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Evaluation of Acute Toxicity, Cytotoxicity and Genotoxicity of a Nickel Mining Waste to *Oreochromis niloticus*

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Abstract The pyrometallurgical process of mining for obtaining ferronickel involves a stage of calcinations. At this stage a residue is generated described as a calcination dust of fine black grains. Analysis of this material revealed a significant presence of Fe, around 53,000 ppm and Ni, around 14,000, beyond of other metals as Al, Mn, and Cr. Adults and larvae of *Oreochromis niloticus* were used to evaluate acute toxicity, cytotoxicity and genotoxicity, and histopathological effects. The data obtained show absence of toxicity in concentrations of 5, 10 and 50% but a considerable potential for bioaccumulation in the fish's body.

Keywords Nickel mining · Ecotoxicity · Fish · Industrial waste · Aquatic toxicity

Metals are naturally redistributed in the environment by geological cycles as well as by anthropogenic activities. The biological cycles of metals include bioconcentration by plants and animals and incorporation into food webs. In the organisms, some metals form complexes with proteins, others destroy sulfhydryl proteins that comprise important structural tissue components (Goyer 1986). Mining on an industrial scale is an activity that has impacts on the environment, because mining operations and their waste-disposal methods are considered one of the main sources of environmental degradation (McKinnon 2002). The toxicity of this waste is difficult to generalize due to the wide mixture of chemicals. A great number of metals and other highly toxic chemicals have been characterized as significant waste in the refinement process of several metals of commercial importance (Lottermoser 2007). Wastes from nickel mining present a complex mixture of different metals. The world's nickel production amounts to around 1.5 million ton/year, and Brazil ranks seventh in the list of largest producers, processing, in 2007, 37,000 tons (BNDES 2008). Nickel is produced by different extraction methods. The pyrometallurgical process for obtaining ferronickel involves a stage of calcination where the ore is charred in ovens at 1,000°C (Gupta 2002). At this stage a residue described as a calcination dust of fine black granulated grains is generated. Qualitative and quantitative descriptions of the main components of this material are important to know the risk that this material represents for underground water and streams near the area. Our previous studies have revealed that *Oreochromis niloticus* is a suitable fish species for use as a bioindicator of water contaminants (Palhares and Grisolia 2002; Grisolia et al. 2009). Although several papers show the effects of metals on fish species (Shukla and Pandey 1985; Brix et al. 2004) in the environment, fish are not exposed to a single metal but to a mixture of different contaminants, and so the objectives of the present study were to characterize the composition of this material and investigate the toxic and genotoxic effects of the calcination dust from nickel mining on *Oreochromis niloticus* adults and larvae through body exposure.

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Materials and Methods

Determination of quali-quantitative composition of the residues was carried out according to the International Organization for Standardization – ISO. Total contents of elements were evaluated in the waste after extraction with aqua regia plus HCl and HNO_3 (v/v 1:3) in inductively coupled plasma optical emission spectroscopy (ICP-OES), Thermo Jarrel Ash, model IRIS AP. To guarantee quality assurance and quality control of determinations, all reagents and standards were of analytical grade (Merck, Darmstadt, Germany). Blanks, duplicates, and spiked samples were used too. Calibration coefficients were maintained at least three 9 s before proceeding with samples ($r = 0.999$).

Calcination dust was mixed with synthetic soft water in concentrations of 50 g/1,000 mL (5%), 100 g/1,000 mL (10%) and 500 g/1,000 mL (50%) using a shaker, for 24 h. After this the material was maintained in decantation for 30 min, before introducing the fish. The exposure system was static with continuous aeration. Tests were conducted in a synthetic soft water at a constant temperature of 25 ± 2°C, pH 7.0 ± 0.2 and hardness of 40–48 mg/L as $CaCO_3$. The *O. niloticus* (adult and larvae) used in this study were obtained from the local municipality's fish farm in Brasília, the capital city of Brazil, where breeding conditions were controlled and monitored constantly. The criteria for adult selection were that they should have a body length of 9–12 cm and be 3 months old. Fish were acclimatized in the Genetic Laboratory of the University of Brasília for a week in tanks of 400 L volume, aerated continuously with synthetic soft water, under a photoperiod of 12 h light/12 h dark regime. The ammonium level in water was constantly monitored and maintained in concentrations lower than 2 ppm, with the periodical change of water.

Two groups of eight *O. niloticus* were exposed to a suspension of calcination dust (10%) for 72 h in a glass aquarium, and were fed twice a day with Purine® fish chow purchased from a commercial supplier. A third group served as control. Cytotoxicity was investigated through nuclear abnormalities in peripheral erythrocyte cells, classifying the nuclei as blebbed, lobed, notched and also binucleated cells. One thousand cells were scored per fish. The different frequencies of classes of nuclear deformities observed in treatments and control were analyzed by non-parametric statistical Mann-Whitney—U test, with significance of 95%, using the software Sigmastat 3.0. Micronucleus test was carried out as described by Hooftman and Raat (1982) for fish erythrocyte cells. Peripheral erythrocytes were drawn from the gills with a heparinized syringe and immediately smeared. They were dried at room temperature for 24 h, fixed with methanol for 15 min and Giemsa stained. Three thousand cells with complete cytoplasm were analyzed per fish and the presence or absence of MN was recorded. The criteria for the identification of fish micronucleated erythrocytes were as follows: (a) MN should be smaller than one-third of the main nuclei, (b) MN must not touch the main nuclei and (c) MN must not be refractive and should be the same color and intensity as the main nuclei. Both nuclear abnormalities and micronucleus were evaluated from the same microscope slide.

Two groups of 15 five-day-old larvae of *O. niloticus* were exposed to the mining waste at dilutions of 5%–50% in 2 L glass aquaria as well as a control group, in a static system of exposure. Aquaria were kept continuously aerated; temperature was maintained at 25 ± 2°C with a photoperiod of 12 h light and 12 h darkness. During the experiments, larvae were fed twice a day with a powdered Purine® fish chow. Fish were killed after 96 h exposure time, the gills and the digestive tract were quickly removed for histological procedures to be analysed on light microscopy. Digestive tract and gills were fixed in Davidson fixative, processed according to the routine for light microscopy and stained with Haematoxylin and Eosin (HE). The system for capturing images consisted of camera CCD-Iris and capture plate Pixel View-Station, Image Pro Express-4.0, Media Cybernetics, LP and Scopephoto.

Results and Discussion

Metallic characterization of the calcination dust is summarized in Table 1 showing the following proportions: Fe (6.0%), Ni (1.4%), Al (0.5%), Mn (0.3%) and Cr (0.2%).

No mortality was observed in adults and larvae of *O. niloticus*. This nickel mining residue, at the tested concentration (10%), did not cause cytotoxicity or genotoxicity to adults of *O. niloticus*. There were no statistically significant differences between control and exposed adult groups considering evaluations of micronucleous and nuclear abnormalities (Table 2, $p < 0.05$). In larvae of *O. niloticus*, results showed the presence of calcination dust along the digestive tract, especially in the intestine of the larvae exposed to concentrations of 5%–50% (Fig. 1). Changes in the coating of the gill epithelium, as well as congestion of

Table 1 Metallic composition in two different samples of calcination dust

Collected samples	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni
Point 1	4,963	6.7	464.7	1,886	111.1	54,200	2,652	14,780
Point 2	4,712	6.6	446.6	1,895	98.9	52,400	2,538	13,905
Mean	4,837	6.6	455.6	1,940	105.0	53,300	2,595	14,343

Values are presented in ppm (mg kg^{-1})

the capillaries, were observed in the respiratory structure of larvae exposed to both concentrations (Fig. 2).

In the digestive tract, mining waste accumulation occurred in the stomach as well as intestine. Particles of calcination dust could be seen added in the brush border epithelial cells affecting the microvilli. Positive relationships were also observed between concentration and accumulation (Fig. 1). Obviously, exposure at 50% showed higher stomach and intestinal accumulation than at 5%. The lamellar height decrease and hypertrophy of the lamellae epithelium in the gills were the main observed effects (Fig. 2).

The fish gill is a multifunctional organ performing vital functions, including respiration, osmoregulation, acid-base balance and nitrogenous excretion. Gills are the first organ to come in contact with environmental pollutants and have frequently been used in assessment of impact of aquatic pollutants in both marine and freshwater habitats (Evans et al. 2005). Metals are one of the most deleterious environmental toxicants affecting gills by changing their morphology and ultra structure (Evans 1987; Mallatt 2007; Pandey et al. 2008). Pandey et al. (2008) reported the same kind of alterations in the gills of a freshwater fish, *Channa punctata*, exposed to a mixture of trace metals at low concentrations. Nickel exposure via water significantly

Table 2 Results of micronucleus evaluation and nuclear abnormality analysis on peripheral erythrocytes in adults of *O. niloticus*

	MN (mean)	Nuclear abnormalities (mean)
Control	0	2.57 ± 1.8
Exposed	0.5 ± 0.2	4.00 ± 2.1

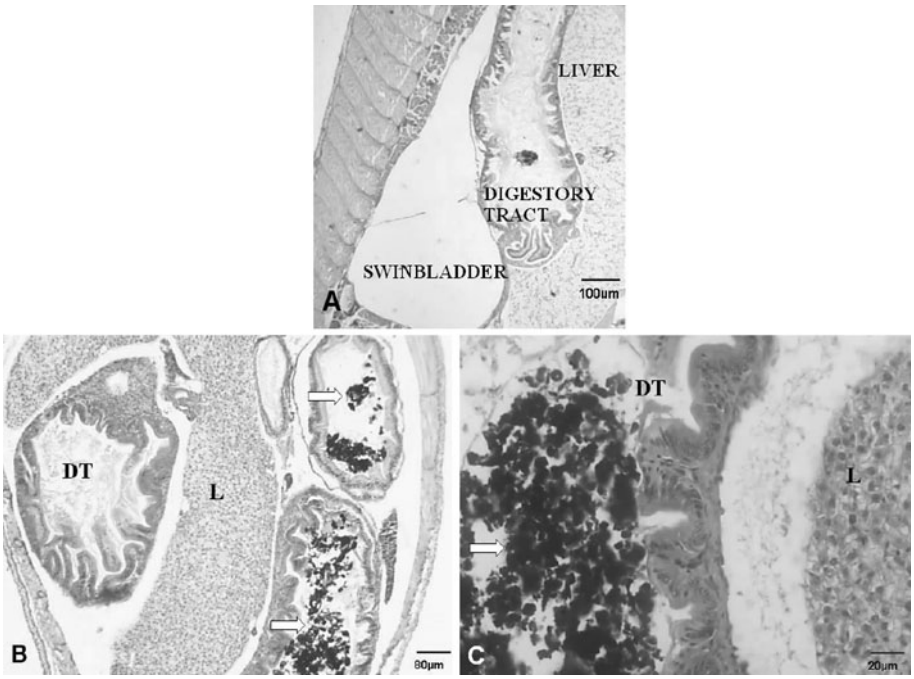

Fig. 1 Histological sections of digestory tract. **a** Larval section showing different organs, magnification of 50×. **b** Individual concentration above 1:1, magnification of 100×. **c** Intestine, magnification of 400×. *L* liver, *DT* digestory tract, and *arrows* indicate the waste in the intestine

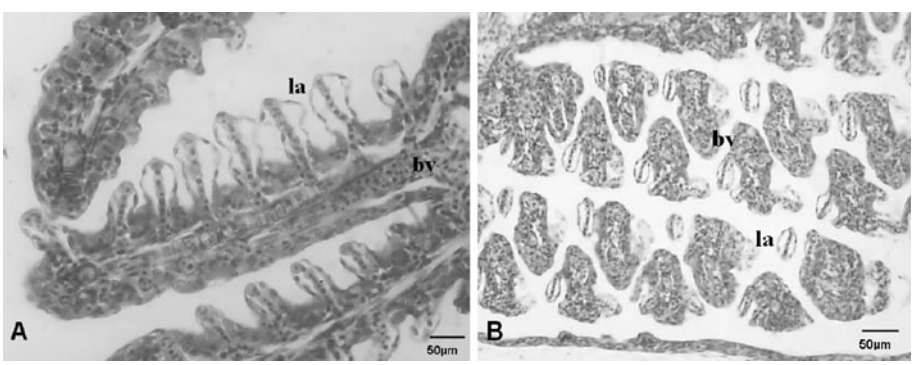

Fig. 2 Histological sections of gills. Congestion of the capillaries and changed lamellae epithelium, magnification of 200×. **a** Slitting section and **b** Cross section. *bv* blood vessel, *la* lamellae

altered the gill ultrastructure of rainbow trout, showing increases in the lamellar swelling and decrease in lamellar height (Pane et al. 2004). Such an alteration can induce respiratory deficiency, even when no residues of mining wastes were found in the gills.

It should be noted that calcination dust presents various toxic metals in its composition (Table 1) and the lethal concentrations (LC_{50} 96 h) of these metals for fish observed in other studies (Shukla and Pandey 1985; Brix et al. 2004; Oliveira-Filho et al. 2004) are much lower than the quantities presented in the studied material. The absence of mortality observed in the present study suggests that the metals in this waste has low water solubility and therefore are not available to cause acute lethality in this short time of exposure. Despite the absence of mortality, injuries observed are considered serious in literature because they can affect respiratory capacity (Pane et al. 2004). The presence of material in suspension in the intestine suggests that, within an increased period of exposure, the absorption of food will also be compromised.

However, special attention should be given to the fact that the absorption of metals occurs mainly in the intestine and gills. In both cases the surface epithelium of the simple type, proximity of blood and plenty of vascularization make these two organs the sites of preferential absorption. Moreover, the intestine and gills have characteristics and processes associated with absorption and transport of ions. In the case of the intestine, as was shown for the species *Oncorhynchus mykiss*, there are different types of absorption along the alimentary tract, with more intense absorption in the intestine and variation in different portions depending on the metal in question. Nickel, for example, is preferentially absorbed in the posterior portion (Ojo and Wood 2007). Histopathological lesions were also observed in kidney and liver of adult lake whitefish (*Coregonus clupeaformis*) subject to diets containing Ni. Altered bile ducts and areas of focal necrosis in liver were observed. Alterations in kidneys, in glomeruli, tubules, collecting ducts, and hematopoietic tissue were also observed (Ptashynski et al. 2002). To explain gill damage observed in the present study Evans (1987) showed several relationships between pollutants and fish gills damage, and also should be pointed that the material studied has large quantities of mineralogical particles in its composition that could contribute to the effects observed in gills. Caillat (2008) reports in her work the presence of several minerals, including silicates and quartz, which might have physical characteristics to cause mechanical effects in the gills.

The concentrations tested of the nickel calcination dust were not enough to cause mortality, cytotoxicity or genotoxicity in larvae and adults of *O. niloticus*, suggesting that fish were not acutely affected by the presence of this mining residue in water. The absence of mortality, cytotoxicity or genotoxicity could be related to the potential low water solubility of the metals presented in the waste, but the hypertrophy of the lamellae epithelium in the gills may be attributed to the metallic composition as well as to the presence of other mineral particles into the studied residue.

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