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ECOLOGY

Aftershocks of the Samarco disaster: diminished growth and increased metal content of *Raphanus sativus* cultivated in soil with mining tailings

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ABSTRACT. The collapse of the Fundão tailings dam of the Samarco mining complex in Mariana, Brazil, was the largest mining disaster in the world to date with many socio-economic and environmental impacts. Soil affected by mining tailings was severely altered with negative impacts for agriculture. We tested whether diluting mining tailings with organic soil would eliminate or at least attenuate the ecotoxic effects on plant development and performance. We cultivated radish, *Raphanus sativus*, in substrates containing different proportions of mining tailings and organic soil: pure tailings ($T_{100\%}$); 2) tailings$_{75\%}$ + soil$_{25\%}$ ($T_{75\%}$); 3) tailings$_{50\%}$ + soil$_{50\%}$ ($T_{50\%}$); 4) tailings$_{25\%}$ + soil$_{75\%}$ ($T_{25\%}$), and 5) pure organic soil (Soil$_{100\%}$, control). There were large differences in soil quality parameters between the 100% tailings treatment ($T_{100\%}$) and the control (Soil$_{100\%}$), as well as for some parameters in the most diluted treatment - $T_{25\%}$ (Ca^{2+}, Fe, Mn) in relation to the control treatment. Although dilution of the tailings soil improved radish development, there was lower radish productivity (leaf area, total biomass, and root/tuber biomass) than for pure soil (control). There were also significantly higher amounts of bioaccumulated metals in radish tubers grown with tailings, even when grown in $T_{25\%}$ for Fe content and in $T_{75\%}$ for Mn content. These results present a worrisome scenario for human communities in the region of the Doce river, as human consumption of crops produced in soil contaminated with tailings is not recommended due to potential toxicological effects from high metal concentrations.

Keywords: doce river disaster; ecotoxicology; food security; mining tailings; plant nutrition; radish.

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Introduction

Even though the collapse of the Fundão tailings dam of the Samarco Mariana Mining Complex (Mariana, Brazil) occurred over six years ago in 2015, its socio-economic and environmental consequences still persist. This unprecedented disaster released ca. 50 million cubic meters of mining tailings down 663.2 km of the Doce river until the Atlantic Ocean. The disaster negatively affected 41 municipalities and millions of human lives along the way (Fernandes et al., 2016; Fernandes & Ribeiro, 2017; Hatje et al., 2017; Coimbra, Alcântara, & de Souza Filho, 2020). It is estimated that 1,176.6 ha of non-forest areas and 457.6 ha of forest were flooded by the tailings, including parts of the megadiverse Brazilian Atlantic Forest (Omachi et al., 2018). Agriculture, livestock, and fisheries are the main economic activities in the region of the Doce river and were most intensely practiced on the fertile lands alongside river banks (Silva Júnior et al., 2018). With the collapse of the Fundão dam, mining tailings were mixed with the soil, profoundly changing its structure (physically and chemically) and biological composition (Segura et al., 2016; Guerra et al., 2017).

Analysis of the Fundão dam tailings showed high particle density and silt proportion (particles of 1 to 200 µm in size) and low organic matter (OM) concentration (Segura et al., 2016; Silva et al., 2016). Iron mining tailings are mainly composed of Aluminum (Al), Iron (Fe), Manganese (Mn), Barium (Ba), Strontium (Sr), Lead (Pb), Arsenic (As), and Cadmium (Cd). Among these, Al, Fe, Mn, Ba, and Sr were identified as having potential for mobility (Segura et al., 2016). Hatje et al. (2017) found Fe, As, Mercury (Hg), and Mn levels in the Fundão tailings to be at concentrations above the Upper Effect Limit (UET), that is, above which toxicity is predicted.

While the tailings deeply affected soil chemistry, other sources of soil contamination in the region were acknowledged, such as the processing of other ores, agro-industry, domestic wastes, and silviculture (Oliveira & Quaresma, 2017).

The combination of soil compaction, high metal concentrations, low OM, and possible interactions with other sources of contamination are highly undesirable for the agricultural practices taking place alongside the banks of the Doce river (Andrade et al., 2018). In fact, studies have consistently shown that the main limiting factor for plant growth in the affected region is the low fertility of the Fundão dam tailings (Andrade et al., 2018; Coelho et al., 2020; Cruz, Gomes, Bicalho, Della Torre, & Garcia, 2020; Esteves et al., 2020). However, high concentrations of metals were also found in plants grown in tailings (Coelho et al., 2020; Esteves et al., 2020; Cruz, Gomes, Bicalho, & Garcia, 2021). When present at high concentrations in the soil, some metals can become toxic and affect important metabolic processes (e.g., inhibition of enzymatic activity, initiation of chlorosis, and lowering plant development/yield capacity) (Påhlsson, 1989; Nagajyoti, Lee, & Sreekanth, 2010; Onakpa, Njan, & Kalu, 2018). In addition, plants growing in soils with mining tailings may bioaccumulate these metals and transfer them through the food chain, possibly affecting the entire ecosystem (Gilberti et al., 2014; Raj & Maiti, 2019). Metal bioaccumulation could ultimately reach humans and trigger or aggravate a wide range of health disorders (Arantes, Savassi, Santos, Gomes, & Bazzoli, 2016; Onakpa et al., 2018).

There is an urgent global need to develop and test reliable strategies that assist in the remediation of soils impacted by mining dam disasters. The development of recovery strategies and native and agronomic plant species with potential for growth in the soil affected by mining tailings have been intensified in recent years to help mitigate impacts. Among the alternatives to minimize the effects of nutritional deficiency and compaction of soil affected by tailings from the Fundão dam mining is the application of chemical fertilizers and/or the addition of OM. Chemical fertilization was found to reverse the deleterious effects of mining tailings on the growth of native Atlantic Forest plants (Cruz et al., 2020). The addition of vermicompost to mining tailings improved soil physical properties, increased nutrient input into the soil, and favored the development of corn plants (Esteves et al., 2020).

Thus, this study evaluated whether diluting mining tailings with soil rich in OM improves crop development (i.e., growth and macro/micronutrient content) and suitability for human consumption based on the toxicity (Tolerable Upper Intake Level - TUIL) of plants cultivated in contaminated substrate. The plant species *Raphanus sativus* L. (common radish, Brassicaceae) was chosen for the study due to its great economical and nutritional (vitamins and minerals) importance as an agronomic species (Manivannan, Kim, Kim, Lee, & Lee, 2019). In addition, *R. sativus* is well-known for its bioaccumulation capabilities (Manivannan et al., 2019), thus being a potential biosafety indicator for growing crop species in soils contaminated with mining tailings (Martin, Vollenweider, Butter & Günthardt-Goerg, 2006; Xu, Zhang, Song, Brewer, & Gao, 2019). The roots (tubers) of *R. sativus* are consumed *in natura*, which represents a worst-case scenario for human health depending on how much metal has bioaccumulated in the tuber. The study addressed the following questions: 1) Does diluting mining tailings with organic soil solve or attenuate the negative effects of mining tailings on radish development? 2) Do radishes cultivated in soil with different proportions of tailings bioaccumulate metals present in the substrate? 3) If radishes do indeed bioaccumulate metals when grown in diluted tailings, does it exceed the tolerable upper intake levels of toxicity for human consumption?

Material and methods

Plant cultivar

The radish cultivar chosen for this study was Crimsom *Gigante* (Feltrin®). The germination time of this cultivar is from four to six days and its development cycle (from sowing to harvest) can vary from 30 days to three months, depending on environmental conditions (Moreira et al., 2019). Radish culturing is nutritionally demanding, requiring high nutrient contents, especially of N and P, in a short period of time for development (Coutinho Neto, Orioli Junior, Cardoso, & Coutinho, 2010).

Sampling design

The soil affected by mining tailings, here simply referred to as tailings (T), was collected on the margin of the Gualaxo do Norte river (20°15'23.09"S; 43°25'7.06"W) in Antônio Pereira, a district of the municipality of

Ouro Preto, Minas Gerais, Brazil. The Gualaxo do Norte river is a tributary of the Doce river. Five treatments were prepared (dilutions in proportion of dry mass) to evaluate the effectiveness of diluting the mining tailings with soil rich in OM (organic soil - Terral®). The organic soil used is non-toxic, sterile, and odorless, free from pests and weeds. It is composed of 50% soil and 50% bovine manure, to improve soil aeration and drainage, thus favoring plant development. Tailings were sifted and homogenized according to the composition of the five treatments: 1) pure tailings ($T_{100\%}$); 2) $tailings_{75\%}$ + $soil_{25\%}$ ($T_{75\%}$); 3) $tailings_{50\%}$ + $soil_{50\%}$ ($T_{50\%}$); 4) $tailings_{25\%}$ + $soil_{75\%}$ ($T_{25\%}$), and 5) pure organic soil ($Soil_{100\%}$). Hence, the treatments were categorized following a progressive dilution of mining tailings in the substrates with organic soil.

Radish seeds were sown in a germination tray containing vermiculite and kept in a Bio-oxygen Demand Incubator (BOD; Ethiktechnology 411D) on a 12 hour photoperiod at 25°C for six days. After germination (6th day), seedlings were transferred from the trays to thirty polyvinyl chloride pots (13 cm high and 7.5 cm diameter) (n = 6 per treatment; total = 30). The pots were taken to a greenhouse laterally surrounded by a 30% light reduction screen (Nortene®) at the *Universidade Federal de Minas Gerais* (UFMG) (19°86'90.73"S; 43°96'65,60"W), Belo Horizonte, Minas Gerais, Brazil. According to the Köppen-Geiger classification, the climate of the region is Cwa - humid subtropical. Temperature varied between 16.44 and 26.81°C during the study period (April–July 2019) [climatological data provided by the Instituto Nacional de Meteorologia INMET, Brazil] (Instituto Nacional de Meterologia [INMET], 2020)[1].

Pots were rotated weekly (12) on the greenhouse's stand to prevent any environmental influence on radish development due to light exposure/microsite effects. Watering occurred on alternate days and each plant received 100 ml of water.

Physical and chemical analyses of tailings

For physical and chemical characterization of the substrates, samples (ca. 500 g) from each pot (n = 6 per treatment; total = 30) were analyzed at the Soil Department at the *Universidade Federal de Viçosa*. The evaluated physical attributes were proportions of clay (kg kg^{-1}), sand (kg kg^{-1}), and silt (kg kg^{-1}), as well as particle density (Teixeira, Donagemma, Fontana, & Teixeira, 2017). According to the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), soils are classified into three categories regarding water retention capacity: sandy (Type 1); medium texture (Type 2); and clayey (Type 3), as provided in the Normative Instruction n. 2 of October 9th, 2008 (Brasil, 2008). Soil chemical attributes were characterized by the available content of Phosphorus (P), Sodium (Na), Potassium (K), Iron (Fe), Zinc (Zn), Manganese (Mn), Cooper (Cu), Cadmium (Cd), and Lead (Pb) using the Mehlich 1 solution (Teixeira et al., 2017). The exchangeable content of Aluminum (Al^{3+}), Calcium (Ca^{2+}), and Magnesium (Mg^{2+}) were extracted using a 1.0 mol L^{-1} solution of potassium chloride (KCl). Hot water was used for Boron (B) extraction. Determination of H+Al used a 0.5 mol L^{-1} calcium acetate solution at pH 7.0. From these data, total exchangeable bases (TEB) and effective cation exchange capacity (ECEC) were calculated. In addition, OM was determined using the Walkley-Black method (Teixeira et al., 2017).

Radish growth and macro/micronutrient analyses

To assess whether the dilution of mining tailings with organic soil solves or attenuates the negative effects of tailings on radish development, the following parameters of plant development were recorded: number of leaves, total leaf area (cm^2), shoot dry mass (g), root dry mass (g), total dry mass (g), and root/shoot ratio. When plants were 90 days old, all individuals (n = 6 per treatment; total = 30) were removed from their pots and gently washed under running water. To obtain total leaf area, all leaves were counted, removed, and photographed for later measurement in ImageJ. To evaluate shoot, root, and total biomass per individual, plant material was left for five days in an air circulation oven at 65°C until weight stabilized and subsequently measured for dry weight using a high-precision scale (0.0001 mg accuracy).

To assess whether radishes cultivated in soil with different proportions of tailings bioaccumulate metals present in the substrate, we determined the macro and micronutrient contents of radish tubers. After drying, samples containing 1g of grounded dry matter were analyzed at the Soil Department at the *Universidade Federal de Viçosa*. The nitric perchloric digestion method was used to evaluate the contents of P, K, Ca^{2+}, Mg^{2+}, Zn, Fe, Mn, Cu, and B.

[1] Retrieved on May 2020, from http://www.inmet.gov.br/webcdp/climatologia/normais

Statistical analysis

The Shapiro-Wilk test was used to assess data distribution, which was not normal. Thus, nonparametric Kruskal-Wallis tests followed by Dunn's method for pairwise comparisons (Quinn & Keough, 2002) were used to compare the following: substrate physical attributes (proportion of clay, sand, silt, and particle density), substrate chemical attributes (OM, P, K, Ca^{2+}, Mg^{2+}, B, Zn, Mn, Cu, Pb, Cd, H+Al, TEB, and ECEC), plant growth parameters (number of leaves, total leaf area, shoot dry mass, root dry mass, total dry mass, and root/shoot ratio) and tuber macro and micronutrient contents (N, P, K, Ca^{2+}, Mg^{2+}, Zn, Fe, Mn, Cu, and B). All statistical analyses were performed using SigmaStat software (version 3.5) and the graphs were prepared using SigmaPlot (version 10.0).

Results and discussion

Physical and chemical analyses of tailings

Substrate physical characteristics and soil type varied according to dilution of tailings and classifications were made considering the United States Department of Agriculture (USDA) (Figure 1), MAPA and the Brazilian Society of Soil Science (acronym in Portuguese, SBCS) (Table 1). Comparing the extreme treatments (i.e., $Soil_{100\%}$ vs. $T_{100\%}$), the substrate of $Soil_{100\%}$ presented three times more silt (0.15 kg kg^{-1}), ten times more clay (0.68 kg kg^{-1}), ten times less sand (0.08 kg kg^{-1}), and smaller particle density (2.44 g cm^{-3}) compared to the substrate of $T_{100\%}$ (Table 1). In general, all treatments showed low proportions of silt, while there was a reduction in clay and an increase in sand proportions in treatments with mining tailings (Figure 1).

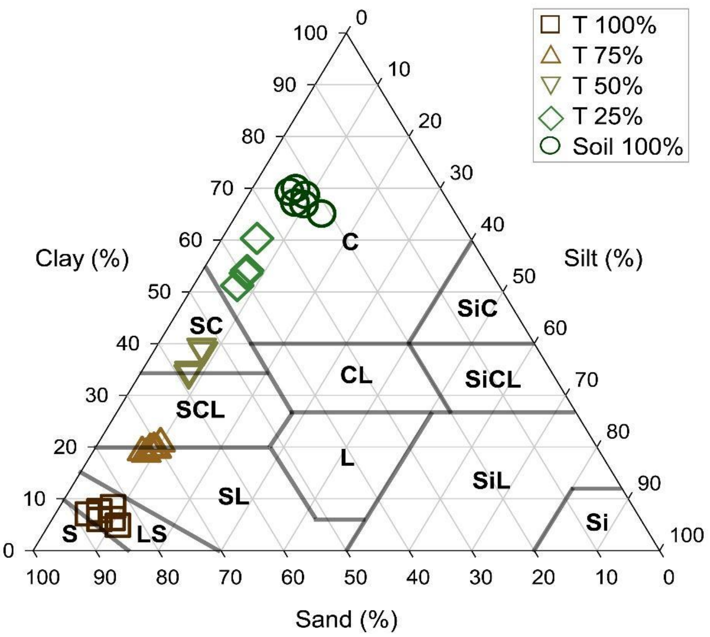

Figure 1. Proportion of sand, silt and clay found in substrates used for *Raphanus sativus* cultivation with different proportions of mining tailings from the Fundão dam in Mariana, Brazil. Treatments are: Soil 100%: pure organic soil; T 25%: $tailings_{25\%}$ + $soil_{75\%}$; T 50%: $tailings_{50\%}$ + $soil_{50\%}$; T 75%: $tailings_{75\%}$ + $soil_{25\%}$; T 100%: tailings. Soil texture classification according to USDA (Soil Survey Division Staff 1993): C: clay; CL: clay loam; L: loam; LS: loamy sand; S: sand; SC: sandy clay; SCL: sandy clay loam; Si: silt; SiC: silty clay; SiCL: silty clay loam; SiL: silty loam; SL: sandy loam.

Table 1. Substrate physical characteristics ($\bar{x}$ ± SE) containing different proportions of mining tailings from the Fundão dam in Mariana, Brazil. Different lowercase letters denote statistically significant differences between treatments (p <0.05).

Substrates physical attributes	$T_{100\%}$ ($\bar{x}$ ± SE)	$T_{75\%}$ ($\bar{x}$ ± SE)	$T_{50\%}$ ($\bar{x}$ ± SE)	$T_{25\%}$ ($\bar{x}$ ± SE)	$Soil_{100\%}$ ($\bar{x}$ ± SE)	P-value
Silt (kg kg^{-1})	0.049^{e} ± 0.002	0.065^{d} ± 0.002	0.088^{c} ± 0.002	0.117^{b} ± 0.003	0.15^{a} ± 0.005	< 0.001
Sand (kg kg^{-1})	0.802^{a} ± 0.008	0.647^{b} ± 0.007	0.467^{c} ± 0.014	0.268^{d} ± 0.016	0.08^{e} ± 0.004	< 0.001
Clay (kg kg^{-1})	0.067^{e} ± 0.005	0.195^{d} ± 0.003	0.368^{c} ± 0.013	0.548^{b} ± 0.019	0.68^{a} ± 0.007	< 0.001
Particle density (g cm^{-3})	2.988^{a} ± 0.019	2.833^{b} ± 0.019	2.693^{c} ± 0.025	2.598^{c} ± 0.032	2.44^{d} ± 0.016	< 0.001
Soil type[1]	1	2/1	2/3	3	3	-
Soil texture classification[2]	Loamy Sand	Sandy Clay Loam	Sandy Clay	Clay	Clay	-

As predicted, the addition of organic soil improved the overall quality of substrates (Table 2). Comparison of the treatments $Soil_{100\%}$ vs. $T_{100\%}$ revealed that all of the chosen substrate quality parameters differed significantly, with the exception of Cu and Cd contents (Table 2). There was a drastic reduction in OM (38.5 times; p <0.001; Table 2), in macronutrients Ca^{2+} and Mg^{2+} (12.4, and 8.1 times lower, respectively; p <0.001; Table 2), and micronutrients B and Zn (2.8, and 3.4 times lower, respectively; p <0.001; Table 2). In addition, the $T_{100\%}$ treatment also had 12 times lower ECEC than the $Soil_{100\%}$ treatment (p <0.001; Table 2), which indicates severe fertility loss in relation to the ideal condition for crop cultivation. Furthermore, the treatment with minimum dilution ($T_{75\%}$) marginally improved substrate quality by increasing OM, K, Zn, TEB, and ECEC to a point that it did not differ significantly from $Soil_{100\%}$. Similarly, substrate fertility also improved with tailings reduction by half ($T_{50\%}$). Otherwise, there was still 2.7 times less Ca^{2+} and 1.5 times less Mg^{2+}, 1.02 times more Fe (p <0.009), and 3.3 times more Mn in relation to the $Soil_{100\%}$ treatment (p <0.001; Table 1). Finally, even though the $T_{25\%}$ treatment had the minimum amount of mining tailings, there was still 1.4 times more Fe (p <0.009), 3.3 times more Mn, and 2.2 times less Ca^{2+} than for the $Soil_{100\%}$ treatment (p <0.001; Table 2).

Table 2. Fertility of substrates ($\bar{x}$ ± SE) containing different proportions of mining tailings from the Fundão dam in Mariana, Brazil. Different lowercase letters denote statistically significant differences between treatments (p <0.05).

Substrate fertility attributes	$T_{100\%}$ ($\bar{x}$ ± SE)	$T_{75\%}$ ($\bar{x}$ ± SE)	$T_{50\%}$ (x ± SE)	$T_{25\%}$ ($\bar{x}$ ± SE)	$Soil_{100\%}$ ($\bar{x}$ ± SE)	p-value
OM (dag kg^{-1})	0.171^{b}±0.042	1.683^{ab}±0.051	3.228^{ab}±0.151	5.340^{a} ±0.398	6.59^{a}±0.148	< 0.001
P (mg dm^{-3})	3.333^{b}±0.109	3.616^{b}±0.087	5.350^{ab}±0.237	7.075^{ab} ±0.111	10.85^{a}±0.848	< 0.001
K (mg dm^{-3})	2.667^{b}±0.422	8.00 ^{ab}±1.633	10.50^{ab}±1.500	17.50^{a}±2.500	21.17^{a}±7.405	< 0.001
Ca^{2+} ($cmol_c$ dm^{-3})	0.633^{c}±0.016	2.56^{b}±0.035	2.893^{b}±0.119	3.553^{b}±0.169	7.85^{a}±1.487	< 0.001
Mg^{2+} ($cmol_c$ dm^{-3})	0.09^{d}±0.003	0.375^{c}±0.012	0.490^{b}±0.020	0.663^{a}±0.023	0.730^{a}±0.044	< 0.001
B (mg dm^{-3})	0.09^{b}±0.005	0.11^{b}±0.010	0.25^{a}±0.036	0.22^{a}±0.020	0.25^{a}±0.008	< 0.001
Zn (mg dm^{-3})	1.391^{b}±0.079	2.236^{ab}±0.136	3.095^{ab}±0.303	4.428^{a}±0.100	4.76^{a}±0.282	< 0.001
Fe (mg dm^{-3})	85.0^{a}±10.404	55.51^{ab}±2.210	54.70^{ab}±2.997	74.05^{a}±6.643	53.73^{b}±6.314	= 0.009
Mn (mg dm^{-3})	67.11^{a}±0.024	78.78^{a}±1.891	71.65^{a}±1.785	69.8^{a}±2.31	21.42^{b}±2.606	< 0.001
Cu (mg dm^{-3})	0.75^{b}±0.020	1.12^{b}±0.032	1.30^{ab}±0.028	1.56^{a}±0.033	1.17^{b}±0.102	< 0.001
Pb (mg dm^{-3})	0.77^{b}±0.108	1.45^{b}0±.131	2.23^{ab}±0.028	2.61^{ab}±0.101	5.03^{a}±0.451	< 0.001
Cd (mg dm^{-3})	0.37^{b}±0.071	0.49^{b}±0.056	0.70^{ab}±0.063	0.79^{a}±0.073	0.55^{b}±0.057	= 0.009
H + Al ($cmol_c$ dm^{-3})	0^{b}±0	2.60^{b}±0.077	5.8 ^{ab}±0.191	8.425^{ab}±0.149	9.95^{a}±0.450	< 0.001
TEB ($cmol_c$ dm^{-3})	0.733^{b}±0.016	2.968^{ab}±0.045	3.445^{ab}±0.135	4.313^{ab}±0.192	8.69^{a}±1.553	< 0.001
ECEC ($cmol_c$ dm^{-3})	0.733^{b}0±.016	2.968^{ab}±0.045	3.445^{ab}±0.135	4.51^{ab}±0.192	8.8^{a}±1.469	< 0.001

The results showed that the tailings from the Fundão dam of the Samarco Mariana Mining Complex were characterized as sandy and of low porosity; thus, confirming other studies that described the characteristics of the tailings from the Fundão dam (Segura et al., 2016; Silva et al., 2016; Andrade et al., 2018). The lower water and nutrient retention capacities of substrates containing tailings is associated with high fine sand content and low porosity, which can be detrimental to plant development (Mengel, Kirkby, Kosegarten, & Appel, 2001). In addition, substrates with low porosity offer higher resistance to root penetration and reduced growth pressure (Passioura, 2002; Tracy, Black, Roberts, & Mooney, 2011; Edrris, Al-Gaadi, Hassaballa, Tola, & Ahmed, 2020). These substrate characteristics in treatments containing tailings may partially explain the observed diminished tuber development in *R. sativus*.

Radish development

The presence of mining tailings in the substrate was detrimental to *R. sativus* development, regardless of the proportion of tailings in the substrate ($T_{100\%}$, $T_{75\%}$, $T_{50\%}$, $T_{25\%}$). In other words, individual radish plants cultivated in the substrate without mining tailings ($Soil_{100\%}$) developed better than they did in any treatment containing tailings (Figure 2). Comparison of the extreme treatments ($Soil_{100\%}$ vs $T_{100\%}$) revealed that radishes cultivated in $Soil_{100\%}$ had more leaves and larger total leaf area. In addition, individual *R. sativus* plants cultivated in $Soil_{100\%}$ presented greater biomass than those cultivated in $T_{100\%}$; which was mainly allocated to their roots (root/shoot ratio).

Raphanus sativus cultivated in $Soil_{100\%}$ had, on average, seven leaves per plant. This number of leaves was significantly greater than all other treatments (p = 0.007), even when compared to plants cultivated in the lowest tailings proportion ($T_{25\%}$), which had four leaves. Individuals cultivated in $Soil_{100\%}$ had 2.3 times more leaves than radishes cultivated in $T_{100\%}$ and 1.75 times more than individuals cultivated in the other treatments ($T_{25\%}$, $T_{50\%}$, $T_{75\%}$) (P = 0.007; Figure 3a). Similar results were observed for *R. sativus* leaf area, with individuals cultivated in $Soil_{100\%}$ having an average total leaf area of 111.67 cm². Leaf area was significantly greater for individuals cultivated in $Soil_{100\%}$ than for individuals cultivated in all other treatments (p <0.001; Figure 3b), specifically, 2.8 times greater than the leaf area of individuals cultivated in $T_{25\%}$ (39.85 cm²); 2.9 times greater than in $T_{50\%}$ (38.73 cm²); 6.9 times greater than in $T_{75\%}$ (16.19 cm²); and 19.0 times greater than in $T_{100\%}$ (5.87 cm²) (p <0.001, Figure 3b).

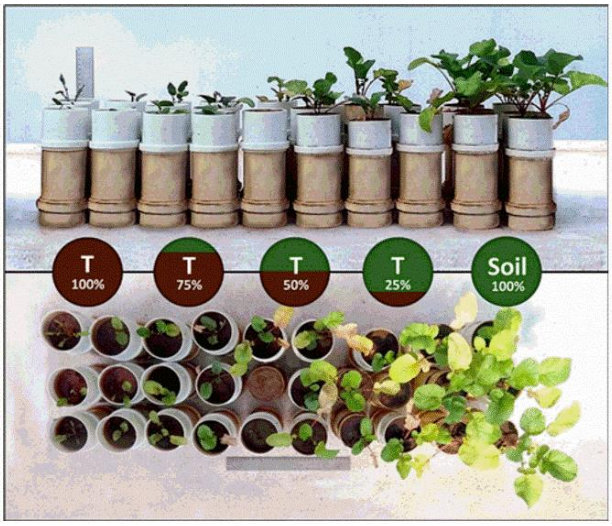

Figure 2. *Raphanus sativus* individuals cultivated in substrates containing different proportions of mining tailings from the Fundão dam in Mariana, Brazil. From left to right: 1) pure tailings ($T_{100\%}$); 2) $tailings_{75\%}$ + $soil_{25\%}$ ($T_{75\%}$); 3) $tailings_{50\%}$ + $soil_{50\%}$ ($T_{50\%}$); 4) $tailings_{25\%}$ + $soil_{75\%}$ ($T_{25\%}$), and 5) pure organic soil ($Soil_{100\%}$).

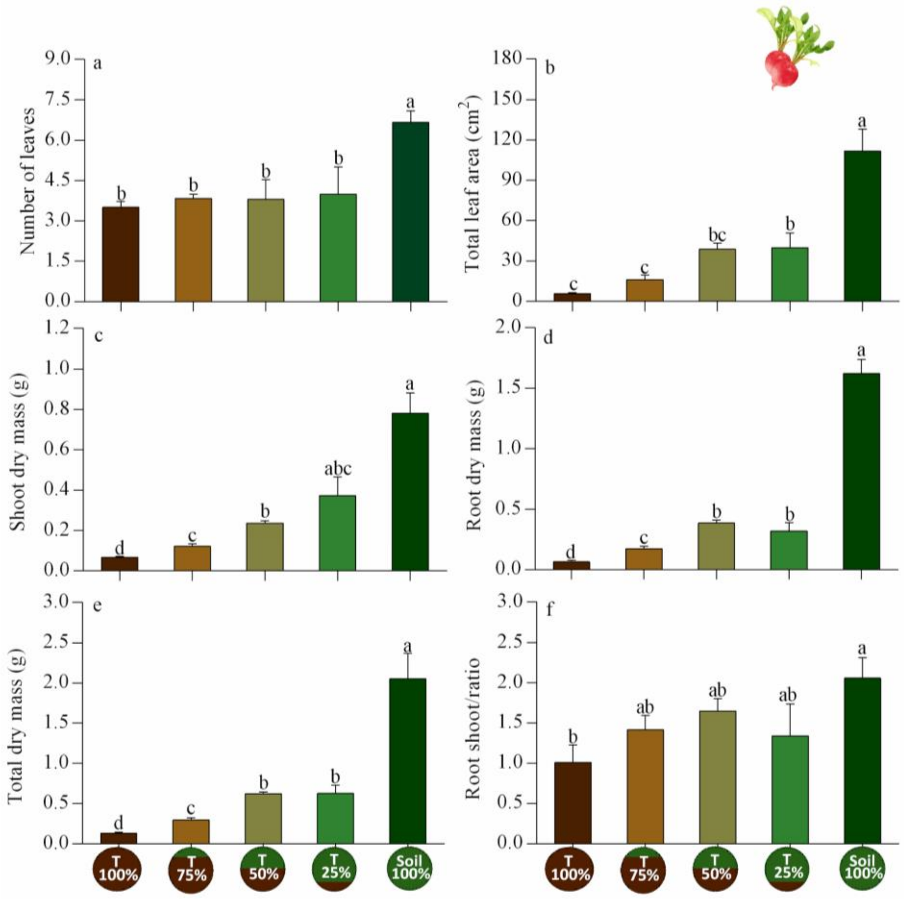

Figure 3. Number of leaves (a), total leaf area (b), shoot dry mass (c), root dry mass (d), total dry mass (e), and root/shoot ratio (f) ($\bar{x}$ ± SE) of radishes cultivated in substrates containing different proportions of mining tailings from the Fundão dam in Mariana, Brazil. Different lowercase letters indicate statistically significant differences between treatments (p <0.05).

Individual radishes cultivated in the $Soil_{100\%}$ treatment had significantly greater shoot dry mass (SDM) than those cultivated in any other treatment, with the exception of $T_{25\%}$ ($p < 0.001$; Figure 3c). Specifically, SDM was 1.9 times greater for radishes cultivated in $Soil_{100\%}$ than for those cultivated in $T_{25\%}$, 2.5 times greater than in $T_{50\%}$, 4.6 times greater than in $T_{75\%}$, and 8.71 times greater in $T_{100\%}$ ($p < 0.001$; Figure 3c). Similarly, root dry mass (RDM) was greatest for radishes in $Soil_{100\%}$ ($p < 0.001$, Figure 3d). RDM was 3.9 times greater for individuals in $Soil_{100\%}$ than for those in $T_{25\%}$, 3.4 times greater than in $T_{50\%}$, 7.2 times greater than in $T_{75\%}$, and 18.7 times greater than in $T_{100\%}$ ($p < 0.001$; Figure 3d). Likewise, *R. sativus* in $Soil_{100\%}$ had the greatest total biomass among treatments, being 2.9 times greater than for plants in $T_{25\%}$, 2.9 times greater than in $T_{50\%}$, 6.2 times greater than in $T_{75\%}$, and 13.7 times greater than in $T_{100\%}$ ($p < 0.001$; Figure 3e). Finally, root/shoot biomass ratio only differed between the extreme treatments ($Soil_{100\%}$= 2.06, $T_{100\%}$= 1.01) ($p = 0.0497$; Figure 3f).

Radish macro and micronutrient analyses

In comparison with radishes cultivated in $Soil_{100\%}$, the different concentrations of mining tailings in the substrates ($T_{25\%}$, $T_{50\%}$, $T_{75\%}$, $T_{100\%}$) did not change tuber content of the macronutrients P, K, Ca, and Mg ($p > 0.05$; Table 3). Zn content was lower for all tailings treatments, with the exception of $T_{25\%}$ ($p = 0.005$; Table 3). Besides, radishes cultivated in substrates with mining tailings bioaccumulated high concentrations of the micronutrients Fe, Mn, Cu, and B; Fe content presented the greatest difference among micronutrients, even when cultivated in the lowest concentration of tailings ($T_{25\%}$; $p = 0.025$; Table 3). Specifically, the Fe content of radishes cultivated in $T_{25\%}$ and $T_{50\%}$ increased 1.3 and 0.8 times, respectively, compared to radishes grown in $Soil_{100\%}$ ($p = 0.025$; Table 3). Moreover, radishes in $T_{75\%}$ had 1.9 times more Fe than plants grown in $Soil_{100\%}$ ($p = 0.025$; Table 3).

Radish individuals cultivated in $T_{100\%}$ had 13.1 times more Fe bioaccumulated in their tubers than did those cultivated in $Soil_{100\%}$ ($p = 0.025$; Table 3). Moreover, it was even 6.9 times greater than the Fe content found in the treatment with the second greatest amount of tailings ($T_{75\%}$). Tuber bioaccumulation of Mn differed significantly between radishes cultivated in $T_{75\%}$ and $T_{100\%}$ ($p = 0.002$; Table 3). Notably, radishes cultivated in $T_{75\%}$ had 5.2 times higher Mn content than did those cultivated in $Soil_{100\%}$. Moreover, radishes cultivated in $T_{100\%}$ had 71.6 times higher Mn content than did those cultivated in $Soil_{100\%}$ ($p = 0.002$; Table 3). Cu tuber content was significantly higher only in $T_{100\%}$, being 6.1 times greater than that for cultivation in $Soil_{100\%}$ ($p < 0.001$; Table 3). Finally, B tuber content only differed between $Soil_{100\%}$ and $T_{100\%}$ ($p = 0.047$; Table 3).

Table 3. Tuber nutritional quality ($\bar{x} \pm SE$) for radishes cultivated in substrates containing different proportions of mining tailings from the Fundão dam in Mariana, Brazil. Different lowercase letters denote statistically significant differences between treatments ($p < 0.05$).

Plant nutritional attributes	$T_{100\%}$ ($\bar{x} \pm SE$)	$T_{75\%}$ ($\bar{x} \pm SE$)	$T_{50\%}$ ($\bar{x} \pm SE$)	$T_{25\%}$ ($\bar{x} \pm SE$)	$Soil_{100\%}$ ($\bar{x} \pm SE$)	p-value
P ($mg\ g^{-1}$)	$0.772^{a}\pm0.32$	$1.128^{a}\pm0.086$	$1.30\ ^{a}\pm0.209$	$1.740^{a}\pm0.275$	$1.451^{a}\pm0.158$	0.066
K ($mg\ g^{-1}$)	$21.235^{a}\pm1.670$	$22.149^{a}\pm0.548$	$22.35^{a}\pm3.084$	$16.571^{a}\pm0.603$	$20.27^{a}\pm1.036$	0.068
Ca^{2+} ($mg\ g^{-1}$)	$9.987^{a}\pm0.178$	$7.227^{a}\pm0.582$	$6.486^{a}\pm0.538$	$7.332^{a}\pm0.557$	$7.121^{a}\pm0.817$	0.139
Mg^{2+} ($mg\ g^{-1}$)	$1.579^{a}\pm0.079$	$1.928^{a}\pm0.160$	$2.036^{a}\pm0.083$	$2.022^{a}\pm0.319$	$2.039^{a}\pm0.174$	0.710
Zn ($mg\ g^{-1}$)	$0.028^{b}\pm0.003$	$0.025^{b}\pm0.001$	$0.020^{b}\pm0.001$	$0.037^{a}\pm0.004$	$0.029^{a}\pm0.001$	0.005
Fe ($mg\ g^{-1}$)	$25.401^{a}\pm0.626$	$3.657^{b}\pm0.879$	$1.646^{c}\pm0.214$	$2.435^{c}\pm0.367$	$1.939^{c}\pm0.488$	<0.005
Mn ($mg\ g^{-1}$)	$1.074^{a}\pm0.019$	$0.078^{b}\pm0.013$	$0.046^{c}\pm0.006$	$0.037^{c}\pm0.008$	$0.015^{c}\pm0.001$	0.002
Cu ($mg\ g^{-1}$)	$1.9^{a}\pm0.0002$	$0.3^{b}\pm0.0002$	$0.34^{b}\pm0.0005$	$0.29^{b}\pm0.03$	$0.31^{b}0.0003$	<0.001
B ($mg\ g^{-1}$)	$0.071^{a}\pm0.017$	$0.040^{b}\pm0.001$	$0.034^{b}\pm0.001$	$0.038^{b}\ \pm0.001$	$0.036^{b}\pm0.001$	0.047

Naturally, the occurrence of plant physiological disorders is linked to physical and chemical aspects of tailings. In such situations, OM content is an important indicator of soil quality and fertility (Zgorelec et al., 2019). Tailings from the ruptured Fundão dam are extremely poor in OM, which is reflected in the substrate's poor ECEC and nutrient content. Therefore, diluting contaminated substrates with soil rich in OM improves its biophysical properties, increasing its capacity to retain water as well as the presence of microorganisms (e.g., Asmelash, Bekele, & Birhane, 2016; Andrade et al., 2018). Increased nutrient availability has shown positive results for plant development in tailings (Bahia et al., 2020; Esteves et al., 2020). Therefore, the dilution of mining tailings with organic soil could enable agriculture production in contaminated substrates, depending on the dilution required for acceptable crop growth, quality for consumption, and associated costs.

Plants need a minimum content of macro and micronutrients for optimal growth, without exceeding a species' threshold (Mengel et al., 2001). A deficient or excessive concentration of these mineral elements in the substrate may lead to serious losses to crop yield (e.g., Andrade et al., 2018). In the present study, the dilution of mining tailings with organic soil progressively improved the concentration of macronutrients (N, P, K, Ca^{2+}, Mg^{2+}) and micronutrients (B, Zn) in the substrates. The dilution of the mining tailings with organic soil increased radish and tuber development relative to plants grown in treatment $T_{100\%}$. However, even the treatment with only 25% of mining tailings ($T_{25\%}$) produced radishes with almost four times lower root biomass than those cultivated in the treatment without tailings ($Soil_{100\%}$). Therefore, from a strict yield perspective, the costs associated with a 75% dilution of mining tailings may be prohibitive if the outcome is still radishes with a four times lower yield than if in uncontaminated soil.

Although the concentrations of Cu and B in the substrates increased with tailings dilution with organic soil, lower concentrations of these chemical compounds were found in radish tubers. The metal concentration ratio in soil is not directly proportional to the concentration found in plant tissues. Notably, factors such as soil physical structure, organic matter concentration, and nutrient balance can interfere with the mobility of these metals in soil and, consequently, their availability for plants (Cataldo & Wildung, 1978; Mertens, Van Laer, Salaets, & Smolders, 2010; Brdar-Jokanović, 2020). Cu and B are more stable in clayer soils with high OM content (i.e., $Soil_{100\%}$), and thus less available to plants, which is reflected in the low concentration of these elements in plant tissue (Walker, Clemente, Roig, & Bernal, 2003). Furthermore, high concentrations of some metals, such as Fe, can also reduce Cu mobility and decrease its uptake by roots (Shuman, 1991; Fageria, Baligar, & Clark, 2002).

Chemical analysis showed a significantly higher Fe concentration in radish tubers in soils with tailings than in $Soil_{100\%}$, even for the most diluted treatment ($T_{25\%}$). Similar results were found for Mn, although the highest concentrations were detected only in the less diluted treatment ($T_{75\%}$) and in the treatment with pure mining tailings ($T_{100\%}$). These results corroborate those of Coelho et al. (2020), who also reported high Fe, Mn, Cu and Cr contents in other plant species of high economic interest when grown in soils with mining tailings. High Fe concentrations can precipitate to plant roots and limit the absorption of important nutrients, such as Ca, Mg, K, and P, by the plant (Tanaka, Loe, & Navasero, 1966). Furthermore, excessive Fe can alter a plant's nutrient cycle and metabolism, resulting in significant reductions to growth, flowering, and fruiting (Grantz, Garner, & Johnson, 2003; Grotz & Guerinot, 2006). Mn also participates in a variety of plant metabolic and physiological processes, but if it exceeds a threshold, it can also lead to impacts such as chlorophyll biosynthesis impairment, the triggering of oxidative stress, and enzymatic activity impairment (Li et al., 2019). Furthermore, the excessive absorption of metals, such as Fe and Mn, by plants may cause plant tissue chlorosis, leaf necrosis, and further alterations of nutrient translocation and use in plant development (Yang et al., 2015, Chen, Yan, Sun, Tian, & Liao, 2016; Ahammed et al., 2020).

Lastly, elements such as Fe and Mn are also essential to humans as they contribute to amino acid, lipid and carbohydrate metabolism, proteoglycan synthesis, and bone formation, among other processes (Freeland-Graves, Mousa, & Sanjeevi, 2014). However, the consumption of high concentrations of these metals (e.g., through contaminated vegetables) can be extremely detrimental to humans (Padhye, 2003; Lönnerdal, 2017). In fact, Fe bioaccumulation in humans is associated with the formation of reactive oxygen species, DNA mutations, and a variety of mental disorders (Sahu, 1992; Kabat & Rohan, 2007; Onakpa et al., 2018). Excessive ingestion of Mn is associated with psychological and neurological abnormalities linked to the central nervous system (Kwakye, Paoliello, Mukhopadhyay, Bowman, & Aschner, 2015; Haugen et al., 2019). It is because of such dangers to human health that the establishment of a Tolerable Upper Intake Level (UL) is crucial. For instance, the recommended daily UL for Fe, Mn, Cu, and B for men or women older than 19 years of age is 45 mg, 11 mg, 10 mg, and 20 mg, respectively (Padovani, Amaya-Farfán, Colugnati, & Domene, 2006). These maximum levels of daily intake must be carefully considered and investigated before the consumption of plants grown in soil contaminated by mining tailings. Notably, the present study found radishes grown in $T_{100\%}$ to have ca. 25.4 mg g^{-1} of Fe, 1.1 mg g^{-1} of Mn, 1.9 mg g^{-1} of Cu, and 0.07 mg g^{-1} of B.

Conclusion

The physical and chemical properties of soil contaminated by mining tailings from the failed Fundão in the Doce river basin are unsuitable for radish development. Dilution of the tailings with organic soil can improve overall substrate quality and support greater plant development. However, this dilution does not eliminate the detrimental effects of the tailings, as even radishes grown in the most diluted treatment still had tubers with four times lower biomass. Lastly, since radish tubers bioaccumulate some metals present in contaminated soil, further studies should test the suitability of the species as a bioremediation tool.

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