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Does Samarco's spilled mud impair the growth of native trees of the Atlantic Rainforest?

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A R T I C L E I N F O

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A B S T R A C T

The failure of the Fundão dam, the largest environmental disaster in the world's mining sector, was responsible for releasing millions of cubic meters of iron ore tailings into the environment. It affected thousands of hectares of the Atlantic Forest domain, one of the biodiversity hotspots for conservation. Considering the urgency to restore the flora of the affected area, we evaluated the effects that iron ore tailings from the Fundão reservoir have on the germination and initial growth of tree species native to the Atlantic Forest in the Rio Doce basin. We demonstrated that the tailings do not affect the seed germination, but do negatively interfere with plant growth. Lower biomass production, height, leaf area, chlorophyll concentration and photosynthesis as well as high concentration of iron was observed in plants grown in the tailings. Thus, we investigated if these deleterious effects were due to the presence of potentially toxic metals or nutritional deficiency imposed by low fertility of the tailings. We concluded that reduced growth was a result of nutritional limitations due to low nutrient availability, low organic matter content and low cation exchange capacity of the tailings. This conclusion was further supported by the application of fertilization, which reversed the deleterious effect of the waste on the growth of plants, assuring physiological levels of iron and nutrients in the shoot. Thus, this strategy should be considered for *in situ* recovery projects aiming to improve the performance of native plants.

1. Introduction

About 50 million cubic meters of iron ore tailings were released into the environment after the rupture of one of Samarco's containment dams (Fundão dam), located in the municipality of Mariana, Minas Gerais, Brazil (IBAMA, 2015). This event is considered the largest environmental disaster in the world's mining sector, both in terms of volume of dumped tailings and magnitude of damage (Carmo et al., 2017). The large volume of tailings covered a total of 663.2 km of water bodies while passing through the rivers Rio Gualaxo do Norte, Rio do Carmo and Rio Doce until reaching the Atlantic Ocean (IBAMA, 2015). The flood of iron ore tailings destroyed villages, disrupted water supplies in several cities and caused massive mortality of the aquatic and terrestrial biota due to burial and suffocation by mud, resulting in serious environmental and socioeconomic damage to the entire Rio Doce basin (Samarco, 2016; SEDRU, 2016).

Most of the Rio Doce basin (98% of the area) is located within the Atlantic Forest biome (IBAMA, 2015), which possesses high indices for biodiversity and endemism, making it a biodiversity hotspot for conservation (Myers et al., 2000). The rupture of the Fundão dam reached 1587 ha of vegetation cover, including permanent preservation areas and remnants of riparian forests, including 511.08 ha of Atlantic Forest (SEDRU, 2016). According to Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Brazilian Institute of Environment and Renewable Resources; IBAMA, 2015), because it is an inert material with no organic matter, the tailings has the potential to affect soil over time, making it difficult to recover and develop previously established species. In addition, sedimentation of the mud, by burying the soil seed bank, can hinder natural regeneration (IBAMA, 2015).

The physical properties and chemical composition of tailings from

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mining activities depends on the composition of the ore, the products used to extract the metals of interest, the efficiency of the extraction method and the degree of weathering during tailings storage (Kossoff et al., 2014). Soils contaminated with iron ore tailings generally have low organic carbon content and basic nutrients, and contain large amounts of fine particles and high concentrations of some metals (Wong and Tam, 1977; Hudson-Edwards et al., 2003). A previous characterization of the tailings from the Mariana mines (including the Fundão reservoir) showed that the residue is basically composed of goethite and has trace elements at levels below limits established by Associação Brasileira de Normas Técnicas (Brazilian Association of Technical Standards; Pires et al., 2003; Silva et al., 2006).

A study of the tailings from the Fundão dam disaster conducted by an independent group for the Environmental Impact Assessment (GIAIA - Grupo Independente de Avaliação do Impacto Ambiental, 2015), found levels of manganese (Mn), arsenic (As) and lead (Pb) to be higher than that recommended by Brazilian governmental regulations and, although there was no legislation standard, considered iron (Fe) and aluminum (Al) levels to be extremely high. However, other studies have shown that the tailings do not contain trace elements at potentially toxic concentrations, thus indicating controversial results (EMBRAPA, 2015). According to Segura et al. (2016), concentrations of the chemical elements analyzed in the tailings were found to be in agreement with levels allowed by Brazilian environmental agencies and SiO_2, Fe, Mn, copper (Cu), calcium (Ca) and chromium (Cr) were the most abundant components in the tailings. According to Hatje et al. (2017), the concentrations of Fe, As, mercury (Hg) and Mn in the Rio Doce basin after the tailings spill exceeded quality guidelines for sediments. In addition, the force of the volume of tailings discharged into rivers may have stirred up and suspended sediments from the bottom of watercourses, which contain trace metals from historical use that may have been spread along the path of the mud (SEDRU, 2016; Hatje et al., 2017). Queiroz et al. (2018) found the tailings to be composed mainly of feoxyhydroxides (goethite, hematite), kaolinite and quartz, and although it did cause an increase in the concentrations (mg kg^{-1}) of Fe (45,200), Mn (433), Cr (63.9), zinc (Zn) (62.4), nickel (Ni) (24.7), Cu (21.3), Pb (20.2) and cobalt (Co) (10.7) on the banks of Rio Doce they did not exceed the limit concentrations of 75, 300, 30, 60, 72 and 25 mg kg^{-1} for Cr, Zn, Ni, Cu, Pb and Co, respectively, established by Brazilian environmental legislation (CONAMA, 2009) (there are no established values for Fe and Mn).

Since the Brazilian Institute of Environment and Renewable Natural Resources held the liable to Samarco's for environmental restoration (IBAMA, 2015) and considering the need to recover the flora of the affected area using native tree species of the region of the Rio Doce basin, this study aimed to evaluate how iron ore tailings from the Fundão reservoir affect seed germination and initial growth of seedlings of native Atlantic Forest tree species occurring in the region of the disaster. In a pioneering way, we aimed to elucidate whether the effects of the waste are related to the presence of metals at toxic levels or to nutritional deficiency imposed by their low fertility. Towards these objectives, samples of the waste and of soil not impacted by the waste (control) were collected in the region where the disaster occurred. Parameters related to fertility (texture, pH, cation exchange capacity, organic matter and nutrient bioavailability) were analyzed, and the available forms of chemical elements with toxic potential quantified. Plant growth as well as their physiological responses were evaluated in the waste, the control soil as well as in fertilized tailings and soil. Our study provides important evidences for the use of fertilization as a tool for recovery programs of the affected areas.

2. Material and methods

2.1. Plant material

Five native tree species of the Atlantic Forest were selected for this study. The species were selected according to their natural occurrence in the Rio Doce basin (Lombardi and Gonçalves, 2000; França, 2013) and availability of seeds for performing experiments. Germinability and initial growth was investigated for the following species: two species of the family Fabaceae — *Albizia polycephala* (Benth.) Killip ex Record and *Peltophorum dubium* (Spreng.) Taub.; and three species of the family Bignoniaceae — *Cybistax antisyphilitica* (Mart.) Mart, *Handroanthus heptaphyllus* (Vell.) Mattos, *Handroanthus impetiginosus* (Mart. ex DC.) Mattos.

2.2. Soil and tailings sampling

Substrate samples were collected randomly at 10 points along three transects of 100 m (20 cm deep) with six samples/point for a total of 60 samples. Samples were collected on the banks of the Carmo River (20°21′26.45″S, 43°7′4.69″W), in the municipality of Barra Longa (MG, Brazil) in April 2017, 18 months after the rupture of the Fundão dam. Samples were obtained from two distinct areas classified as follows: 1) no affected area – area with no tailing at a distance of 5 m from the tailings collection site, under the same transect (to assure that this was the soil in the area where plants were growing just before the tailing deposition) and 2) area with iron ore tailings deposition. After sampling, the substrates were subsequently homogenized and stored separately in plastic bags at 4 °C to preserve physicochemical and biological characteristics until the experiments were carried out.

2.3. Physical and chemical properties of substrates

Soil and tailings samples were analyzed for primary particle and organic matter (OM) contents. Primary particle composition was determined by sieving, and classified as follows (Camargo et al., 2009): coarse sand (2–0.5 mm), medium-sized sand (0.50–0.21 mm), fine sand (0.21–0.053 mm), silt (0.053–0.002 mm) and clay (< 0.002 mm). Organic matter content was determined by the extent of reduction of a strong oxidizing agent (potassium dichromate) (Camargo et al., 2009). The analyses of pH (determined in $CaCl_2$), potential acidity (H + Al) and cation exchange capacity (CEC) were performed according to EMBRAPA (2011).

The concentration of chemical elements on substrates available for plants were evaluated according to Raij et al. (2001). Total nitrogen (organic + ammoniacal) was quantified using the Kjeldahl method. Potassium, phosphorus, calcium and magnesium were extracted in ion exchange resin. Copper, iron, manganese, zinc, cadmium, chromium, nickel and lead were extracted with DTPA-TEA solution at pH 7.3, while boron was extracted by hot water.

After physicochemical characterization (reported in the Results section) soil samples showed the same characteristics than that present in soils predominating in the Rio Doce Basin (Dystrophic Red-Yellow Latosols/Argisols), such as low pH and low concentration of nutrients (EMBRAPA and Rio de Janeiro, 2006; Coelho, 2009). Therefore, the soil samples used are representative of those occurring in areas where native species were growing before the disaster.

2.4. Experiment I: seed germination

Seeds of *Handroanthus impetiginosus* were provided by Fundação Zoobotânica de Belo Horizonte (Belo Horizonte, MG, Brazil), while *A. polycephala, P. dubium, C. antisyphilitica* and *H. heptaphyllus* seeds were provided by Instituto Espinhaço - Biodiversidade, Cultura e Desenvolvimento Socioambiental (Gouveia, MG, Brazil). Due to their physical dormancy, seeds of *Albizia polycephala* (Dos Santos et al., 2015) and *Peltophorum dubium* (Zuffo et al., 2017) were previously scarified with sandpaper to allow imbibition and germination. This treatment was not required, however, for seeds of *C. antisyphilitica, H. heptaphyllus* and *H. impetiginosus* which do not show physical dormancy. Prior to assembling the germination tests, the seeds were surface disinfested with a solution of 5% sodium hypochlorite (NaClO) for 10 min and then washed with distilled water.

Germination tests comprised three treatments: 1) filter paper (two layers of Whatman No. 1 as a standard control treatment used in seed germination studies); 2) soil; and 3) iron ore tailings from the rupture of the Fundão dam. For each treatment, four replicates of 25 seeds of each species were placed to germinate in transparent germination boxes. The boxes containing filter paper were moistened with distilled water at a proportion of 3.5 times the weight of the filter paper (Brasil, 2009). The substrates (soil and tailings) were moistened with distilled water, maintaining 75% of their field capacity. The replacement of moisture to the substrates was performed according to the initial weight of the germination boxes with the substrate. The experiments were conducted in B.O.D germination chambers at optimum germination temperature for each species (25 °C for *C. antisyphilitica* and 30 °C for *A. polycephala*, *H. heptaphyllus*, *H. impetiginosus* and *P. dubium*) under 12 h photoperiod (white light, 40 μmol de fótons m^{-2} s^{-1}). The number of germinated seeds was monitored daily until the stabilization of germination response. The criterion used for germination was the emergence of 2 mm of the primary root.

2.5. Experiment II: initial growth of seedlings

The seedling's initial growth experiment was carried out using all species: *A. polycephala*, *P. dubium*, *C. antisyphilitica*, *H. heptaphyllus* and *H. impetiginosus*. Plant growth was evaluated in the following treatments (substrates): 1) tailings; 2) soil; 3) fertilized tailings; and 4) fertilized soil. Mixed mineral fertilizer was applied to the soil via "Osmocote Plus 15-9-12" (15% N, 9% P_2O_5, 12% K_2O, 1.3% Mg, 6% S, 0.05% Cu, 0.46% Fe, 0.06% Mn and 0.02% Mo; Dublin, USA – with a nutrient release duration of six months). "Osmocote" fertilizer was used because it is a slow-release fertilizer capable of increasing the efficiency of nutrient use by plants and reducing losses by physical processes (Shaviv and Mikkelsen, 1993), as well as being practical to handle and the possibility of a single application. The concentration used, as suggested by the manufacturer for native tree species, was 7.5 g L^{-1}.

Freshly germinated seeds in filter paper were transplanted into 180 cm^3 polyvinyl chloride tubes containing one of the four substrates using a sample of 24 plants per treatment for each species. The growth experiment was carried out at the Instituto Espinhaço - Biodiversisdade, Cultura e Desenvolvimento Socioambiental, located at Universidade Federal de São João Del Rey, Sete Lagoas campus (MG, Brazil). Plants were randomly arranged and kept in a nursery shaded by a 50% screen with 15-min of micro sprinkler irrigation four times a day. We removed invasive plants manually on a weekly basis.

2.6. Growth parameters

After ten weeks, ten seedlings of each treatment were randomly harvested, washed in distilled water and the shoot and main root length was measured. Then, plants were separated into roots, stem and leaves, which were dried until constant weight (96 h) using a forced air circulation oven at 65 °C to obtain dry mass. The dry mass fraction of the organs was calculated according to Poorter and Nagel (2000) as:

Root weight fraction: $RWF = W_R / W_T$

Stem weight fraction: $SWF = W_S/W_T$

Leaf weight fraction: $LWF = W_L / W_T$

where, W_R = root weight; W_S = stem weight; W_L = leaf weight; W_T = total weight.

2.7. Photosynthetic pigment and chlorophyll a fluorescence

Photosynthetic pigment was measured on samples from the first, second and third fully expanded leaves (physiologically mature leaves) in five seedlings per treatment, for a total of three measurements per plant. Four readings using a SPAD-502 portable chlorophyll meter (Minolta, Japan) were obtained for each leave. Chlorophyll fluorescence measurements were performed in dark acclimated leaves for 30 min to obtain the maximal photochemical efficiency of PSII (FV/FM) (Butler, 1978). The chlorophyll fluorescence emission was assessed using a pulse-amplitude modulation (PAM) fluorometer (model PAM-2500, WALZ, Effeltrich, Germany) with a light saturation pulse of ~10,000 μmol photons m^{-2} s^{-1}. These measurements were performed on fully expanded leaves in five plants per treatment.

2.8. Nutritional analyses

Shoots and roots of five samples from each treatment was ground and dried together, constituting one replicate, with a total of four replicates/treatment. For phosphorous, calcium, magnesium, iron, copper, zinc and manganese evaluations, plant tissues (roots or shoots) were digested at 200 °C using 10 ml of the 4:1 nitric acid + perchloric acid mixture (Sarruge and Haag, 1974). After digestion, the solutions were cooled, filtered through Whatman n° 40 filter paper, and brought to a final volume of 25 ml with ultra-pure water. The filtered extracts were preserved under refrigeration (4 °C) until analysis. Phosphorus concentration was measured colorimetrically using the ascorbic acid method (Braga and Defelipo, 1974), while calcium, magnesium, iron, copper, zinc and manganese were determined using an inductively coupled plasma optical emission spectrometry (ICP-OES PerkinElmer model Optima 8300 DV). For nitrogen, samples were digested at 350 °C using 5 ml of sulfuric acid and after been cooled, filtered and completed to 10 ml with ultra-pure water, total N was determined by the Kjeldahl method. Due to the lack of enough plant material, nutritional evaluations were not performed in the roots of *P. Dubium* (all nutrients) and *H. Heptaphyllus* (only N).

2.9. Statistical analyses

Generalized linear models (GLMs) were created to assess differences in fertility parameters (Gaussian distribution) between substrates (tailings and soil), percentage of germination (binomial distribution) between treatments (filter paper, tailings, soil), growth/photosynthetic parameters and nutrient concentration (both Gaussian distribution) between substrates (soil and tailings) for each species and organs separately. For growth, photosynthesis and nutrient data, interaction between substrate (tailings and soil) and fertilization (absence or presence) were included in the model. Full models were constructed and subsequently simplified by removing non-significant explanatory variables until obtaining the minimal adequate model. Residual analysis was conducted to test the suitability of the minimal model for error distribution. We tested each model against a null model using analysis of variance. In cases where the interaction between the explanatory variables was present, we subjected the levels of the explanatory variables to a contrast analysis, enabling separation of significantly different levels ($P < 0.05$) and lumping of significantly similar levels (Crawley, 2012). Furthermore, data of nutritional analyses were submitted to a multivariate analysis by applying the values of each variable to a Principal Component Analysis (PCA) based on correlation. Statistical analyses were performed using R software (R Development Core Team, 2014).

3. Results

3.1. Chemical and physical properties of substrates

pH and the concentrations of the macronutrients nitrogen (N), potassium (K) and magnesium (Mg), and the concentrations of the available forms of the micronutrient boron, copper, iron, manganese and

Table 1
Soil properties and bioavailability of chemical elements (OM is the organic matter and CEC is the cationic exchange capacity). Values of averages ± SD of four replicates . P values < 0.05 indicate significant differences by analysis of variance between soil and tailings. na = not applicable.

Parameters	Soil	Tailings	F	P value
pH ($CaCl_2$)	4.8 ± 0.08	6.1 ± 0.14	253.5	< 0.001
OM (g/dm^3)	14 ± 0.82	0.75 ± 0.50	766.09	< 0.001
N (g/kg)	0.88 ± 0.05	0.15 ± 0.10	168.2	< 0.001
P (mg/dm3)	3.75 ± 0.50	9.48 ± 1.73	40.692	< 0.001
K (mmol$_c$/dm^3)	2.65 ± 0.06	0.23 ± 0.05	4032.4	< 0.001
Ca^{2+} (mmol$_c$/dm^3)	9.75 ± 0.96	12.25 ± 0.96	13.636	< 0.05
Mg^{2+} (mmol$_c$/dm^3)	6.25 ± 0.50	0	625	< 0.001
H + Al (mmol$_c$/dm^3)	23 ± 2.16	7 ± 0.00	219.43	< 0.001
CEC (mmol$_c$/dm^3)	41.65 ± 2.94	19.5 ± 0.95	205.48	< 0.001
Fe (mg/dm^3)	35 ± 2.94	9.25 ± 0.50	297.45	< 0.001
Mn (mg/dm^3)	33.48 ± 1.59	18.55 ± 0.47	322.93	< 0.001
Zn (mg/dm^3)	1.03 ± 0.26	0.05 ± 0.06	52.448	< 0.001
Cu (mg/dm^3)	0.85 ± 0.06	0.2 ± 0.00	507	< 0.001
B (mg/dm^3)	0.39 ± 0.02	0.32 ± 0.01	52.418	< 0.001
Cd (mg/dm^3)	0	< 0.1	na	na
Cr (mg/dm^3)	0	< 0.1	na	na
Ni (mg/dm^3)	0.2 ± 0.03	< 0.01	216.6	< 0.001
Pb (mg/dm^3)	1.53 ± 0.07	0.28 ± 0.03	1191.4	< 0.001
Clay (%)	29.9	6.9	na	na
Silt (%)	20.4	44.1	na	na
Coarse sand (%)	10	0.9	na	na
Medium sand (%)	17.9	2.8	na	na
Fine sand (%)	21.9	45.4	na	na

zinc, were significantly higher ($P < 0.05$) in the soil in relation to the tailings (Table 1). While lead concentration (1.5 mg/dm^3) was significantly higher ($P < 0.05$), cadmium and chromium were not found in the soil and were found below 0.1 mg/dm^3 in the tailings. The concentration of nickel was below 0.1 mg/dm^3 in the tailings and 0.2 mg/dm^3 in the soil (Table 1).

The cation exchange capacity of the tailings (19.5 mmolc/dm^3) was two times lower and the organic matter concentration (0.75 g/dm^3) 18 times lower than in the soil (41.7 mmolc/dm^3 and 14 g/dm^3, respectively). The iron ore tailings presented high silt (44.1%) and very fine sand (26.6%), and only 6.9% clay, being classified as sandy loam soil (Table 1). In contrast, the soil was classified as sandy-clay loam, presenting higher percentage of clay (29.9%), and lower percentage of silt (20.4%) and very fine sand (7.1%).

3.2. Seed germination

Germinability of *A. polycephala*, *H. heptaphyllus* and *H. impetiginosus* seeds did not differ among treatments ($P > 0.05$; Fig. 1). Germination of *C. antisyphilitica* seeds was similar in the tailing and filter paper ($P > 0.05$). For seeds of *P. dubium,* however, lower germinability was observed in the tailings ($P < 0.05$; Fig. 1).

3.3. Initial plant growth

Seedlings of *A. polycephala* and *H. heptaphyllus* presented lower shoot length in the tailings compared to the soil ($P < 0.001$; Fig. 2). A significant interaction ($P < 0.05$) was observed between substrate and fertilization for *P. dubium* shoot length (Fig. 2). Regardless of the substrate, fertilization increased shoot length. Moreover, while the shoot length did not differ in fertilized-plants between the substrates, non-fertilized plants grown in tailings were smaller. The seedlings of *C. antisyphilitica* and *H. impetiginosus* were smaller in the non-fertilized treatments, and their shoot length did not differ ($P > 0.05$) between soil and tailings. Seedlings of *C. antisyphilitica* and *H. impetiginosus* showed higher root lengths in the treatments without fertilization ($P < 0.05$). The main root of the *A. polycephala* seedlings presented longer length in the tailing. ($P < 0.001$; Fig. 2).

A significant interaction ($P < 0.001$) was observed between substrate and fertilization for the leaf area of *P. dubium*, *C. antisyphilitica*, *H. heptaphyllus* and *H. impetiginous* ($F = 51.862$; 42.005; 32.301; 13.4998, respectively; Table 2). For all the species, the total leaf area was significantly lower ($P < 0.001$) in seedlings grown in the tailing and the fertilization of substrates significantly increased ($P < 0.001$) the total leaf area in relation to non-fertilized soil or tailings. For *P. dubium* and *H. impetiginous* seedlings, the total leaf area did not significantly differ ($P < 0.05$) between the fertilized treatments (Table 2).

3.4. Allocation of biomass

The total dry mass of the seedlings of the five species studied was lower when they grew in the tailings (Fig. 2; $P < 0.001$). Seedlings grown on fertilized substrates (soil and tailings) had higher dry mass compared to the same substrates without fertilization ($P < 0.05$). With exception of *A. polycephala,* a significant interaction ($P < 0.05$) between substrate and fertilization was observed for dry mass (Fig. 2). While for *C. antisyphilitica, H. heptaphyllus and H. impetiginosus* the dry mass of the seedlings was higher when grown in the fertilized soil than in the fertilized tailings ($P < 0.05$), in *P. dubium* plants had higher dry mass in the fertilized tailings treatment ($P < 0.05$).

Seedlings of *H. heptaphyllus* and *H. impetiginosus* grown in the tailings without fertilization proportionally allocated more biomass in the roots than the seedlings that grew in the other treatments (Fig. S1). With exception to *A. polycephala*, the fertilization of substrates (soil and taillings) favored the allocation of biomass in leaves (Fig. S1). *A. polycephala* showed a similar biomass partition among the treatments regardless of the studied organs (leaves, stems and roots).

3.5. Chlorophyll concentration and photosynthesis

The fertilization increased chlorophyll concentration in *H. impetiginosus* ($P < 0.001$; $F = 142.04$) and *P. dubium* ($P < 0.001$; $F = 405.624$), in which high chlorophyll concentration was observed in seedlings grown in soil than in tailings (Table 2). A significant interaction ($P < 0.05$) between substrate and fertilization was observed for chlorophyll concentration in seedlings of *A. polycephala*, *C. antisyphilitica* and *H. heptaphyllus* ($F = 14.408$; 7.8004; 18.403, respectively). For these species, chlorophyll concentration was lower ($P < 0.05$) in non-fertilized seedlings grown in the tailings in relation to those grown in soil (Table 2). With exception of *H. heptaphyllus,* fertilized seedlings grown in tailings had similar chlorophyll concentration than those grown in soil.

A significant interaction ($P < 0.01$) was observed between substrate and fertilization for *C. antisyphilitica* and *H. heptaphyllus* photosynthesis, as evaluated by the maximal photochemical efficiency of PSII (F_V/F_M) ($F = 9.1587$ and 13.506, respectively; Table 2). For these species, F_V/F_M was lower ($P < 0.01$) in non-fertilized seedlings grown in the tailings in relation to those grown in soil (Table 2). This was also observed in fertilized seedling of *H. heptaphyllus*, while in *C. antisyphilitica* the fertilization resulted in similar F_V/F_M between seedlings grown in soil and tailings. For *H. impetiginosus* and *P. dubium* species, seedlings grown in fertilized substrates showed great ($P < 0.01$) F_V/F_M in relation to seedlings grown in non-fertilized substrates ($F = 11.169$ and 15.017, respectively; Table 2). F_V/F_M values were higher in *A. polycephala* seedlings grown in soil than in the tailings ($P < 0.05$; $F = 6.2405$, Table 2).

3.6. Mineral concentration

Fertilization significantly increased ($P < 0.05$) N (exception to *H. heptaphyllus*) and P concentrations in shoots (Fig. 4). With exception of *C. antisyphilitica,* a significant interaction ($P < 0.05$) between substrate and fertilization was observed for shoot P concentrations. In relation to plants grown in non-fertilized tailing, P concentrations in shoots were

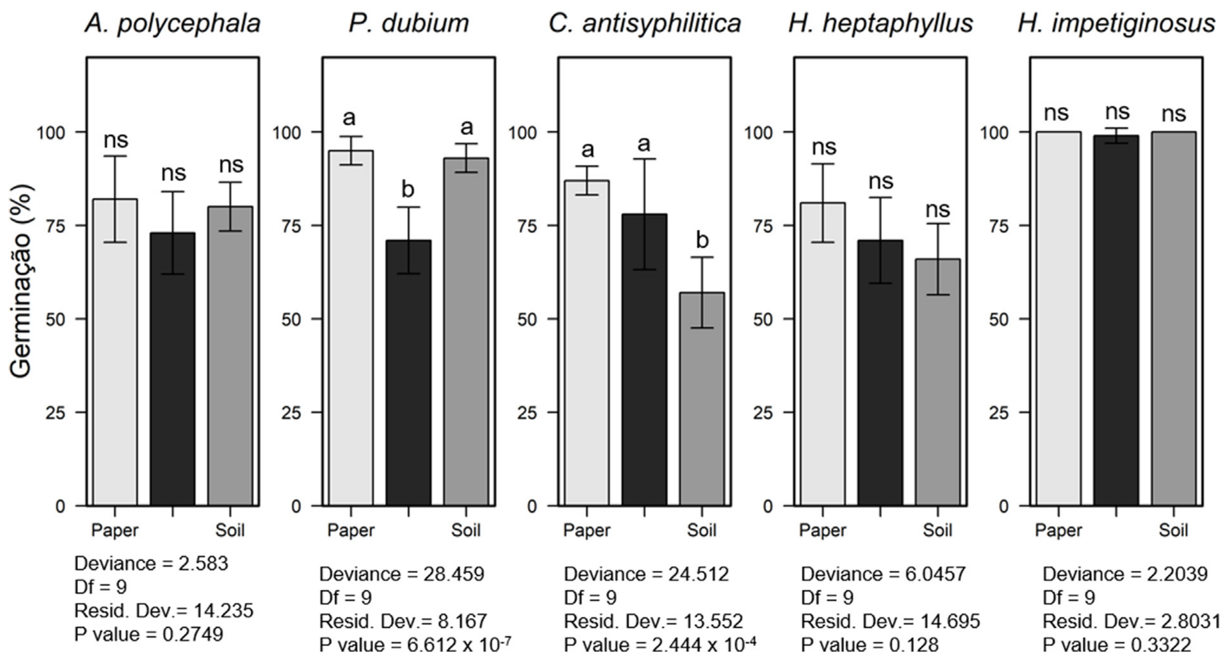

Fig. 1. Germination percentage for five tree species submitted to different substrates (filter paper, tailings, soil). Bars represent means ± SD of four replicates. Different letters indicate significant differences by chi-squared test between treatments ($p < 0.05$). ns = not significant.

greater in plants from the fertilized-tailing treatment (Fig. 3). For all the studied species, root concentrations of N and P were greater ($P < 0.05$) in fertilized compared to non-fertilized plants. A significant interaction ($P < 0.05$) between substrate and fertilization was also observed for root N (for *C. antisyphilitica* and *H. impetiginosus*) and P (for *H. impetiginosus*) concentrations. N and P concentration in roots of *C. antisyphilitica* was greater while in *H. impetiginosus* N concentration did not significantly differ between fertilized-plants grown in tailings and soil. For *A. polycephala*, *P. dubium and H. impetiginosus*, K concentration was greater in shoots from fertilized than unfertilized substrates (Fig. 3). With exception to *H. heptaphyllus*, K concentrations in roots were greater in plants from fertilized than in unfertilized tailings (Fig. 4).

In *A. polycephala* and *C. antisyphilitica* greater Mg concentrations in shoots and roots were observed in plants from fertilized in relation to unfertilized tailings (Figs. 3 and 4). In contrast, *H. impetiginosus* seedlings showed greater Mg concentrations in shoots when grown in unfertilized substrates. A significant interaction ($P < 0.05$) was observed between substrate and fertilization for Ca concentrations in shoots and roots (with exception of *A. polycephala*). Ca concentrations was decreased by fertilization, regardless of the substrate (except for roots of *C. antisyphilitica* grown in tailings) (Figs. 3 and 4).

Copper (Cu) concentrations in shoots were lower ($P > 0.05$) in *C. antisyphilitica* and *H. heptaphyllus* plants grown in tailings than in the soil (Fig. 3) and with exception for *H. heptaphyllus* grown in soil, Cu concentration in roots was greater in fertilized than unfertilized plants (Fig. 4). For all the species, Fe and Mn concentrations in roots and shoots were greater in plants grown in tailings than soil (Figs. 3 and 4). A significant interaction ($P < 0.05$) was observed between substrate and fertilization for Fe concentrations in shoots (with exception of *C. antisyphilitica*) and roots (*H. heptaphyllus* and *H. impetiginosus*). In shoots, the fertilization decreased shoot Fe concentration in plants grown in tailings (Fig. 3). A significant interaction ($P < 0.05$) between substrate and fertilization was also observed for Mn concentrations in shoots (*A. polycephala*) and roots (*C. antisyphilitica* and *H. impetiginosus*). In those plants, the fertilization increased Mn concentrations in shoot and roots of plants grown in tailings (Figs. 3 and 4).

The main trends of nutritional composition in roots and shoot of all species can be visualized by the principal component analysis (PCA). PCA evaluates variations in the values of experimental parameters and derives new complex variables that reflect maximal changes in the parameter data set. This approach allows transforming the set of nutritional parameters into fewer variables that determine the changes in plant physiology (Jolliffe, 2002). The PCA analysis showed here that the two main axes explained 52%, 72%, 72%, 64%, 70% and 69% of the data variability for *A. polycephala*, *P. dubium*, *H. heptaphyllus*, *H. impetiginosus* and *C. antisyphilitica*, respectively (Fig. 5). Axis 1 (all species included) ordered plants in accordance with increased nutrient concentration, thus the tailing treatment is at one end of the gradient (less concentration of nutrients) while the soil fertilized is at the opposing end (greater concentration of nutrients). The same may be seen in analyzes made with the species separately. Axis 2 (all species) represented 23% of the data variability and Ca, N and Mg ordered roots and shoots in two groups.

Axis 2 represented 27%, 27% and 29% of the data variability for *A. polycephala, P. dubium*, *H. heptaphyllus,* respectively (Fig. 5). These axes completely ordered roots and shoots in two distinct groups, which P and Mn were the most important variables for this grouping. Calcium was also an important variable for this grouping in *A. polycephala* plants, N for *P. dubium* and Fe for *H. heptaphyllus*. Regarding *H. impetiginosus*, the Mg and Ca concentration were the most important variables to separate roots and shoots.

4. Discussion

The tailings had a negative effect on seed germination only for *Peltophorum dubium.* Due to its limited porosity, the tailings resembled the filter paper treatment by also forming a thin layer of water around the seeds. Substrates with a high proportion of silt have greater potential for forming superficial crusting, which consequently decreases water infiltration (Lemos and Santos, 1996). The thin layer of water formed around the seeds in the tailings provided more water availability in comparison to seeds germinating in soil, where there is greater infiltration due to lesser propensity for compaction. Thus, a presumed greater imbibition velocity in the filter paper and tailings treatments seems to explain the greater germinability for *C. antisyphilitica* in these two conditions (Fig. 1). Depending on the species, trace elements at low concentrations such as Cd at 0.1 mg/dm^3, may

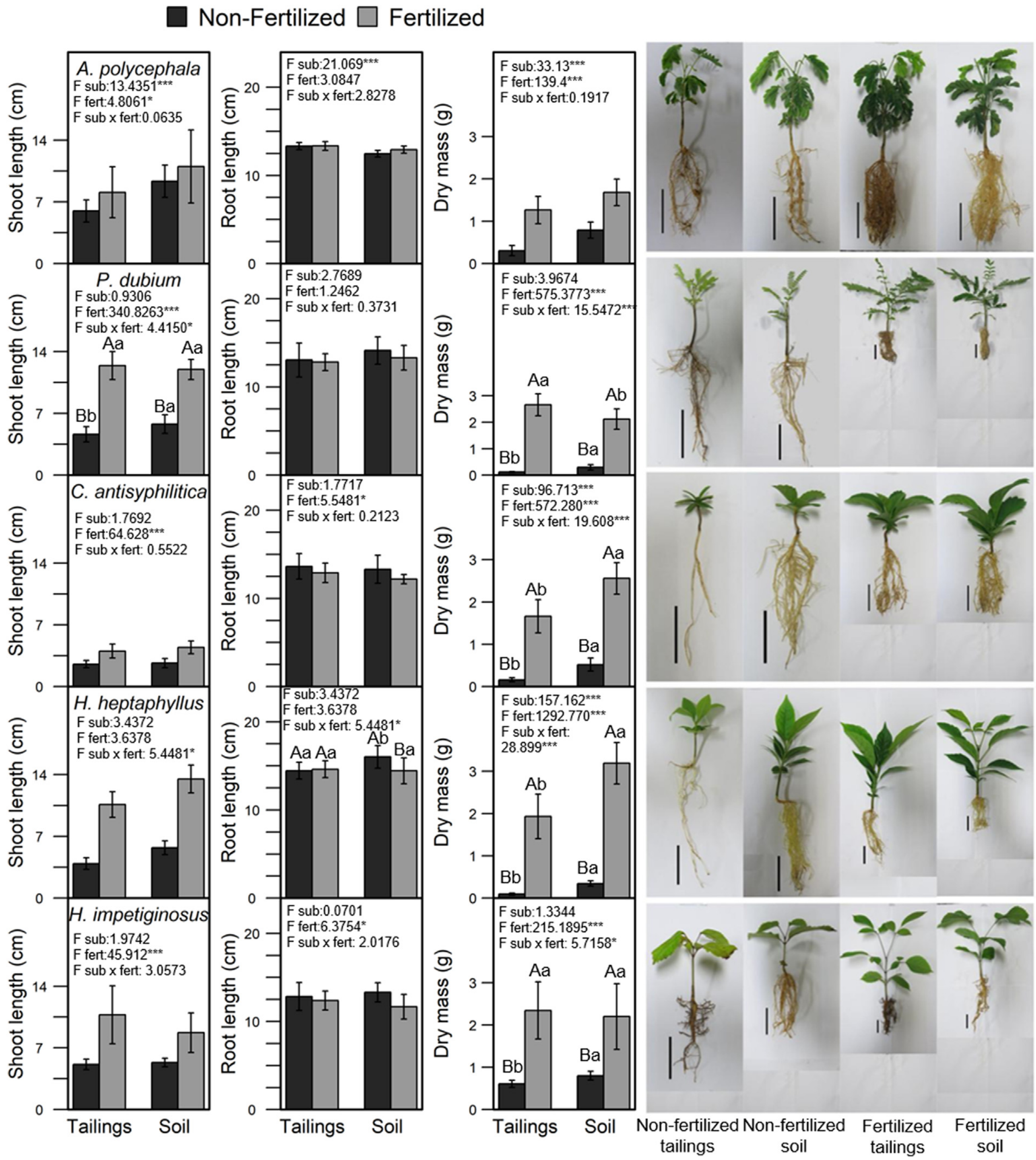

Fig. 2. Dry mass, shoot length and root length of five tree species grown with and without fertilization in different substrates (tailings and soil). Bars represent means ± SD of ten replicates. Capital letters indicate significant differences between fertilization (non-fertilized and fertilized plants) within the same substrate, while lowercase letters indicate significant differences between substrates for the same fertilization condition, by contrast analysis ($P < 0.05$). The scale bar in the photos is 5 cm.

inhibit, not have an effect, or even stimulate seed germination (Duarte et al., 2012; Lefèvre et al., 2009). The high seed germination (71% of *P. dubium*) found for the tailings treatment indicates the absence of chemical elements in concentrations deleterious to the germination itself in this substrate.

Although seed germination of most of the studies species was not affected, seedling growth was reduced when cultivated in the tailings. Nutritional limitation explains the reduced growth of plants cultivated in the tailings relate to soil, since soil is more fertile than the tailings. One can argue that the presence of toxic metals or high concentrations of metals in the tailings could also result in decreased growth of plants. However, among the potentially toxic elements found, concentrations of Cd and Cr (not found in the soil) were below 0.1 mg/dm^3 in the tailings, which is below the toxicity threshold for plants (CONAMA, 2009; CETESB, 2014). Furthermore, concentrations of Ni and Pb in the soil ($\leq$0.2 mg Ni/dm^3 and 1.5 mg Pb/dm^3) were greater than in the tailings ($\leq$0.1 mg Ni/dm^3 and 0.3 mg Pb/dm^3). These data, associated with the results of the seed germination tests, allow discarded the hypothesis that toxic metals in the tailings are responsible for deleterious effects to plant growth. The results of this study corroborate those obtained by Segura et al. (2016), demonstrating that the mining tailings is chemically poorer, and thus less fertile, than the soil in the region.

Table 2
Leaf area (LA) in cm^2, chlorophyll content (SPAD) and potential of quantum yield of photosystem II (F_V/F_M) of trees species seedlings submitted to four treatments (tailings, soil, fertilized tailings and fertilized soil). Values of averages ± SD of ten (LA) or five (SPAD and F_V/F_M) replicates. Capital letters indicate significant differences between fertilization at the same substrate, while lowercase letters indicate significant differences between substrates for the same fertilization condition, by contrast analysis ($P < 0.05$).

	Tailings		Soil	
	Non-fertilized	Fertilized	Non-fertilized	Fertilized
Albizia polycephala				
LA (cm^2)	27.01 ± 2.89	92.88 ± 10.02	72.54 ± 3.99	140.6 ± 11.81
SPAD	27.6 ± 5.14Bb	63.17 ± 5.62Aa	46.73 ± 4.28Ba	62.49 ± 7.74Aa
F_V/F_M	0.69 ± 0.03	0.69 ± 0.02	0.72 ± 0.02	0.71 ± 0.02
Peltophorum dubium				
LA (cm^2)	7.51 ± 0.57Bb	274.78 ± 14.88Aa	18.37 ± 1.63Ba	222.1 ± 13.87Aa
SPAD	17.22 ± 1.68	44.53 ± 4.52	23.65 ± 1.55	48.29 ± 2.73
F_V/F_M	0.58 ± 0.07	0.69 ± 0.03	0.63 ± 0.05	0.68 ± 0.03
Cybistax antisyphilitica				
LA (cm^2)	5.21 ± 0.39Bb	120.47 ± 8.38Ab	20.67 ± 1.64Ba	189.4 ± 10.79Aa
SPAD	40.53 ± 3.43Bb	52.45 ± 3.56Aa	50.38 ± 4.04Aa	53.02 ± 3.80Aa
F_V/F_M	0.75 ± 0.02Bb	0.79 ± 0.01aAa	0.79 ± 0.01Aa	0.78 ± 0.02Aa
Handroanthus heptaphyllus				
LA (cm^2)	7.68 ± 1.44Bb	225.65 ± 18.51Ab	39.16 ± 2.98Ba	349.4 ± 26.51Aa
SPAD	27.77 ± 1.87Bb	46.57 ± 3.01Ab	43.52 ± 2.78Ba	50.87 ± 3.91Aa
F_V/F_M	0.75 ± 0.00Bb	0.79 ± 0.01Ab	0.8 ± 0.01Ba	0.81 ± 0.02Aa
Handroanthus impetiginosus				
LA (cm^2)	39.92 ± 2.59Bb	287.32 ± 24.65Aa	68.94 ± 2.75Ba	264 ± 31.06Aa
SPAD	18.72 ± 2.23	35.55 ± 2.59	19.63 ± 1.89	32.49 ± 3.65
F_V/F_M	0.69 ± 0.02	0.76 ± 0.01	0.7 ± 0.03	0.73 ± 0.05

According to the classification of Raij (1996), the bioavailability of Cu and Zn is low in the tailings (high and average in the soil, respectively), while the bioavailability of Mn and B did not differ between soil and tailings (being high and medium, respectively). In addition, the tailings had a much lower organic matter content than the soil (Table 1). Extremely low organic matter content was also observed in assessments made shortly after the dam rupture (SEDRU, 2016; Andrade et al., 2018). The low content of organic matter and clay (6.9%) of the tailings resulted in low cation exchange capacity (CEC). Compared to soil, the low availability of nutrients, limited organic matter content and low CEC of the tailings demonstrate that the low fertility of the tailings was the main limiting factor for the growth of the studied plant species.

In addition to low fertility, the iron ore tailings had high contents of silt (44.1%) and very fine sand (26.6%) (Table 1), which favors compaction. Since the physical conditions of a substrate are directly related to the mechanical resistance that a matrix can impose of root growth (Clark et al., 2003), compaction could be an additional limiting factor for plant growth. Andrade et al. (2018) observed that the texture of tailings limited the advancement of rice roots. However, in the present study, the reduction in root length of species cultivated in the tailings was, when significant, very low (3–10%) (Fig. 2). These results indicate that, in general, the roots did not have difficulty growing in the tailings corroborating nutritional limitation as a preponderant factor affecting the plant growth. To test this assumption, we evaluated the growth in tailings and soil with the addition of mineral fertilizer. Indeed, fertilization significantly increased biomass incorporation, leaf area and seedling height of all species (except *A. polycephala* in relation to shoot length) compared to the non-fertilized tailings (Fig. 2). These results demonstrated that limitation to growth in the tailings was due mainly to the lack of essential nutrients for plant growth. Similarly, Andrade et al. (2018) showed that the changes induced in rice plants growing in different concentrations of tailings were due to nutritional deficiency and not to metal toxicity.

In addition to affecting total biomass production, nutritional condition interferes with the partitioning of biomass among organs of the plant (Fig. S1). This became evident in the present study when seedlings of all species were found to allocate greater biomass to the roots when grown in tailings without fertilization and to the aerial part when grown in fertilized tailings (Fig. S1). The leaf dry mass fraction of plants (LWF) increases with nutrient availability (Poorter et al., 2012), while plants with a low nutrient supply allocate more to roots (Brouwer, 1963). Poorter et al. (2012) observed that plants in soils with low nutritional resources generally allocate proportionally more biomass to the roots to the detriment of the stems and leaves. It is interesting to note that all seedlings of the studied species invested proportionally more in leaves when they grew in the soil than in the tailings, both without fertilization, reaffirming the impact of the tailings on soil fertility for vegetation in the affected region. In the fertilized substrates there was proportionally lower root biomass, but these roots were more efficient at the absorption of nutrients, thus facilitating a greater allocation of resources to the aerial part. These results show an optimization of root growth with availability of nutrients, which is in agreement with the concept of functional balance between roots and the aerial part. According to this concept, root growth is under control of photoassimilated production, while the aerial part is limited by nutrients available to the roots (Thornley, 1995; Bangerth et al., 2000). Plants of tree species cultivated in nurseries often allocate more than 50% of their biomass to leaves (Poorter et al., 2012). *Albizia polycephala* was the only species that did not allocate 50% of its biomass to leaves and showed no difference in patterns of biomass allocation to the three organs between treatments, which may be related to symbiosis with nitrogen fixing bacteria (nodulation), which is common among species of the family Fabaceae (Denison, 2000). Higher nodulation on substrates without fertilization is expected since nodulation is inversely proportional to nitrogen availability (Parsons et al., 1993). Although more studies are needed, it should be emphasized that the tailings were not limiting the growth of diazotrophic organisms or the development of symbiosis between them and the plants.

The plants grown in the fertilized tailings had higher concentration of essential nutrients than those grown in the tailings without fertilization (Figs. 3–5). Macro- and micronutrients are vital elements due to their involvement in metabolic, physiological, and developmental processes in plants, and adequate amounts of nutrients are necessary to sustain the plant growth. PCA analysis showed the benefit of fertilization to improve plant mineral nutrition, regardless the substrate (soil or tailings). Moreover, the improvement on macronutrient nutrition (N, P and K), as a result of substrate fertilization, increased biomass in plants grown in the fertilized tailings (Figs. 2–4). Particularly, higher

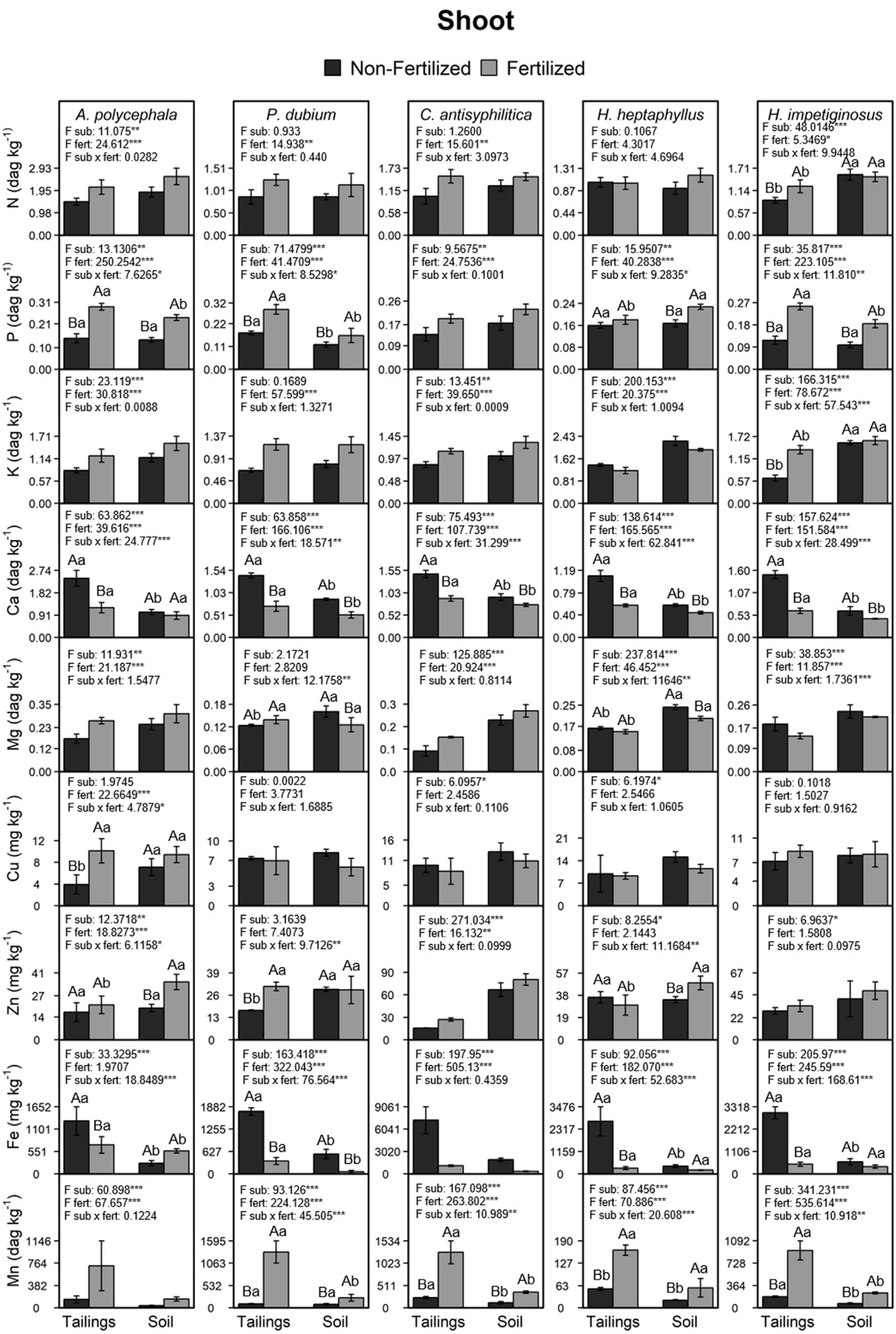

Fig. 3. Macro- and micronutrients concentration in the shoots of fiveseven tree species. T = tailings; S = soil; FT = fertilized tailings; FS = fertilized soil. Bars represent means ± standard deviationSD of four replicates. Different letters indicate significant differences between treatments ($p < 0.05$) for each species. Capital letters indicate significant differences between fertilization at the same substrate, while lowercase letters indicate significant differences between substrates for the same fertilization condition, by contrast analysis ($P < 0.05$). ns = not significant.

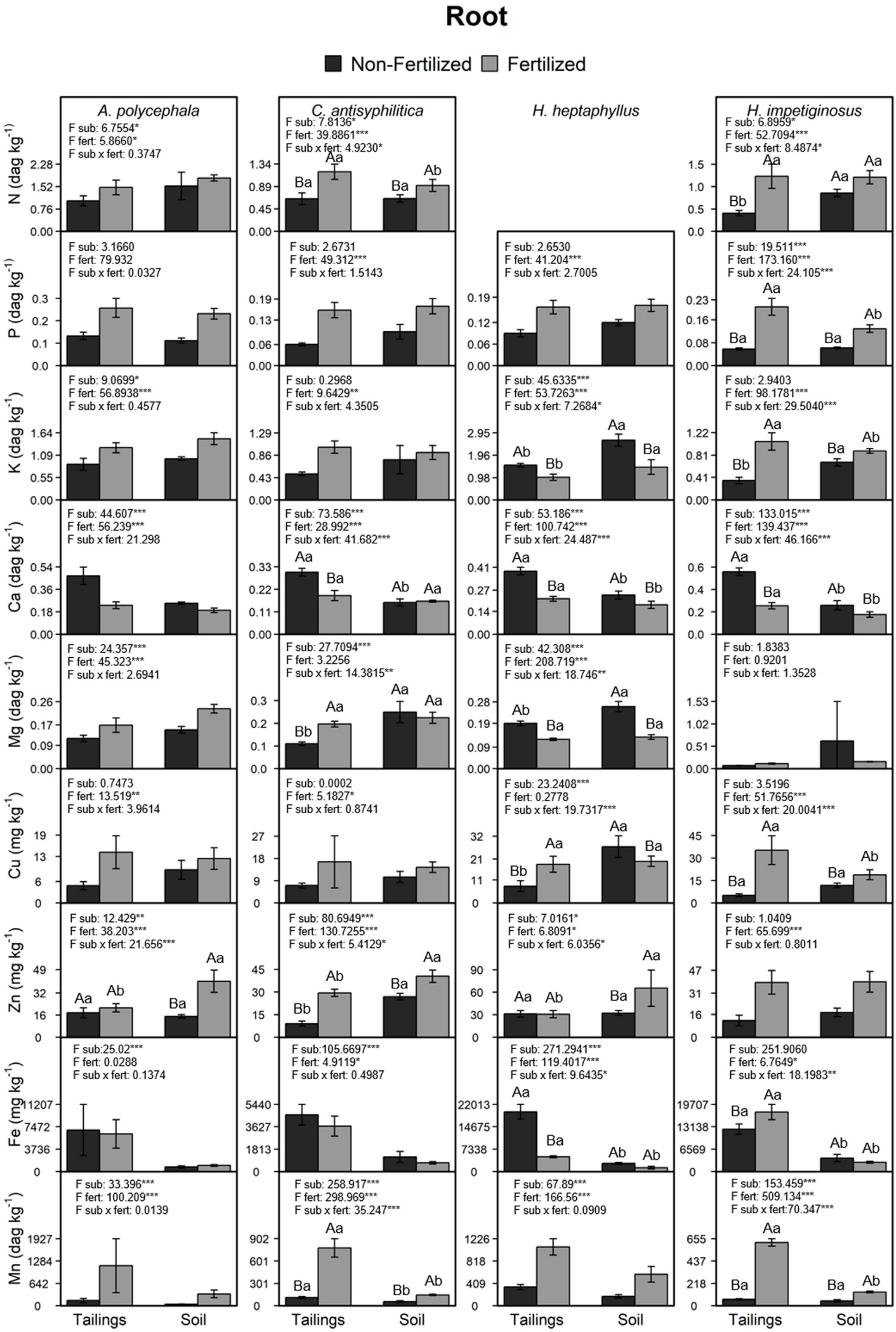

Fig. 4. Macro- and micronutrients concentration in the roots of four tree species. Bars represent means ± SD of four replicates. Capital letters indicate significant differences between fertilization at the same substrate, while lowercase letters indicate significant differences between substrates for the same fertilization condition, by contrast analysis (P < 0.05).

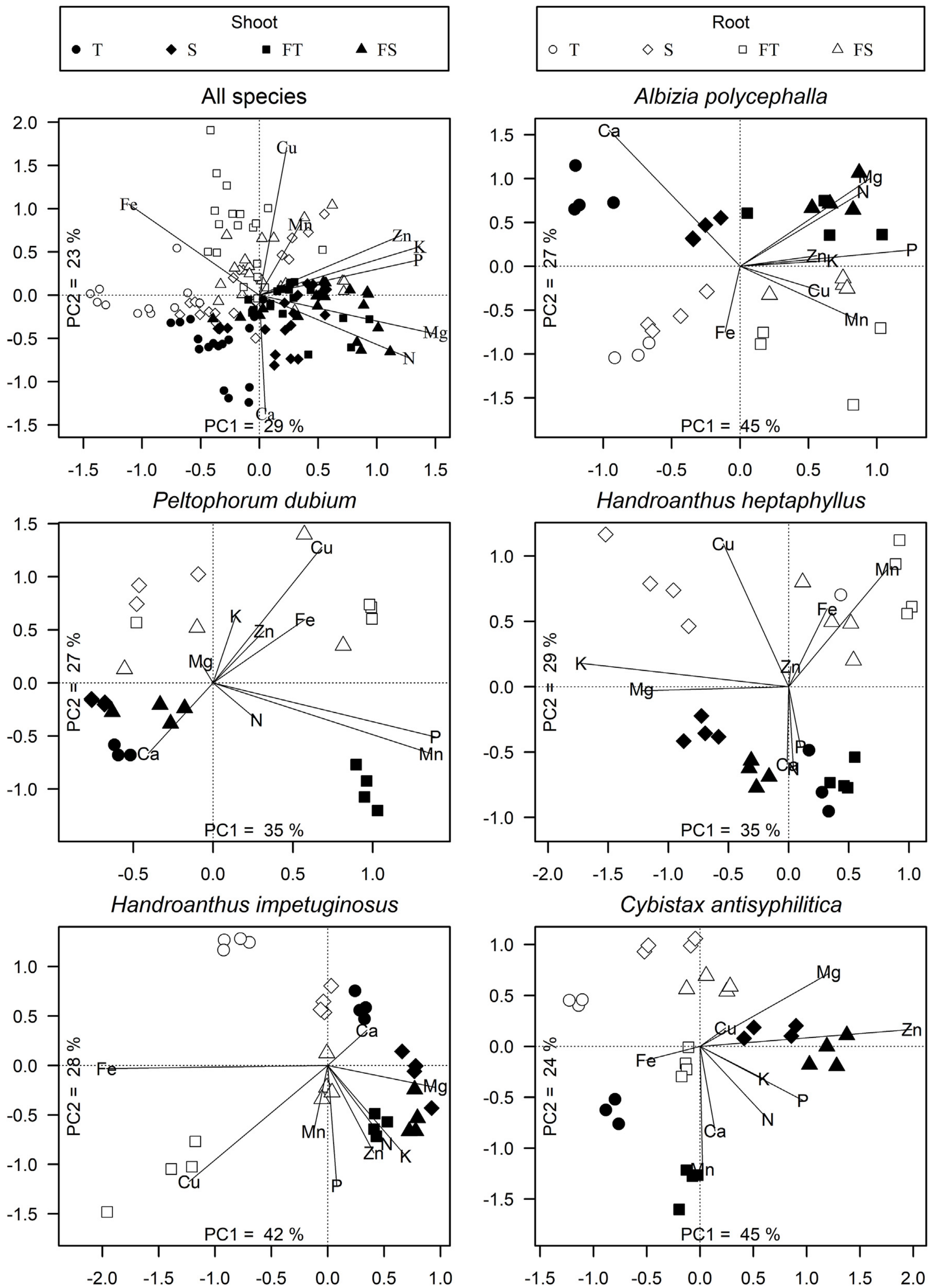

Fig. 5. PCA biplot diagram showing the concentration of macronutrients and micronutrients in the parts of five tree species (submitted to different treatments (tailings, soil, fertilized tailings and fertilized soil).

concentration of N, an essential constituent of chlorophylls (Kjellbom and Larsson, 1984), assures high photosynthesis activity through the mineral allocation to Rubisco, and photosystem components (such as D1 protein and chlorophyll) (Gastal and Lemaire, 2002; Lawlor, 2002). Therefore, greater biomass production of fertilized plants should be associated with better N nutrition, as a result of their higher chlorophyll concentrations and quantum yield of photosystem II (F_V/F_M) (Table 2).

Interestingly, although Fe was more bioavailable in the soil than in the tailings, plants grown in the tailings showed higher Fe concentration in their tissues (Figs. 3 and 4). Lower competition between Fe and other cations for adsorption sites in the tailings may explain the high concentration of the metal (Genon et al., 1994; Lombi et al., 2002) in plants tissues, since the other substrates showed higher cation concentrations. In addition, the high ground compaction in tailings may decreases the amount of available oxygen in the substrate and, thus, limits the oxidation of Fe^{2+} to its insoluble form Fe^{3+}, favoring the metal uptake by plants. In the same way, roots of plants from tailing may have lower ability to oxidize Fe^{2+} to Fe^{3+} due to lower K concentration observed in this substrate, which also favors Fe uptake by plants (Trolldenier, 1973). Besides higher accumulation of Fe in shoot coincided with lower growth of plants in tailings (mainly for *C. antisyphilitica*, *H. impetiginosus* e *H. heptaphyllus*), it is important to point out that the fitoextraction is a characteristic well-recommended for revegetation of mining degraded areas.

In addition to Fe, plants grown in the tailings without fertilization also had high concentration of Ca in their tissues (Figs. 3 and 4). This can evidence the function of Ca as defense signaling under stress conditions imposed by excess of Fe. Calcium plays central role in cell membrane stabilization, nutrient uptake and enzymatic and hormonal regulations during abiotic stress (Parvin et al., 2019). Under metal-stressed environment, Ca difficult the metal accumulation through controlling membrane permeability and movements of divalent cations across cell membrane, as well as competing for transporter sites (Hirschi, 2004; Farzadfar et al., 2013). Gai et al. (2017) observed increased Ca concentrations in leaves of *H. impetiginosus* submitted to Zn toxic concentrations. Similarly, Ca supplementation decreased the toxicity of Cd (Ahmad et al., 2015), nickel (Mozafari, 2013) and As (Rahman et al., 2015) in plants. The PCA analysis showed greater concentration of Ca in shoots of plants grown in unfertilized tailings treatments, in which greatest Fe concentration was found (Figs. 3 and 4). Therefore, high Ca concentration in shoots may assure lower toxic effects of excessive metals, such as Fe, to photosynthetic apparatus, although this strategy was not sufficient to allow normal growth and photosynthesis activity in plants grown in unfertilized tailings.

The fertilization of the tailings ensured physiological levels of Fe and Ca in the plants (Fig. 3). Thus, the concentration of iron in plant tissues was equal to or less than it was found in plants grown in the natural soil of the region. Therefore, with the fertilization of the tailings, Fe ceases to be a potential stressor for the plants. On the other hand, fertilization also increased the ability of plants to absorb and accumulate Mn, which resulted in potentially toxic levels (> 700 mg kg^{-1} in the shoot of four species: *A. polycephala*, *P. dubium*, *C. antisyphilitica* and *H. impetiginosus*). Nonetheless, during the experiments, no visible symptoms of Mn toxicity were observed, and thus, future experiments are needed to clarify the exact effect of Mn on plants.

Three species (*A. polycephala*, *C. antisyphilitica* and *H. heptaphyllus*) had greater seedling dry mass when grown in fertilized soil than in fertilized tailings (Fig. 2). The low clay content and very little organic matter of the tailings, provides less negatively charged particles available for cationic adsorption compared to the soil. Consequently, less nutrients may be absorbed by plants in the tailings compared to those in the soil, even though they received the same concentration of fertilizer. Curiously, two species had the same (*H. impetiginosus*) or greater (*P. dubium*) biomass in fertilized tailings compared to fertilized soil, even with high concentrations of Mn in the shoot (*P. dubium* 1328 mg kg^{-1}; *H. impetiginosus* 934 mg kg^{-1}). Regarding *P. dubium* this higher biomass in the fertilized tailings was due to a higher accumulation of P and for *H. impetiginosus* was due to a similar accumulation of N and K in the two fertilized treatments (Fig. 5).

Our results demonstrate that the iron ore tailings released by the rupture of the Fundão dam interfere with the growth of native Atlantic Forest plant species. Reduced plant growth was due to nutritional limitation, caused by low nutrient availability, low organic matter content and low CEC of the tailings. In our experiments, fertilization reversed the deleterious effect of the tailings on the plants and so should be considered in *in situ* recovery projects aimed at better initial growth and development of native plants. Furthermore, we showed that some species are more suitable for planting in regions affected by the rupture of the dam, and reinforces the importance of studies to select species to be planted in the area in order to achieve the best performance of the plants and the recovery of overall diversity. Moreover, the germination of most of the tested species was not affected by the tailings, and fertilization of the tailings resulted in a significant increase in plant growth, the present study suggests the use of both seeds and seedlings for the reestablishment of the flora at the area affected by the tailings spill.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.110021.

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