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Potential toxicity of iron ore tailings after the overflow of a mining dam in Nova Lima (Minas Gerais State, Brazil)

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A B S T R A C T

In January 2022, the Pau Branco Mine dam in Nova Lima (MG), under the responsibility of Vallourec, overflowed after intense rainfall and iron ore tailings impacted surrounding soils and aquatic ecosystems. These tailings can alter the natural composition of soils and rivers, poising risks on soil biota. This study consists of a preliminary evaluation of potential toxicity of terrigenous materials impacted by tailings from the Pau Branco Mine, based on acute bioassays with earthworms (*Eisenia andrei*) and germination tests with lettuce (*Lactuca sativa*). The physical and chemical characterization and determination of toxic metals in the samples supported the interpretation of the ecotoxicological data. The results indicated that the tailing deposition induced the increase in particle density, due to the increase in iron concentrations in the samples. As a consequence, the water holding capacity (WHC) of the materials and organic matter concentrations were dramatically reduced. Given that the mining tailings exhibit fine texture, their depositions on soils increased the percentage of fines particles (silt) in the samples. Metal determination revealed geochemical anomalies for nickel, copper and chromium, whose concentrations exceeded the threshold limits defined by Brazilian law for soil and aquatic sediment quality. The bioassays with earthworms showed no significant earthworm mortality, indicating low acute toxicity. However, earthworm biomass was significantly reduced, suggesting that they were submitted to some stress induced by the exposure to contaminants and/or by the reduction of the organic matter contents (food scarcity) and WHC (water scarcity). The bioassays with lettuce showed no significant effects on germination levels. However, the seedling biomass was significantly reduced due, possibly, to the reduction of WHC and especially due to the increase of fines particles in the samples, which tends to inhibit the establishment of the root systems.

Keywords: earthworm, lettuce, contamination, metal, soil.

Potencial tóxico de rejeitos de minério de ferro após o transbordamento de uma barragem da mineração em Nova Lima (Minas Gerais, Brasil).

R E S U M O

Em janeiro de 2022, a barragem da Mina Pau Branco em Nova Lima (MG), sob responsabilidade da empresa Vallourec, transbordou após fortes chuvas e rejeitos de mineração de ferro atingiram solos e ecossistemas aquáticos vizinhos. Tais rejeitos podem alterar a composição natural de solos e rios, colocando em risco a saúde da biota de solo. O presente trabalho consiste na avaliação preliminardo potencial tóxicos de materiais terrígenos impactados pelos rejeitos da Mina

Pau Branco, com base em bioensaios agudos com minhocas (*Eisenia andrei*) e de germinação com alface (*Lactuca sativa*). A caracterização física, química e determinação de metais pesados nos materiais subsidiou a interpretação dos dados ecotoxicológicos. Os resultados apontam que a deposição da lama induziu aumento da densidade de partículas, em virtude do incremento das concentrações de ferro nos materiais. Como consequência, a capacidade de retenção de água (CRA) dos materiais e as concentrações de matéria orgânica reduziram dramaticamente. Visto que os rejeitos de mineração são de granulometria fina, a deposição dos rejeitos aumentou o percentual de finos (silte) nas amostras. A determinação da concentração de metais pesados revelou que anomalias geoquímicas para níquel, cobre e cromo, cujos teores excederam os limites definidos pela lei brasileira para qualidade de solos e sedimentos aquáticos. Os bioensaios com minhocas acusaram ausência de mortalidade significativa de organismos, indicando baixa toxicidade aguda. Porém, os organismos perderam significativamente biomassa, sugerindo que os mesmos estavam submetidos a algum tipo de estresse induzido por contaminantes e/ou pela redução do teor de matéria orgânica (escassez de comida) e da CRA (escassez de água). Os bioensaios com alface mostraram ausência de efeitos significativos sobre a germinação. Contudo, a biomassa das plântulas foi significativamente reduzida devido, possivelmente, à redução da CRA e, sobretudo, ao aumento do percentual de finos nas amostras, que tende a inibir do estabelecimento do sistema de raízes.

Introduction

In January 2022, the Pau Branco dam (in Nova Lima, Minas Gerais State, MG, Brazil), under the supervision of Vallourec company, overflowed after intense rainfall and iron ore tailings reached roads, rivers and rainforest areas. The tailings mobilized many other materials on the way, and caused the temporary interruption of an important state road (BR 040). Moreover, the tailings impacted the Macaco river, which drains to Das Velhas river and whose mouth is the São Francisco river (one of the most important Brazilian watersheds). Given the recent events in Mariana (MG, in 2017) (Buch et al. 2020; Cesar et al. 2022) and Brumadinho (MG, in 2019) (Thompson et al. 2020; Siqueira et al. 2022), this event in Nova Lima shows that the management of mining dams is in fact a challenge in Brazil, especially in the Minas Gerais State. The overflow of the Pau Branco dam did not cause human victims, but the tailings may have caused relevant on soils and fluvial systems, which should be carefully studied.

The deposition of mining ore tailings on soil and aquatic systems may alter the natural physical, chemical and mineralogical properties of these materials, thus impacting biodiversity. This scenario may include relevant changes on soil/sediment texture, particle density, pH, water holding capacity (WHC), clay minerals, anomalies in toxic metal concentrations, and others (Esteves et al. 2020; Siqueira et al. 2022). The understanding of such processes (including their ecotoxicological implications) is crucial for decision-makers to identify critical areas of contaminations and ecological receptors at most risk. In addition, local populations should be empowered by accessing this type of information ("the right to know"), since it supports their effective participation in decision-making, minimizing environmental injustices.

Ecotoxicological tests with earthworms (*Eisenia andrei*, *Eisenia fetida*) have been widely applying to monitor potential toxicity of mining tailings. The use of those organisms in bioassays are due to their importance for soil food web, sensibility to contaminants and relative short life cycle (Edwards et al. 2022). In addition, earthworms are abundant in tropical and temperate systems, influence nutrients biogeochemical cycles and are the most used organism in soil ecotoxicology (Niva et al. 2016; Adeel et al. 2021; Li et al. 2020). Lettuce (*Lactuca sativa*) is also commonly used as test organism in toxicity tests, since its germination is sensitive to disturbances in soil ecosystems (Rede et al. 2019; Pinheiro et al. 2020). Also, lettuce is a producer organism and adverse effects on *Lactuca sativa* may be a good indicator of disturbances on the basis of the trophic chain.

This study consists of a preliminary evaluation of acute ecotoxicological effects related to the overflow of the Pau Branco mining dam. To achieve this purpose, bioassays with *Eisenia andrei* and *Lactuca sativa* two samples of surface materials were collected immediately after the dam overflow close to a river channel. Metal determination and physical and chemical characterization of the samples were determined to support the understanding of the ecotoxicological impacts. Our working hypotheses are: (i) the tailings were able to modify physical and chemical aspects of surrounding soils and sediments, especially in respect to the particle density and anomalous Fe-enrichment; (ii) the tailings exhibit anomalous concentrations of metals due to tailings deposition; and (iii) the tailings are toxic to earthworms and lettuce.

Materials and méthods

Study area

Nova Lima (Minas Gerais State, Brazil) is located in the Iron Quadrangle area, where mining activities has been occurring since Portuguese colonial period. Due to intense metamorphism in the geological past and hydrothermalism phenomena, regional geochemistry e pedology naturally exibit materials containing relatively high metal contents (i.e., high metal background) (Gomes 2009; Vicq et al. 2015). The iron extraction is performed in Itabirite rocks, a type of banded iron formation from the Neoproterozoic age, which are mainly composed of hematite and quartz.

Local climate is wet tropical, and is strongly influenced by the topography (ranging from 707m to 1664m high). Nova Lima is located at a transition area between two important Brazilian biomes: Cerrado (Brazilian Savanah) and Mata Atlântica (Atlantic Rainforest), which are considered hot-spots of biodiversity (Ab'saber 1977). Regional pedology is mainly characterized by oxisols, i.e., highly weathered materials.

Samples

Two surface samples (P1 and P2; ~20cm depth) were collected in the fluvial valley of the Macacos river (Figures 1 and 2). The sampling was performed one day after the dam overflow (January 9, 2022). The samples were obtained by using a plastic shovel and stored into plastic bags. At the laboratory, the samples were air dried, disaggregated, sieved (at 2mm) and homogenized. A reference soil sample (S 200 13' 49.5''; W 440 07' 19.4'') collected in Brumadinho (MG; close to Nova Lima) by Siqueira et al. (2022) was used as a geochemical background. Siqueira et al. (2022) highlight that such reference sample was collected in a non-contaminated rainforest area.

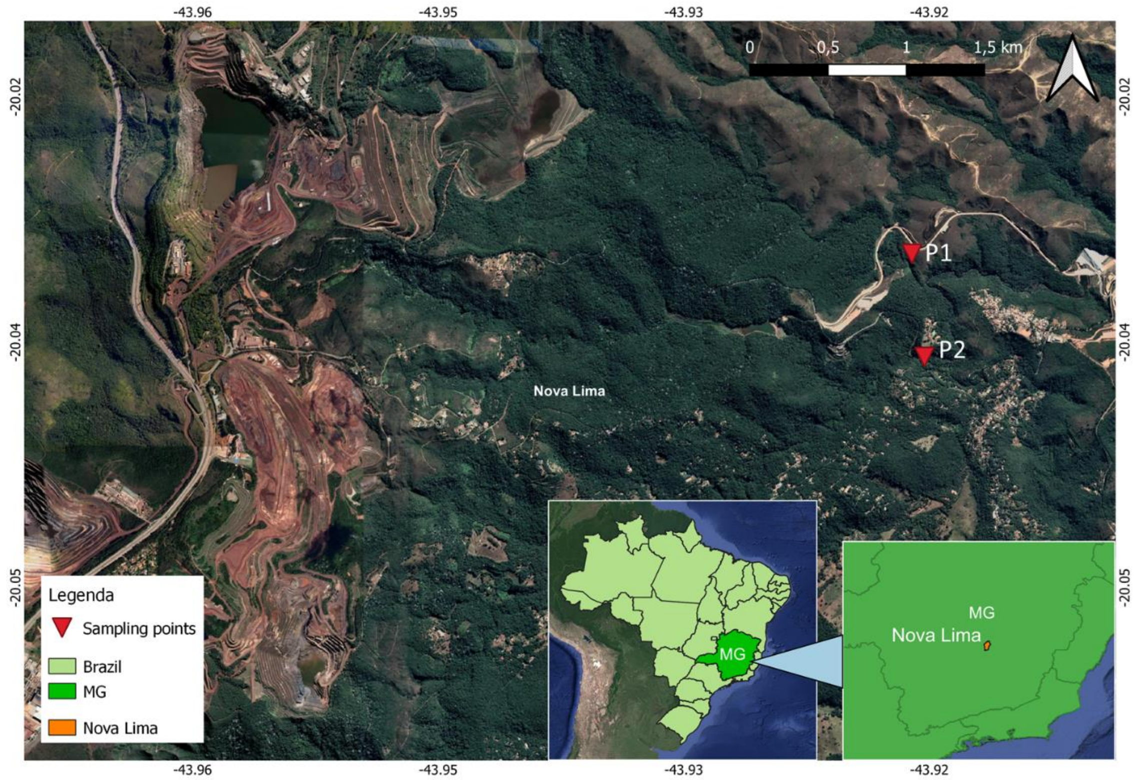


Figure 1. Sampling points (P1 and P2) in the surroundings of the Pau Branco mining dam (Nova Lima, Minas Gerais State, Brazil

Physical and chemical characterization

Physical and chemical characterization of the samples included the determination of texture (grain size), particle density, pH, water holding capacity (WHC), total iron and total phosphorus (indicator of organic matter). The procedure adopted for iron and phosphorus determination are reported in the 2.4 section. The texture was determined using the pipet method, in accordance with EMBRAPA (1997) recommendations. The percentages of sandy were determined by wet sieving. Silt and clay contents were quantified by using a dispersant solution, that induces silt precipitation, while clay contents remain in suspension.

The determination of the particle density was performed by weighting a given mass of soil into a 50mL volumetric flask. After that, the flask was filled with ethanol 98% using a 50mL buret, and particle density was determined by checking the volume occupied by the sample into the volumetric flask (EMBRAPA 1997). Before determining such volume, the volumetric flask should be weakly shaken to remove bubbles that could eventually mask the results. The determination of pH was performed using a solution composed of 10g of sample and 25mL of distillated water, in accordance with EMBRAPA (1997). The values of pH were determined 30 minutes after the mixture water:soil being shaken. After this step, the eletrod was inserted into the solution and an ANALYSER equipment (model 350M) was used for pH determination.

The water holding capacity (WHC) was determined by saturating a soil column with distillated water (through capillarity phenomena), in accordance with Natal-da-Luz et al. (2009). The mass of water capable to saturate the soil column was determined by gravimetry. The results were reported in percentage (%) of water related to the dried soil mass.

Metal determination

The same procedure adopted to determine total metal concentrations (Zn, Cu, Pb, Ni, Cd, Cr, Hg and As) was the same one used by Siqueira et al. (2022). The method consists of solubilizing 1g of sample using mixture composed of $3HCl:1HNO_3$ (Aqua Regia extractor). Metals in solution were determined by ICP-OES equipment. The same method used for determining total metal concentrations was used to determine total phosphorus concentrations (an indicator of organic matter). All the samples were analyzed in triplicate and certified samples were also used to guarantee the quality of the results.

Total metal concentrations were assessed by adopting the following criteria: (i) Brazilian threshold limits for metal contaminations in soils (Prevention Limits – CONAMA 2009), i.e., the concentrations that are able to cause adverse effects on soil ecological receptors; (ii) Brazilian threshold limits for metal contamination in freshwater sediments (CONAMA 2012), i.e., Levels One and Two, which are limits for low and high probability for adverse effects on aquatic biota; and (iii) the estimation of metal-enrichment factors (EF).

Metal enrichment-factors (EF) were calculated to estimate anthropic contributions related to potentially bioavailable concentrations of metal in the samples. The EF is commonly used as a reference of ecological quality (Selvaggi et al. 2020), and consists of the ratio between metal concentration in the sample and metal concentration in a local background sample (in this case, the reference sample) (Equation I). The concentrations should be previously normalized in regards to an element whose origin is predominatly lithogenic and whose behavior is conservative (Al, Fe ou Sr – in this case, Al – as suggest by Kemp and Thomas (1976)). When FE exceeds two units (FE > 2), one could reasonably suggest that metal concentration is anomalously increased due to anthropic interventions (Kemp and Thomas (1976).

$$EF = \frac{(M_i/Al)_{sample}}{(M_i/Al)_{background}} \qquad \text{Equation I}$$

Where, Mi = concentration of a given metal.

Acute bioassays with earthworms (*Eisenia andrei*)

The acute bioassays with *Eisenia andrei* followed ISO (2012) recommendations. Two tests with different periods of exposure were performed: 14 and 28 days. The tests were performed with cylindrical recipients filled with 500g of soil and 10 adult organisms (with well-developed clitellum) of similar weight (ranging from 400mg to 600mg) per replicate (four replicates per sample). The soil samples had their moisture contents previously adjusted to 40-60% of the WHC. The test was carried out at constant illumination and controlled temperature (20 ± 2 0C). By the end of the tests, the number of surviving worms was counted and their biomass changes were checked by considering their mean weight per replicate before and after the test.

Additional replicates containing artificial soil were added to the experiments to guarantee the quality of the results. The test was considered valid when the mean mortality among the replicates with artificial soil and reference soil sample did not exceed 10%. The composition of the artificial followed the recommendations defined by Garcia (2004): 70% of quartz sandy, 20% of kaolin and 20% of coconut shell dust. Significant different between the mortality levels found in the samples and the ones found in the contaminated samples were checked using Kruskal-Wallis tests. The normality of the data was previously checked using the Shapiro Wilk test. Such statistical analyses were performed by using the software Past 3.

Avoidance bioassays with earthworms (Eisenia andrei)

These bioassays were performed according to ISO (2008) protocol. The test was performed with five replicates per sample and ten adult earthworms (with well-developed clitellum) per replicate. Plastic test recipients were divided in to sections of same size, using a plastic card (Figure 2). One section was filled with control sample (in this case, reference soil), and the one with the sample to be tested. After removing the plastic, the organisms were placed in the interface of the soils (Figure 2). After 48 hours of exposure, under controlled temperature (20 ± 2 0C) and photoperiod of 16:8h (light:dark), the number of earthworms in each section was counted. Missing earthworms were considered dead. When the avoidance response (i.e., percentage of worms found in the control sample) exceeded 80%, one could consider that the test soil exhibited “limited habitat function” (ISO 2008).

Additional replicates containing control sample in both sections were added in the experiments to guarantee the quality of results (Dual Control Test). The distribution of the organisms between the soil sections are supposed to be relatively equal for the test to be considered valid. Significant differences between the control number of organisms found in the sections containing reference and contaminated soils were evaluated by applying the Fisher Exact Test (Natal-da-Luz et al. 2009).

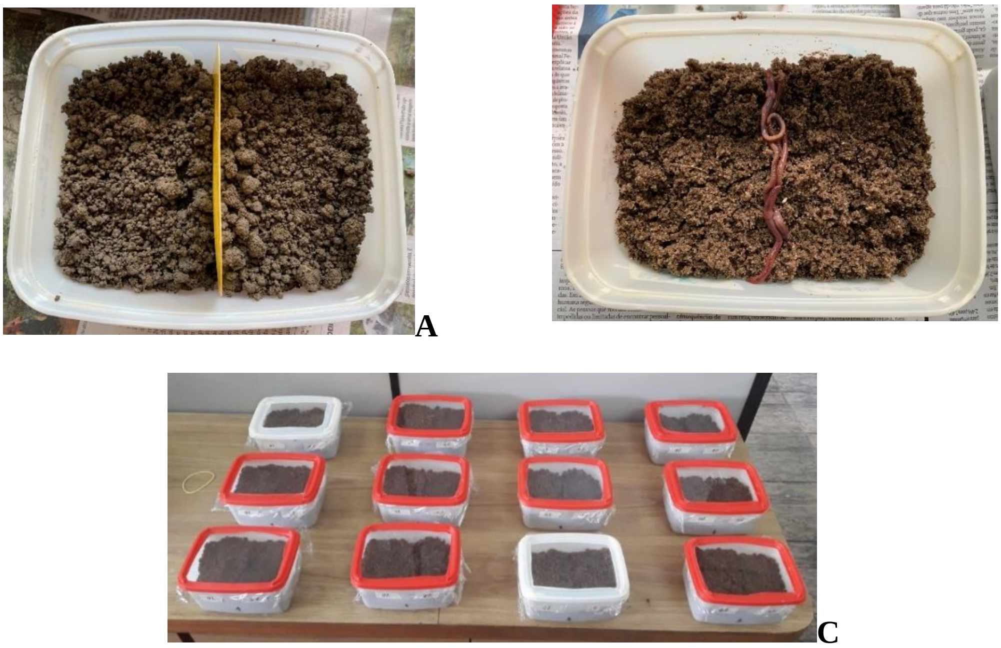
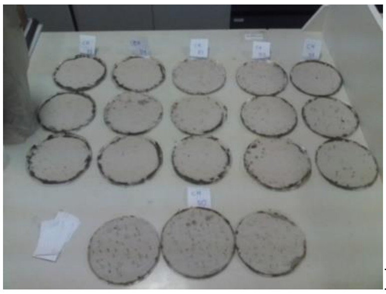


Figure 2. Avoidance bioassay with *Eisenia andrei*: test recipient divided in two equal sections and filled with control sample (reference soil) and contaminated soil (A); earthworms inoculated on the interface of the soils (B); and panoramic view of test (C).

Germination bioassays with lettuce (Lactuca sativa)

The procedure adopted for this test followed the OECD (1984) recommendations. The seeds of L. sativa (Figure 3A) used the tests were obtained without agrotoxicants. The test was carried out with plastic plates (15cm diameter) filled with 100g of soil containing 40 seeds per replicate. About 100g of fine quartz sand was added on soil surface to avoid moisture. The moisture of the soil was previously adjusted to 40-60% of the water holding capacity (WHC), which was determined in accordance with Natal-da-Luz et al. (2009). The test was run with four replicates per sample and photoperiod of 16:8h (light:darkness) and at 22 ± 2 °C. After 7 days of test, the number of germinated seeds was counted and the biomass of the seedlings was determined (Figure 3). The biomass was reported as a ratio between biomass and the number of germinated seeds per replicate. For a better graphic representation, the value of the ratio was multiplied by a factor of 10^3.


A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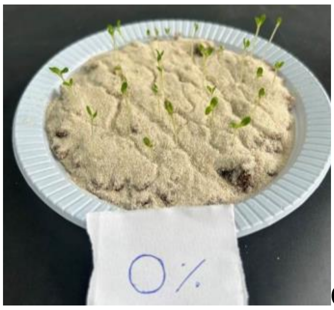

C

Figure 3. Lettuce (*Lactuca sativa*) seeds (A); panoramic view of the test recipients (B); and aspect of seedlings after 7 days of germination test (C).

Results

Physical and chemical characterization

The Table 1 shows some physical and chemical aspects of the samples (P1 and P2) impacted by the dam overflow. The results suggest that the samples are predominantly muddy (mud = silt plus clay) and exhibit higher silt contents compared to the reference soil. The particle density determined in the P1 sample is similar to the value found in the reference soil. In contrast, the value found in the P2 sample is significantly higher compared to the reference sample. Total Fe concentrations were much higher compared to the reference sample, especially the P2 sample, whose value was almost five times higher than the one found in the reference sample. By following an opposite tendency, the WHC levels are significantly lower than the ones found in the reference sample, especially again the P2 sample, whose value twice lower compared to the reference sample. Finally, total P concentration in both P1 and P2 samples were also more than twice lower than one found in the reference sample.

Table 1 Physical and chemical parameters determined in iron ore tailings samples after the overflow of the Pau Branco dam (Nova Lima, MG, Brazil). WHC = water holding capacity. Reference soil = soil from a non-contaminated area.

Parameters	**Reference soil**	**P1**	**P2**
Sand (%)	62.0	35.3	41.9
Silt (%)	24.0	55.1	53.7
Clay (%)	14.0	9.7	4.4
Particle density (g/cm^3)	2.46	2.47	3.39
WHC (%)	60.4	48.66	31.02
Total Fe (%)	0.65	2.41	3.21
Total P (mg/kg)	940.0	460.0	420.0

Metal Determination

The Table 2 shows metal concentrations and their respective EF values found in the samples collected in Nova Lima (MG), including comparisons with the threshold limits defined by Brazilian law for soil (Prevention Limit) and freshwater sediment (Levels One and Two) quality. The concentrations of Cd, As, Hg and Pb are below the minimum detection limits and, due to this reason, they were not recorded in the Table 2. The concentrations of Cu and Cr in the P1 sample exceeded the Prevention and Level One limit. Ni concentration in the P1 sample is close to exceeding the Prevention Limit. In the P2 sample, none of the metals exceeded the limits established by law. None of the samples exceeded the Level Two for sediment quality.

Given the fact that Al was used to estimate the EF values, no EF values of Al are shown in the Table 2. All the metals revealed FE > 2 (the limit for anthropic contributions) for at least one of the samples. The EF values found in the P2 sample were generally higher than the ones obtained with the P1 samples. In fact, the EF values found in the P2 sample can considered extremely high, especially for Ni (828), Fe (113.6), Cu (110.1), Mn (23) and Zn (10.3), since they are much higher than 2 units.

Table 2. Total metal concentrations (in mg/kg) and metal-enrichment factors (EF) determined in tailings samples after the overglow of the Pau Branco mining dam (Nova Lima, MG, Brazil). Prevention limit is the threshold limit for adverse effects on soil ecological receptors (CONAMA, 2009). Level One and Leve Two are the limits for low and high risks on freshwater biota (CONAMA 454). * = higher than the prevention limit; # = higher than the Level One; # = higher than the Level Two. Values marked in bold correspond to EF > 2.

Metal		Ref.	P1	P2	CONAMA 420 (2009)	CONAMA 454 (2012)	
					Prevention Limit*	Level One#	Level Two##
Zn	mg/kg	19.0	28.5	8.5	300	123	315
	EF	-	0.9	10.3			
Cu	mg/kg	2.8	127*#	13.4	60	35.7	197
	EF	-	28.6	110.1			
Ni	mg/kg	< 0.1	27.8	3.6	30	18	35.9
	EF	-	175.2	828.0			
Cr	mg/kg	5.3	79.4*#	<0.2	75	37.3	90
	EF	-	9.4	0.9			
Al	mg/kg	0.92	1.46	0.04	-	-	-
	EF	-	-	-			
Fe	mg/kg	0.65	2.41	3.21	-	-	-
	EF	-	2.3	113.6			
Mn	mg/kg	0.02	0.05	0.02	-	-	-
	EF	-	1.6	23.0			

Note: Brazilian law does not define threshold limits for Al, Fe and Mn.

Acute bioassays with earthworms

The Figure 3 presents survival levels and biomass changes of earthworms after 14 and 28 days of exposure on tailing samples collected in Nova Lima (MG). The tests were considered valid, since mean earthworm mortality found in the artificial soil and reference soil by the end of the tests did not exceed 10%. No significant earthworm mortality was observed after 14 and 28 days of exposure. Significant reduction of earthworm biomass was detected for both P1 and P2 samples after 28 days of exposure. Earthworm cocoons were observed after 28 days of exposure in the reference sample, but no cocoons were detected for the samples P1 and P2 samples.

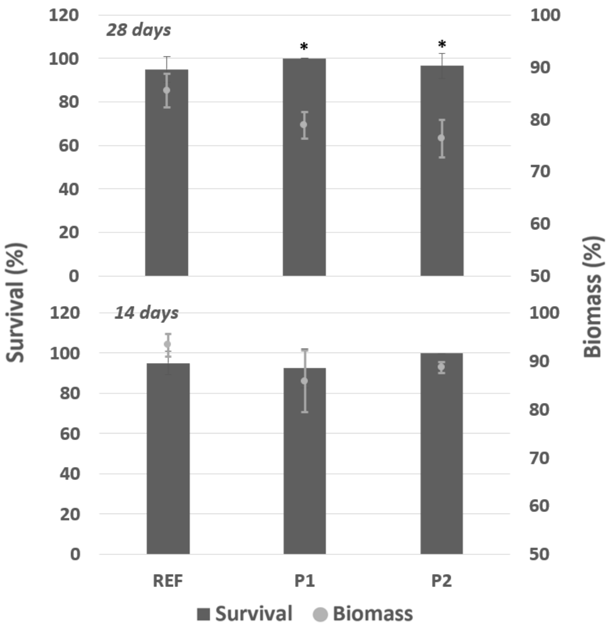

Figure 3. Survival and biomass changes of *Eisenia andrei* after being exposed for 14 and 28 days to iron ore tailing samples collected in Nova Lima (MG) after the overflow of the Pau Branco dam. REF = reference soil sample. * = biomass significantly different from the reference sample (Kruskal-Wallis, $p < 0.05$).

Avoidance bioassay with earthworm

The avoidance responses of *E. andrei* after being exposed to tailing samples collected in Nova Lima are shown in the Figure 4. The valid criteria of the test were fulfilled, since earthworm distribution in the Dual Control Test was relatively equal. The avoidance responses found for both P1 and P2 samples were significant, and both samples were able to limit the habitat function of the soil (avoidance response ≥ 80%).

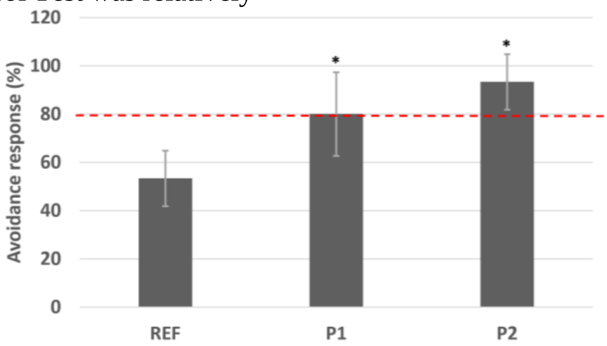

Figure 4. Avoidance responses of *Eisenia andrei* after exposure on iron ore tailing samples collected after a dam overflow in Nova Lima (MG, Brazil). REF = reference soil (Dual Control Test). * = means significant avoidance response (Fisher Exact Test, $p < 0.05$). The red line means the limit defined by ISO (2008) for “limited habitat function”.

Germination bioassay with lettuce

The Figure 5 shows the germination and seedling biomass levels of L. sativa after being exposed to tailings samples collected in Nova Lima (MG, Brazil). The valid criteria of the test were fulfilled, since mean germination in artificial soil was higher than 90%. No significant reduction of germination levels was observed. The germination in the P1 and P2 samples were even higher than the reference soil. However, significant reduction of the seedling biomass levels was observed for both P1 and P2 samples.

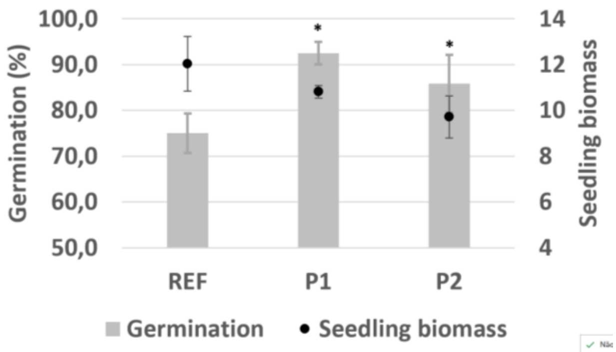

Figure 5. Percentage of germinated seeds (*Lactuca sativa*) and seedling biomass after the exposure on the samples collected in Nova Lima (MG, Brazil). REF = soil reference sample. * = seedling biomass significant different from the reference sample (Kruskall-Wallis, $p < 0.05$).

Discussion

Iron ore tailings often exhibit high hematite contents. After being deposited on surrounding soil and river, these tailings can induce an anomalous increase of the Fe concentrations in soils and fluvial sediments. As a consequence of abnormally high Fe concentrations, the particle density tends to increase (Carmignano et al. 2021). Due to Fe-enrichment, organic matter contents (nutrients) are deeply reduced (Wu et al. 2019). Given the fact that water retention in soils is dependent on organic matter, the WHC levels were strongly decreased. Also, Fe particles are not good water absorbers. The increase of muddy particles in the sample are due to the fine texture of the tailings, since Fe extraction generally requires a primary step that involve the rock crushing and grinding in order to increase contact specific surface, optimizing further chemical extraction steps (Gomes 2009). This scenario involving relevant physical and chemical changes on surrounding soils and river is similar to the one found by Siqueira et al. (2022) in Brumadinho (MG).

Even though most of metal concentration are lower than the limits established by Brazilian law, the EF are definitely very high and are most probably associated with the tailing's deposition. The Fe extraction does not include the use of toxic metals, and metals found in the samples do not necessarily come from the tailings. However, the surroundings of the dam are naturally metal-enriched, since Iron Quandrangle region was submitted to intense metamorphism in the geological past (Vicq et al. 2015), i.e., high metal background levels. In addition to that, old abandoned mining areas are common in Minas Gerais State and such hazards sites are not well-mapped and identified (Cesar et al. 2011; Windmöller et al. 2015). Therefore, the release of the tailings to the surroundings of the dam may have mobilized toxic metals from old mining sites and or from natural sources. Moreover, toxic metals have great affinity with iron oxides (Javed et al. 2022) and the tailings are hematite-enriched. This particular aspect of the tailings may have greatly contributed to alter natural biogeochemical cycle of metals in soils and rivers. Finally, a similar reality in regards to the total metal concentrations was also observed in Brumadinho (Siqueira et al. 2022).

The acute toxicity of the tailings to earthworms can be considered low, since no significant mortality was observed after 14 and 28 days of exposure. Perhaps, a relevant factor in the toxicity is the increase of particle density, of muddy contents and decrease of WHC and organic matter in the soil, which may have induced the significant reduction of earthworm biomass after 28 days exposure in both P1 and P2 samples. This scenario supports the impacts found for earthworms (*Eisenia andrei*) described by Siqueira et al. (2022), who also suggest a decrease of earthworm biomass after acute exposure on tailing samples collected in Brumadinho (MG).

In this sense, when the soil becomes muddier, porosity and permeability levels tend to reduce, thus requiring more energy for the earthworms to move in the soil. If the soil particles also become denser, such scenario gets worse in term of energy spend for the organisms to live in the soil. If on one hand the demand for more energy was increased, on the other hand the availability of food and water was dramatically reduced due to Fe-enrichment (Siqueira et al. 2022). This combination resulted in significant reduction of earthworm biomass, especially after long-term exposures. Last, but not least, the absence of earthworm cocoons by the end of the experiments consists of an issue of concern, since this suggests possible significant effects on the earthworm reproduction. This aspect of the toxicity should further be better investigated by performing reproduction bioassays.

The earthworm avoidance responses found in the test were high and limited the soil as a habitat for the earthworms. This means that some of the ecosystem services provided by the earthworms cannot by be compromised, which includes soil aeration, increase of porosity, permeability, addition of nutrients, reduction of soil erosion susceptibility, and others (Hallam et al. 2020; Yadav et al. 2023). Avoidance responses are generally a more sensitive endpoint compared to mortality, biomass or earthworm reproduction and the results found in this study are in fact an issue of concern.

Although WHC levels are much lower compared to the reference sample, no significant inhibition of the germination was observed. On the other side, the significant reduction of biomass seedling is probably due to the increase of fine particles in the soil (resulting in soil compaction), which cause disturbances in root systems of the seedlings, thus affecting their growing (Azzi et al. 2017). Also, low WHC levels and low nutrients contents (induced by Fe-enrichment) may have also compromise the development of the seedlings.

This study shows a preliminary ecotoxicological evaluation related to the tailings from the Pau Branco mining dam and more sample should be collected in furthers studies in order to clarify our findings and validate tendencies. In this respect, ecotoxicological tests with other soil organisms and the determination of potentially bioavailable metal contents are highly recommended. At this point, the acute effects on L. sativa and E. andrei seem to be low, but the reduction of earthworm and seedling biomass should be better detailed under a chronic perspective in further investigations.

Conclusions

The acute toxicity levels of the tailings exhibited can be considered low to L. sativa e *E. andrei* (i.e., low mortality and low inhibition of germination levels), but the tailings were able to significantly reduce earthworm and lettuce seedling biomass levels, as well as to cause important avoidance responses on the earthworms. The toxicity found in the bioassays are most probably due to important disturbances induced by the tailing deposition, which include not only the increase of Fe concentration, particle density and fine particles-enrichment, but also the reduction of the WHC and organic matter levels. Even though total metal concentrations were below the threshold limit defined by Brazilian legislation, the EF values suggest that such concentrations and are anomalous (especially Ni, Cu and Fe) and me be a result of tailing deposition in surrounding soil and fluvial sediments.

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