

Resumo – O objetivo deste trabalho foi determinar a solubilidade do pó da rocha biotita xisto por diferentes métodos, bem como a potencial toxicidade/acumulação dos seus metais no peixe dulciaquícola *Danio rerio*. A solubilidade foi testada em água ultrapura e em solução de $CaCl_2$ (10 mmol L^{-1}). Analisou-se a presença de metais por espectrometria de emissão óptica por plasma acoplado indutivamente. Os peixes foram separados em diferentes grupos e expostos ao pó da biotita xisto. A solubilidade dos elementos tóxicos na água não é letal, e, 30 dias após o término da exposição, não há acumulação de metais nos peixes.

Termos para indexação: bioacumulação, biodisponibilidade, ecotoxicologia, fertilizantes.

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The Brazilian territory consists, in the most part, of acidic soils, poor in nutrients such as potassium. To make production feasible, it is necessary to use high quantities of fertilizers, which account for about 40% of production costs. Therefore, economic alternatives to traditional fertilizers are needed. Brazil imports most of the K fertilizer used for agriculture, mainly in the form of KCl (Resende et al., 2006). In 2013, the country ranked eleventh among world producers, with a production of 492,100,000 tons of KCl in the same year, corresponding to 311,000 tons of K_2O equivalent (Oliveira, 2014).

Results obtained in previous studies are indicative that rocks containing reasonable amounts of phlogopite or biotite may be alternative K sources for agricultural use. Some of the rocks with potential for agronomic use include alkaline magmatic rocks (e.g. carbonatites, kimberlites, kamafugites, and syenites) and potassic metamorphic rocks and minerals (e.g. biotite schist and phlogopite schist), with a wide and variable distribution throughout the Brazilian territory. The prospection, geochemical characterization, definition of processing methods, and systematic agronomic evaluation of these rocks could lead to the identification of efficient alternative sources of nutrients, mainly K, giving rise to a new group of agricultural inputs (Martins et al., 2008). Souza et al. (2016) observed a potentiation of the effect of conventional sources of nutrients after the use of biotite schist in cassava (*Manihot esculenta* Crantz) crops.

However, there is concern regarding the use of rocks because some elements that are part of their composition can be hazardous to the environment of agricultural production. The harmful effect of these undesirable chemicals depends, basically, on their accumulated quantity and in the chemical forms they appear in the rock and later in the soil. The occurrence of heavy metals in soluble, exchangeable, occluded, precipitated, or complexed forms will define their toxicity potential and impact on soil quality and water, as well on plants and other organisms (Caporale & Violante, 2016). As happens with commercial fertilizers, rock powders and the chemical elements released by them can also reach the aquatic environment through runoff or leaching, causing impacts ranging from nutrient enrichment/eutrophication (Foereid, 2017; Huang et al., 2017) to toxicity to aquatic species (Simplicio et al., 2017). In general, heavy metals are the most toxic elements in fertilizers (Gupta et al., 2014). Metals, because of their low solubility, tend to accumulate in the sediment, where they can be mobilized through physical and chemical factors by several components of the trophic chain, resulting in the process of bioaccumulation (Tchounwou et al., 2012). This shows the importance of evaluating how metals may affect different species.

The objective of this work was to determine the solubility of biotite schist rock powder by different methods, as well as the potential toxicity/accumulation of its metals in *Danio rerio* (Hamilton, 1822) freshwater fish.

The biotite schist rock powder used in the present study originates from an overburdened and unregulated emerald mine (Martins et al., 2008), located in the municipality of Nova Era, in the state of Minas Gerais, Brazil (19°42'00"S, 43°02'30"W). The used rock is a biotite schist composed mainly of biotite (35%), chlorite (20%), amphibole (5%), quartz (20%), K-feldspar (10%), and carbonate (5%). The granulometry of the used rock powder was as follows: > 2.00 mm (0.1%), < 2.00 mm and > 0.84 mm (1.9%), < 0.84 mm and > 0.297 mm (50 %), and < 0.297 mm (48%). The percentages of oxides present in the rock powder, in ascending order, were: 52.74% SiO_2 > 13.91% MgO > 10.45% Al_2O_3 > 10.15% Fe_2O_3 > 5.27% CaO > 2.07% K_2O > 0.22% Cr_2O_3 > 0.18% MnO > 0.06% P_2O_5. The contents of the quantified chemical elements were: 151 ppm for Zn, 142 ppm for Ba, 93.3 ppm for Ni, 53.9 ppm for Cu, and 49.4 ppm for Co. However, concentrations lower than 20 ppm were also registered: 15.9 ppm for Sr, 5.8 ppm for Mo, 10.3 ppm for Pb, and <0.1 ppm for Cd.

In order to determine the potential risk of biotite schist powder to the aquatic environment, tests were carried out to evaluate their solubility and the release of metals in the water. To simulate a leaching process, the powder samples were shaken in two different solutions – ultrapure water and calcium chloride. These solvents have different chemical properties and are used to model processes that occur naturally in the soil. Ultrapure water, which is a solubilizing agent that extracts the hydrophilic elements that are easily released into the environment, was used to simulate rainwater action on biotite schist. The solution of $CaCl_2$ (10 mmol L^{-1}) was used to evaluate the exchangeable contents of the metals present in the rock, simulating the solvent action of the soil solution. As in Houba et al. (2000), this solution has about the same ionic strength as the average salt concentration in many natural soil solutions.

For the solubility tests, 2.0 g rock powder were added to 20 mL ultrapure water or to 20 mL $CaCl_2$ solution, at a ratio of 1:10. The samples were placed on a shaker for 48 hours and then centrifuged at 22221 RCF for 15 min, in order to separate the precipitate from the supernatant. Finally, the supernatant was filtered on a 0.45 µm membrane to obtain dissolved particles. This method has been standardized at the Water Chemistry Laboratory of Embrapa Cerrados, aiming to determine the bioavailability of metals in potential aquatic contaminants (Oliveira-Filho et al., 2013a, 2016), as adapted from Tüzen (2003). For the determination of metal contents, the extracts of the two processes were analyzed in triplicate by inductively coupled plasma optical emission spectroscopy (ICP-OES), using the iCAP 6000 Series spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and following the method of U.S. Environmental Protection Agency (US EPA, 2014).

To simulate water contamination with biotite schist, 700 g rock powder were mixed in 2,800 mL synthetic soft water with a hardness of 40 mg L^{-1} in $CaCO_3$, according to Associação Brasileira de Normas Técnicas (ABNT, 2016), at a ratio of 1:4 (powder:water), based on an adaptation of the method for sediments reviewed by Burton Jr. (1991). Three groups of 13 adult *D. rerio* fish, commonly known as zebrafish, were exposed, in duplicate, to dilution water (control groups 1, 3, and 5), whereas another three groups of 13 fish were exposed, also in duplicate, to the rock powder for 96 hours under constant aeration in different beakers (groups 2, 4, and 6). The exposure of fish was performed in a semi-static system with a water temperature maintained at 25±1°C, a photoperiod of 12 light hours and 12 dark hours, and pH 7.4±0.2 (ABNT, 2016). After the initial 96 hours of exposure time, fish from groups 1 and 2 were removed for the chemical analysis. Fish from groups 4 and 6, after initial exposure, were transferred to clean synthetic soft water and maintained there for 15 and 30 days, respectively. At the end of 19 days, the fish from groups 3 and 4 were removed for the chemical analysis, and, at the end of 34 days, those from groups 5 and 6 were removed. Figure 1 shows the test scheme used during the experiment.

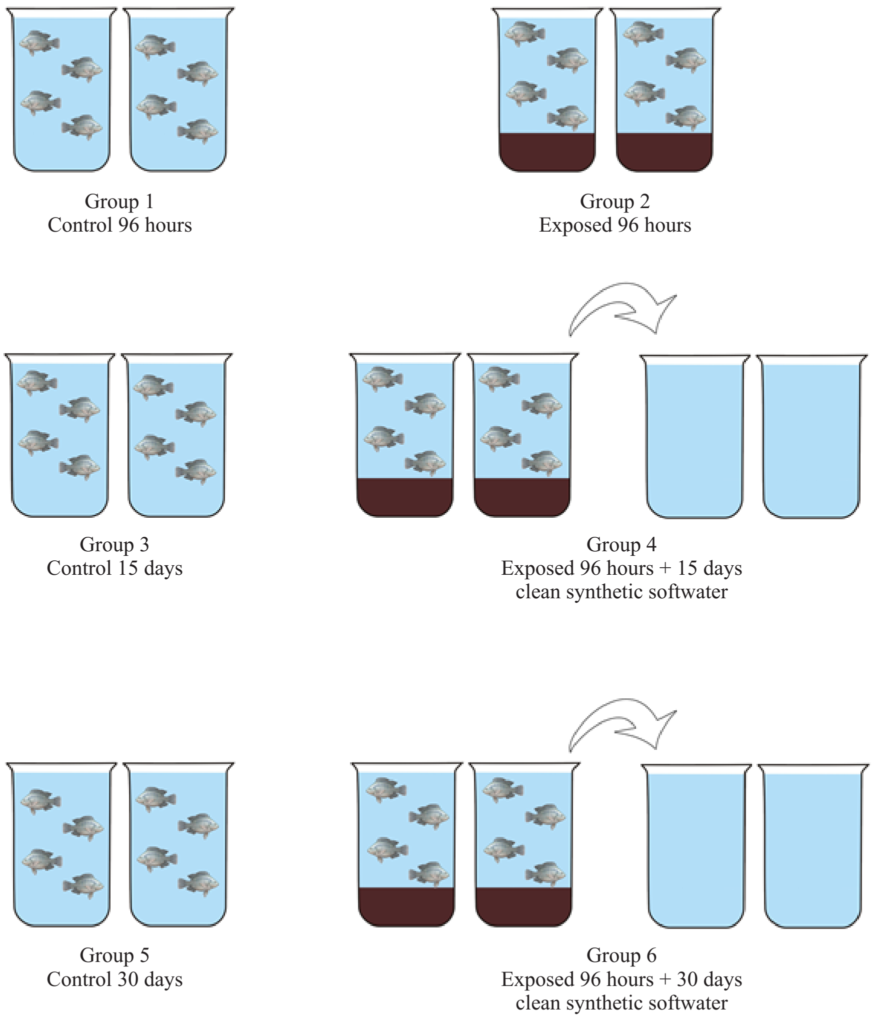

Figure 1. Test scheme developed to evaluate *Danio rerio* freshwater fish during the experiment with biotite schist rock powder.

The assays with zebrafish were approved by the animal use commission of Centro Universitário de Brasília (number 003/15), on March 19, 2015.

For the chemical analysis, the fish were euthanized by freezing in a refrigerator and then oven-dried, at 110ºC, for 24 hours. The material was macerated, and 1.0 g was selected from each fish group and then placed in a muffle oven at 500°C, for 4 hours, to obtain ashes. This residue was digested in 5.0 mL of 25% v/v nitric acid and, when necessary, the mixture was warmed slowly to dissolve the ashes. The solution was diluted to 25 mL in a volumetric flask with ultrapure water. The metal contents in each fish group were quantified by ICP-OES. All presented results represent the means of the replicates analyzed in each determination.

The most solubilized chemical elements in ultrapure water were K (69.4 mg L^{-1}), Cr (0.88 mg L^{-1}), Mn (0.26 mg L^{-1}), and Sr (0.13 mg L^{-1}). Other elements – Co, Ni, and Zn – were not soluble, indicating that they are not easily released from the studied rock into the environment.

Stirring with $CaCl_2$ solution extracted 254 mg L^{-1} K, 1.09 mg L^1 Cr, 0.36 mg L^{-1} Mn, 0.35 mg L^{-1} Ni, and 2.18 mg L^{-1} Sr, which are larger amounts than those found in ultrapure water. This is due to the fact that saline solution extracts exchangeable elements. The $CaCl_2$ solution mobilizes the elements, which are more easily removed from the structure of the rock mineral components. In addition, during the 96 hours of fish exposure, no mortality was observed in the performed dilution, showing that the biotite schist powder did not cause acute toxicity to fish after this time of exposure. Indeed, according to Simplicio et al. (2017), fertilizers and their active ingredients show a relative low toxicity to aquatic species. For zebrafish, the authors found a lethal concentration for 50% of individuals (LC_{50}) of 96 hours of K, equivalent to 509.65 to 993.65 mg L^{-1}.

Table 1 presents the amounts of metals accumulated by the fish when exposed to biotite schist powder. After 96 hours of exposure, higher amounts of Al, Cr, Cu, Fe, K, and Zn were found in fish bodies, in comparison with the control group (water without

Table 1. Amount of metals accumulated by the freshwater fish *Danio rerio* after separated into different groups and exposed to biotite schist rock powder(1).

Group		Al	Co	Cr	Cu	Fe	K	Mn	Ni	Sr	Zn
		(mg kg^{-1})									
Control 96 hours	Mean	43.25	0.26	12.97	3.41	60.90	7700	28.92	1.33	100.8	326.6
	SD	1.21	0.11	1.48	0.24	2.75	111.5	1.19	0.11	1.13	8.53
	CV	0.03	0.37	0.12	0.08	0.04	0.01	0.04	0.08	0.01	0.03
Control 15 days	Mean	42.53	0.21	11.16	2.40	65.90	8700	27.72	1.01	115.7	288.2
	SD	1.61	0.04	1.04	0.37	4.21	150.4	3.74	0.13	8.20	3.61
	CV	0.04	0.17	0.09	0.16	0.07	0.02	0.15	0.13	0.07	0.01
Control 30 days	Mean	46.04	0.22	14.77	3.14	62.95	7300	26.02	0.97	119.1	306.4
	SD	2.20	0.02	0.44	0.15	2.17	183.9	2.34	0.16	3.91	4.02
	CV	0.05	0.09	0.03	0.05	0.04	0.03	0.10	0.15	0.03	0.01
96 hours exposed	Mean	229.3	0.17	23.05	7.49	215.3	17750	23.33	1.23	112.9	550.5
	SD	4.95	0.01	1.21	0.30	8.83	758.3	1.53	0.16	2.41	15.09
	CV	0.02	0.08	0.06	0.04	0.04	0.08	0.07	0.15	0.03	0.03
15 days after exposure in clean water	Mean	58.70	0.23	15.71	5.39	95.25	8350	22.49	1.38	106.8	318.1
	SD	1.24	0.02	1.02	0.31	2.62	199.8	1.58	0.14	2.16	9.15
	CV	0.02	0.07	0.07	0.06	0.03	0.02	0.07	0.11	0.02	0.03
30 days after exposure in clean water	Mean	36.01	0.17	13.86	3.16	63.55	7500	20.98	0.81	90.73	314.0
	SD	1.24	0.02	0.18	0.11	1.64	187.36	1.09	0.06	1.17	2.85
	CV	0.04	0.08	0.01	0.03	0.03	0.03	0.05	0.07	0.01	0.01

(1)SD, standard deviation; and CV, coefficient of variation.

addition of rock powder). The group that remained in clean synthetic soft water for 15 days after exposure showed a considerable reduction in these elements in their body, but the observed values were higher than those of the control group. However, the group that remained in clean synthetic soft water for 30 days after exposure presented levels of all metals similar to those of the control group, evidencing the total elimination of the ingested material from their bodies.

Although the solubility data from biotite schist in ultrapure water indicated the presence of K, Cr, Mn, and Sr, no fish mortality was observed. This can be explained by the fact that the concentration detected in the aqueous extract is lower than that required to cause damage to organisms. Oliveira-Filho et al. (2013b) reported a LC_{50} of 35.9 mg L^{-1} of Cr for zebrafish after 96 hours of exposure, a value greater than that found in the extracts of ultrapure water and $CaCl_2$ solutions.

Regarding the metal contents detected in the whole body of the fish, the results suggest that the observed levels could be related to the possible ingestion of the rock itself and also that, after 30 days, the fish possibly excreted all the material consumed during exposure, which allowed the amounts of metals in their body to return to normal. Therefore, the metabolism of the fish was not able to release and assimilate the metals present in the mineral fractions of biotite schist, which were not accumulated in their bodies. Oliveira-Filho et al. (2010, 2013a) reported absence of acute toxicity, cytotoxicity, genotoxicity, and bioavailability of metals when exposing Nile tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] to Ni mining residue containing high amounts of metals. In this last case, a higher concentration of metals was detected in the fish only when mineral residues were found in their digestive tract.

Based on the obtained results, it can be concluded that the solubility of the potentially toxic elements present in the biotite schist powder is not enough to be lethal to zebrafish, and that, 30 days after the end of the exposure, there is no accumulation of metals in the whole body of the fish, suggesting a low bioavailability of the metals in the rock powder. However, the absence of lethality to the tested fish is not enough to guarantee safety to aquatic ecosystems, especially regarding the potential chronic toxicity to the most susceptible organisms, mainly invertebrate species.

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