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Fertilization assures mineral nutrition but does not overcome the effects of Fe accumulation in plants grown in iron ore tailings

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Abstract

The rupture of Fundão dam was the biggest environmental disaster of the worlds' mining industry, dumping tons of iron ore tailings into the environment. Studies have shown that the Fundão dam's tailings are poor in nutrients and have high Fe and Mn concentration. In this context, our objective was to evaluate the growth performance of two native tree species (Bowdichia virgilioides and Dictyoloma vandellianum) in two treatments: fertilized soil and fertilized tailings. We hypothesize that the high concentrations of iron and manganese in the tailings can impair the growth performance of plants by interfering with the absorption of nutrients made available through fertilization. Soil and tailings samples were collected in the municipality of Barra Longa (MG, Brazil), and then fertilized with mixed mineral fertilizer ("Osmocote Plus 15–9-12" at 7.5 g L^{-1}). The experiment was conducted for 75 days in a greenhouse using 180 cm³ tubes. We evaluate chlorophyll content, maximal PSII quantum yield, root length, shoot length, root:shoot ratio, leaf area, specific leaf area and leaf area ratio, dry mass, macroand micronutrients concentration in the tissues, and metal translocation factor. Although assuring the adequate levels of the main nutrients to plant growth (N, P, K, Ca, and Mg), the fertilization did not reverse the negative effect of tailing on these species. The high concentration of Fe in the tissues associated with less biomass production, lower plant height, smaller leaf area, bigger specific leaf area, and reduced chlorophyll content indicates a probable phytotoxic effect of iron present in the tailings for D. vandellianum. Our results base further field evaluations and longer experiments, which will facilitate the understanding of the performance of tree species submitted to tailings with fertilization. So far, this study suggests that B. virgilioides are more tolerant to excess Fe from the tailings of Fundão dam than D. vandellianum.

Keywords Mineral nutrition · Plant growth · Iron concentration · Fundão dam · Degraded areas · Samarco

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Introduction

Brazil is the world's second largest producer of iron ore (IBRAM 2012). Despite being an indispensable activity for human society, mining can generate major impacts on the environment. Brazil had more than 80 mine-related environmental disasters and it is estimated that at least 126 mining dams in the country are vulnerable to failure in the forthcoming years (Nazareno and Vitule 2016; Garcia et al. 2017). At the end of 2015, the Fundão dam, operated by Samarco Mining Company and located in the municipality of Mariana, Minas Gerais (Brazil), collapsed, launching tons of iron ore tailings into the environment, which traveled over 600 km of watercourses from the Doce River basin until reaching the Atlantic Ocean (IBAMA 2015; Samarco 2016).

Considering the volume of tailings dumped (~50 million m^3) and the magnitude of the socioenvironmental damages, the rupture of Fundão dam was the biggest environmental

disaster of the worlds' mining industry (IBAMA 2015; Carmo et al. 2017). This accident reached the Brazilian Atlantic Forest which given its species richness and endemism levels, it is considered one of the world's biodiversity hotspots, making its restoration considerably important (Myers et al. 2000; Ribeiro et al. 2009; Massad et al. 2011). The ore tailings from Fundão dam flooded and destroyed native riparian vegetation, i.e., 457.6 ha of Atlantic Forest along the river course (Omachi et al. 2018).

The contents of the potential toxic elements (Ag, Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Rb, Ti, Zn, Zr) in the tailings released from the Fundão dam rupture were below the threshold levels defined by Brazilian environmental law (EMBRAPA 2015). However, according to Segura et al. (2016), some days after the disaster, the sediments in suspension in the river water affected by the tailings contained iron (Fe) and manganese (Mn) at concentrations approximately 4 (Fe) and 1.5(Mn)-fold higher than the maximum Brazilian limit for water bodies quality assessment. Furthermore, the concentration of Fe and Mn found in the tailing samples were very high compared to the recommended concentration for plant growth in Minas Gerais' soils (Zago et al. 2019) and were above the values allowed by the CONAMA (National Environment Council) (Esteves et al. 2020a). Despite Fe and Mn are mainly associated with less bioavailable fractions (oxides and hydroxides), environmental physical-chemical conditions and plant activity may solubilize these forms, thus releasing their associated metals and increasing its bioavailability (Andrade et al. 2018; Queiroz et al. 2018). Additionally, the tailings are deficient in nutrients, poor in organic matter, and have less clay content and less cation exchange capacity than the regions' natural soils (SEDRU 2016; Segura et al. 2016; Cruz et al. 2020). Therefore, the tailings have the potential to affect soil over time, making it difficult to recover and develop previously established plant species (IBAMA 2015).

It is difficult to estimate how many years it will take for the affected flora to recover (Lyra 2019). An accident of this magnitude, where the long-lasting effects are still unknown, requires studies to mitigate environmental damage and restore the functioning of ecosystems (Segura et al. 2016; Hatje et al. 2017). Revegetation plans, erosion control, and restoration of the ciliary strip aiming at the recovery of the affected areas (EMBRAPA 2015) are demanded by the Federal Public Ministry of Brazil. One of the main issues of a reforestation project is the diversity of plant species (Massad et al. 2011). Studies have shown that plant species perform better in mixed plots than in monoculture, and, therefore, biodiversity may improve productivity in tropical reforestation (Montagnini 2001; Erskine et al. 2006; Potvin and Gotelli 2008). Indeed, it is very important to choose the correct species for the success of the restoration process (Balestrin et al. 2019). In addition to support biodiversity,

the chosen species may tolerate the adverse conditions to be effectively established in the recovering area. In this context, the study of the performance of potential species to be used in areas affected by the Fundão dam tailings is a priority.

There are some studies on the performance of plants in Samarco's mining tailings. In a field study at impacted areas by the Fundão tailings, Santos et al. (2019) related the mortality of tree species due to the presence of high amounts of sodium and ammonium and low nutrient contents in the tailings. The deleterious effects in plant growth were associated to nutrient deficiency as well as to physical properties (high density and silt percentage) of the Fundão tailings (Andrade et al. 2018; Esteves et al. 2020a). In that context, the nutritional amendment of tailings improved biomass production of three species of aromatic grasses (Chrysopogon zizanioides, Cymbopogon citratus, and Cymbopogon winterianus) (Zago et al. 2019), three agronomic species (Pennisetum glaucum L., Sorghum bicolor, and Zea mays L.) (Esteves et al. 2020b), and five tree species (Albizia polycephala, Cybistax antisyphilitica, Handroanthus heptaphyllus, Handroanthus impetiginosus, and Peltophorum dubium) (Cruz et al. 2020).

The initial coverage with grasses and legumes and the planting of native tree species are two measures included in the Integrated Environmental Recovery Plan (PRAI), prepared by the Renova Foundation-the organization responsible for conducting environmental restoration measures in the Doce River Basin (Renova 2017). The best performance of this initial vegetation cover was observed in areas where inorganic fertilizer was used in comparison to areas without fertilization (Renova 2017). According to the Renova Foundation (Renova 2020), the tailings from the rupture of the Fundão dam are inert (composed mainly of dirt, iron, and manganese) and its removal from the places where it was deposited would cause a second wave of environmental impacts. The next steps focus on direct planting of seedlings of native tree species, which include items such as irrigation, ant control, and fertilization (Renova 2020).

Given the presence of iron ore tailings in some sites to be reforested, the fact that the region's soil and the tailings deposited are poor in nutrients (EMBRAPA 2006; Coelho 2009; SEDRU 2016) and fertilization is a practice adopted in projects for the recovery of degraded areas, it is necessary to select species to revegetate the affected area. *Bowdichia virgilioides* Kunth ["sucupira preta," Fabaceae] and *Dictyoloma vandellianum* Adr. Juss. ["tingui," Rutaceae] were the two tree species selected for this study. Both are naturally occurring in the region affected by the collapse dam (Lombardi and Gonçalves 2000; Lopes et al. 2002) and recommended in programs for recover of degraded areas (Montagnini 2001; Flavio and Paula 2010; Lima et al. 2013; Filho and Sartorelli 2015; Colmanetti et al. 2016). *Bowdichia virgilioides* is classificated as late secondary (Oliveira et al. 2011) and *D. vandellianum* as a pioneer species in ecological succession (Balestrin et al. 2019). Furthermore, these species are found in ferruginous ecosystems (Messias et al. 2012; Pereira et al. 2013; Andrino et al. 2020), known as *canga*, which have high concentrations of oxides and hydroxides of iron (Simmons 1960; Maciel et al. 2017). These metal-rich environments such as *canga* are expected to harbor metal-tolerant and hyperaccumulator plant species (Jacobi et al. 2007).

Therefore, the objective of this study was to evaluate the growth performance of these two native tree species (Bowdichia virgilioides and Dictyoloma vandellianum) in two treatments: fertilized soil and fertilized tailings. We hypothesize that the high concentrations of iron and manganese in the tailings can impair the growth performance of plants by interfering with the absorption of nutrients made available through fertilization. For this purpose, we evaluate several physiological parameters: chlorophyll content, maximal PSII quantum yield (F_V/F_M) , root length, shoot length, root:shoot ratio, leaf area, specific leaf area and leaf area ratio, dry mass, macro- and micronutrients concentration in the tissues, and metal translocation factor. The present research contributes to a physiological understanding of tree species that are potentially be used in restoration programs in areas affected by iron ore tailings, as in the case of the Fundão dam rupture.

Material and methods

Soil and iron ore tailings sampling

Substrates were collected (20 cm deep) on the riverside 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. Soil samples (dystrophic red-yellow latosols/argisols) were obtained from not affected area (with no tailing at 5 m from the tailings collection site, under the same transect) and tailings samples from area with iron ore tailings deposition. After sampling, the substrates were homogenized and packed in plastic bags until the experiments were carried out. Soil and tailings properties and bioavailability of chemical elements of the collected substrates can be seen in our previous studies (Cruz et al. 2020).

Conditions of plant growth

Bowdichia virgilioides and *D. vandellianum* seeds were provided by the Instituto Espinhaço, Biodiversidade, Cultura e Desenvolvimento Socioambiental (Gouveia, MG, Brazil). Physical dormancy imposed by hard seed coat in *B. virgilioides* was broken by chemical scarification with 7-min

immersion in sulfuric acid (Albuquerque et al. 2007). Physical or physiological dormancy was not reported for *D. vandellianum* seeds, and, therefore, no breaking dormancy treatment was applied. Afterwards, seeds of each species were placed to germinate in transparent Gerbox boxes containing filter paper (Whatman No. 1) moistened with distilled water (Brasil 2009). Freshly germinated (emergence of 2 mm of the primary root) seeds were transplanted into 180 cm³ polyvinyl chloride tubes containing one of the two treatments: fertilized soil or fertilized tailings. One replicate was defined as one tube containing one plant, totaling 24 replicates per treatment for each species.

Substrates were fertilized with mixed mineral fertilizer applied to the soil/tailings via "Osmocote Plus 15–9-12" (15% N, 9% P₂O₅, 12% K₂O, 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 6 months). The concentration used was 7.5 g L⁻¹, as suggested by the manufacturer for native tree species. "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 assuring the possibility of a single application.

The growth experiment was conducted for 75 days in a greenhouse located at the Espinhaço Institute–Biodiversity, Culture and Socioenvironmental Development, situated at the Federal University of São João Del Rey, Sete Lagoas campus (MG, Brazil). Plants were arranged in a complete randomized design for each species (2 substrate treatments \times 24 replicates). During the experiment, the average humidity was 82.4% and the temperature ranged between 19.6 and 28.8 °C, averaging at 23.4 °C. The plants were exposed to natural sunlight with a 50% shade screen (from November to February) and irrigated by micro-sprinkler irrigation for 15 min, four times a day. Invasive plants were removed manually every week, when necessary.

Photosynthetic pigment and chlorophyll a fluorescence

We measured the photosynthetic pigment in the leaves using a SPAD-502 portable chlorophyll meter (Minolta, Japan). These measurements were performed on fully expanded leaves in five plants per treatment. The result for each plant was the average of four readings on three different physiologically mature leaves from each repetition.

The minimal fluorescence level (F_0) and the maximal fluorescence yield (F_M) were determined in five plants per treatment using a pulse-amplitude modulation (PAM) fluorometer (model PAM-2500, WALZ, Effeltrich, Germany). We measured chlorophyll *a* fluorescence on the first, second, and third fully expanded leaves (physiologically mature

leaves), for a total of three measurements per plant. Before these measurements, the leaves of the plants were adapted to darkness for 30 min to completely re-oxidize the primary electron acceptors of photosystem II (PSII). The maximal PSII quantum yield (F_V/F_M) was evaluated following Butler (1978):

$$F_v/F_M = (F_M - F_0)/F_M$$

where $F_{\rm M}$ is the dark-adapted maximal fluorescence and F_0 is the minimal fluorescence.

Growth parameters

After 75 days, ten plants of each treatment were collected and washed with water. The shoot and main root length were measured with a measuring tape (cm). For leaf area (LA) determination, all leaves of ten plants per treatment were scanned and then the LA (in cm²) was calculated using the ImageJ software. Subsequently, the leaf, stem, and root dry biomass production were assessed after drying plant organs in an oven with forced air circulation at 65 °C until constant weight. Biomass was weighed in an analytical scale (0.001 g precision). To evaluate biomass partition among root and shoot, the root/shoot ratio were calculated following Hunt (1982):

root/shoot ratio : $RS = W_R/(W_L + W_S)$;

where W_R is the root dry weight; W_S is the stem dry weight; and W_L is the leaf dry weight.

The specific leaf area (SLA) and the leaf area ratio (LAR) were calculated according to the following equations (Beadle 1985):

 $SLA = LA/W_L$ LAR = LA/W_T

where LA is the leaf area; W_L is the leaf dry weight; and W_T is the total dry weight.

Concentration of nutrients in the plant tissues

Shoots or roots of six plants from each treatment were mixed, constituting one replicate, with a total of three replicates/treatment (fertilized soil of *D. vandellianum* has four replicates). The dry plant material was sent to the Laboratory of Soil, Vegetable Tissue and Fertilizer analysis at Universidade Federal de Viçosa (Minas Gerais, Brazil). For nitrogen, samples were digested at 350 °C using sulfuric acid and after, they have been cooled, filtered, and completed with ultra-pure water, and then total N was determined by the Kjeldahl method. For P, K, Ca, Mg, Fe, Cu, Zn, and Mn 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 digesting and filtering the extracts (Whatman n° 40 filter paper), phosphorus concentration was measured colorimetrically using the ascorbic acid method (Braga and Defelipo 1974). The other nutrients (Ca, Mg, Fe, Cu, Zn, and Mn) were determined using an inductively coupled plasma optical emission spectrometry (ICP-OES PerkinElmer model Optima 8300 DV). The limit of quantification (LOQ) was 1.5 mg kg⁻¹ for Mg, 2 mg kg⁻¹ for Fe, and 0.5 mg kg⁻¹ for Ca, Cu, Mn, and Zn.

Translocation factor

Translocation factor (TF) was calculated as the ratio between the metal (Ca, Cu, Fe, K, Mg, Mn, and Zn) concentration in shoots and roots (Bao et al. 2009).

Statistical analyses

Generalized linear models (GLMs) were created to assess differences in growth parameters (dry biomass, root length, shoot length, root:shoot ratio, leaf area, specific leaf area, leaf area ratio), chlorophyll content, nutrient concentration, and translocation factor (all Gaussian distribution) between treatments (fertilized tailings and fertilized soil) for each species and organs (nutrition data) separately. A GLM was also made for the maximal PSII quantum yield (F_V/F_M), which has a quasi-binomial distribution. Tests with *P* values lower than 0.05 were considered significantly different. Statistical analyses were performed using R software (R Development Core Team 2020).

Results

Chlorophyll concentration was 15% lower in *D. vandellianum* plants growing in the fertilized tailings compared to fertilized soil (P < 0.01; Fig. 1). For the two species, maximal photochemical efficiency of PSII (F_V/F_M) was not significantly different (P > 0.05) between treatments (Fig. 1). Compared to fertilized tailings, *D. vandellianum* plants grown in the fertilized soil were 4.9% higher (P < 0.001) and no difference in main root length was observed between the treatments for both species (Fig. 2). *Bowdichia virgilioides* had 60.9% higher root/shoot ratio in the fertilized tailings than in fertilized soil (P < 0.001; Fig. 2).

Lower total leaf area was observed in plants of both species growing under the fertilized tailing treatment (P < 0.001; Fig. 3). The leaf area of *D. vandellianum* plants grown in the fertilized tailings was 52.2% smaller than that found in the plants in the fertilized soil, whereas *B. virgilioides* plants had a 41.1% smaller leaf area in fertilized tailings than in the fertilized soil. *Dictyoloma vandellianum* plants grown in the fertilized tailings





Fig. 1 Chlorophyll content (SPAD) and the maximal PSII quantum yield (F_V/F_M) of two tree species grown in different substrates (fertilized soil and fertilized tailings). (a), (b)=*Bowdichia virgilioides* and (c), (d)=*Dictyoloma vandellianum*. Bars represent means ± SD of

five replicates (n=10). The symbols *, **, or *** indicate significant differences between treatments at P < 0.05, P < 0.01, and P < 0.001, respectively

showed high specific leaf area (P < 0.01; Fig. 3). While for *B. virgilioides* plants, the leaf area ratio was higher in the fertilized soil (P < 0.05), for *D. vandellianum*, it was greater in plants growing in the fertilized tailings (P < 0.01) (Fig. 3). Greater dry biomass was observed for both species growing in the fertilized soil than in the fertilized tailings (P < 0.01 for *B. virgilioides*; P < 0.001 for *D. vandellianum*; Fig. 4). The plants of the species *B. virgilioides* accumulated 50% more biomass in the fertilized soil than in the fertilized tailings and those of the



<Fig. 2 Root length, shoot length, and root:shoot ratio of two tree species grown in different substrates (fertilized soil and fertilized tailings). (a), (b), (c)=*Bowdichia virgilioides* and (d), (e), (f)=*Dictyoloma vandellianum*. Bars represent means \pm SD of ten replicates (*n*=20). The symbols *, **, or *** indicate significant differences between treatments by analysis of variance at *P*<0.05, *P*<0.01, and *P*<0.001, respectively

species *D. vandellianum* 160% more in the fertilized soil than in the fertilized tailings.

The N, P, and K concentrations found in the shoots and roots did not significantly differ between treatments for both species (P > 0.05; Figs. 5 and 6). Only D. vandellianum showed a difference between treatments in the concentration of Ca, where plants grown in the fertilized tailings had 86.5% higher shoot concentration of Ca than those grown in the fertilized soil. A 32.1% and 37.5% lower root Mg concentration (P < 0.05; Fig. 5) was observed in D. vandellianum and B. virgilioides plants, respectively, grown in the fertilized tailings compared to fertilized soil. Similarly, shoot Mg concentration was 28.1% lower in D. vandellianum plants grown in fertilized tailings (P < 0.05; Fig. 6). Shoot Fe and Mn concentrations were greater in plants growing in the fertilized tailings (P < 0.05; Fig. 6). Dictyoloma vandellianum shoots from the fertilized tailings had a 10 times higher concentration of iron and five times higher of manganese compared to the shoots from the fertilized soil treatment. For B. virgilioides, this difference was less expressive, three times higher iron concentration and 3.75 times more manganese in the plants grown in the tailings compared to those grown in the soil. Similar results were observed for root Fe and Mn concentration of D. vandellianum, while in B. virgilioides roots, it differs only for iron root concentration and not for manganese (Fig. 5). There was no significant difference (P > 0.05) in copper concentration, neither in the roots nor in the shoots, for both species. In the shoots, the zinc concentration was 34.3% lower for D. vandellianum and 47.4% lower for B. virgilioides in plants grown on fertilized tailings compared to fertilized soil (Fig. 6). Similarly, the zinc concentration in the roots was 2.9 and 4.6 times higher (D. vandellianum and B. virgilioides, respectively) in the plants grown in the fertilized soil (Fig. 5).

The Ca, Cu, Fe, K, Mg, and Mn translocation factor did not significantly differ (P > 0.05) between treatments and Zn translocation factor was higher in the fertilized tailings for *B. virgilioides* plants (Table 1). In contrast, for *D. vandellianum*, the plants exposed to fertilized tailings showed Ca, Cu, Fe, Mn, and Zn translocation factor greater than those from the fertilized soil (1.62, 2.46, 2.56, 1.84, and 2.03 times as much, respectively) (P < 0.01; Table 1).

Discussion

Lower biomass production of D. vandellianum and B. virgilioides plants was observed in fertilized tailings than in fertilized soils. The difference between treatments were more accentuated for D. vandellianum in which total dry mass was 2.6 times greater in fertilized soil in relation to fertilized tailings, while for B. virgilioides, this relation was of 1.5 times. The lack of nutrients is the main factor responsible for the negative effect of iron ore tailings from the rupture of the Fundão dam to plant growth (Andrade et al. 2018; Cruz et al. 2020). Although physical and chemical characteristics such as particles size (silt 44.1% and clay 6.9%), organic matter, and cation exchange capacity are unfavorable in the tailings compared to the soil (Cruz et al. 2020), there was no difference in the concentrations of N, P, K, Ca, and Mg in the shoots of the B. virgilioides plants grown on both fertilized substrates. The same occurred for D. vandellianum, except for Mg, which was present in higher concentrations in plants grown in the fertilized soil. These results demonstrate that even assuring the levels of important nutrients to plants (N, P, K, Ca, and Mg), the fertilization did not reverse the negative effect of tailing on plant growth. The absorption of these macronutrients (except Mg for D. vandellianum) was not affected by iron and manganese as hypothesized. This does not mean that one of these micronutrients may not have impaired plant growth, as discussed below.

Manganese and iron concentrations were higher in plants grown in fertilized tailings. Manganese is required for the activity of several enzymes and participates in the photochemical release of O_2 in photosynthesis (Millaleo et al. 2010). Compared to macronutrients, micronutrients, such as Mn, are needed in small amounts, and at excessive levels, they could become toxic to plants (Nagajyoti et al. 2010). The concentrations of mineral elements in plant tissues vary widely, depending on the species, soil characteristics, and climate (Smith 1962; Han et al. 2011). The usual Mn concentration found in terrestrial plants varies from 5 to 2000 mg kg⁻¹ of dry plant (Sheoran et al. 2010). The total concentration of Mn in plants grown in the fertilized tailings was 2217.83 mg kg⁻¹ for *B. virgilioides* and 1858.13 mg kg⁻¹ for *D. vandellianum*, values close to the usual range. Indeed, the Mn concentration in the shoots, 899.52 mg kg⁻¹ for *B. virgilioides* and 769 mg kg⁻¹ for *D.* vandellianum, is below the critical toxicity concentrations of Mn in the shoots of other species such as sweet potato $(1380 \text{ mg kg}^{-1})$, sunflower $(5300 \text{ mg kg}^{-1})$, and Eucalyp*tus camaldulensis* (6510 mg kg⁻¹) (Reichman et al. 2004; Marschner 2011). Moreover, typical visual symptoms of Mn toxicity, such as chlorosis and necrotic brown spotting on leaves (Millaleo et al. 2010; Reichman 2002), were not



<Fig. 3 Leaf area (cm²), specific leaf area, and leaf area ratio of two tree species grown in different substrates (fertilized soil and fertilized tailings). (a), (b), (c)=*Bowdichia virgilioides* and (d), (e), (f)=*Dictyoloma vandellianum*. Bars represent means \pm SD of ten replicates (*n*=20). The symbols *, **, or *** indicate significant differences between treatments by analysis of variance at *P*<0.05, *P*<0.01, and *P*<0.001, respectively

observed in the plants of this study. Manganese toxicity is accompanied by induced deficiencies of other nutrients such as Ca, Mg, Fe, and Zn and the concentration of these elements in the species of this study was above or within the levels for adequate growth (3–10, 1–3, 100, and 0.01–05 g kg⁻¹ for Ca, Mg, Fe, and Zn, respectively) (Ronquim 2010; Marschner 2011). Enhanced formation of auxiliary shoots ("witches' broom") is another symptom of Mn toxicity (Bañados et al. 2009) that was not observed in the plants. Therefore, it is most likely that Mn did not cause phytotoxicity in the studied species.

In contrast, excess of Fe on plant tissues could explain the decreased growth of plants grown on fertilized tailings in relation to those grown in fertilized soil. Generally, the critical iron toxicity concentrations are above 500 mg Fe kg⁻¹ dry weight (Marschner 2011). Both species accumulate Fe in the shoots (820.98 mg kg⁻¹ for *B. virgilioides* and 2797.17 mg kg⁻¹ for D. vandellianum) in phytotoxic concentrations (Pugh et al. 2002; Kuki et al. 2008; Santana et al. 2014). Zago et al. (2019) also observed high concentrations of iron $(1200-1400 \text{ mg kg}^{-1})$ in plants grown in the tailings from the rupture of the Fundão dam. There is no study about the limit of Fe toxicity for the B. virgilioides and D. vandellianum; however, both species are found in an iron-saturated environment (Versieux et al. 2011; Teixeira and Filho 2013; Guimarães et al. 2019). Nevertheless, the species in this study had higher iron concentration in the shoots (820.98 mg kg⁻¹ and 2797.17 mg kg⁻¹) than found by Schettini et al. (2018) in leaves of 27 native plants from ferruginous ecosystems (90.6 to 696.5 mg kg $^{-1}$).

Iron has a central role in redox reactions (Guerinot and Yi 1994). It is found in components of proteins and catalyzes redox reactions in several fundamental processes such as photosynthesis, respiration, nitrogen metabolism, hormone biosynthesis, and defense against pathogens (Hänsch and Mendel 2009; Nagajyoti et al. 2010). It is necessary for chloroplast development and chlorophyll biosynthesis and is part of several complexes in the electron transport chain of photosynthesis (Hell and Stephan 2003; Nagajyoti et al. 2010). However, iron excess can cause the reduction in the accumulation of dry matter and lower plant height during the vegetative stages (Becker and Asch 2005; Li et al. 2012; Casierra-Posada et al. 2014). Also, Fe excess can increase the production and accumulation of reactive oxygen species (ROS), causing oxidative stress and consequent cellular damage (Kuki et al. 2008; Neves et al. 2009; Jucoski et al.

2013). Once accumulated, ROS can react with chlorophylls leading to their degradation (Gomes et al. 2016), and similarly to D. vandellianum plants grown in the fertilized tailings, several studies have observed that Fe excess decreased the chlorophyll content in plants (Xing et al. 2010; Pereira et al. 2013; Xu et al. 2015). Iron concentrations above the adequate physiological level negatively affect photosynthesis, decreasing the electron transport rate, plant net photosynthetic rate, and F_V/F_M , which culminate in reduction of the plant dry mass production (Nenova 2006; Neves et al. 2009; Pereira et al. 2013; Xu et al. 2015). We, however, did not observe significant differences between treatments for F_V/F_M in both species. Similarly, F_V/F_M was not affected in maize, millet, and sorghum plants grown in the Fundão tailings, although Fe concentrations had exceeded its limit toxicity in these agronomic species (Esteves et al. 2020a, b). Although F_V/F_M is normally useful to attest stress condition to plants (Baker and Rosenqvist 2004), it is important to note that it does not reflect the performance at which the PSII is operating under ambient light (Baker 2008). Therefore, more sensitive chlorophyll a fluorescence parameters such as effective PSII quantum yield and electron transport rate must be used when assessing the impact of high Fe concentration on the leaves of plants grown in the tailings.

The leaf area ratio (LAR) was higher in the fertilized soil treatment for *B. virgilioides* and higher in the fertilized tailings for D. vandellianum. LAR expresses the ratio between the area of leaf and the total plant biomass and reflects the leafiness of a plant or amount of leaf area formed per unit of biomass. As for LAR value, B. virgilioides plants grown in fertilized soil showed bigger leaf area showing their great investment in shoot biomass. On the other hand, for D. vandellianum, although the total leaf area of the plant was also higher in plants grown in fertilized soil, the value of LAR as well as SLA was higher in plants grown in the fertilized tailings, which indicate the production of thinner leaves in plants growing in tailings. The specific leaf area (SLA) measures the ratio of leaf area to leaf dry mass of plants, i.e., light-capture area deployed per unit leaf mass. Thinner leaves (high SLA) have been observed in susceptible cultivars of rice submitted to iron toxic levels (Audebert and Sahrawat 2000), which corroborate our hypothesis of Fe toxicity in plants grown in tailings.

Bowdichia virgilioides had higher root/shoot ratio in the fertilized tailings, indicating a higher biomass allocation to roots. The preferential biomass allocation to roots in *B. virgilioides* plants grown in fertilized tailings also corroborates to the smaller leaf area observed in these plants and consequently, less light interception and less growth. Under conditions of high iron levels, plants allocate proportionally more biomass in the root than in the shoot to tolerate the stressor (Casierra-Posada et al. 2014). The greater allocation of biomass in the root occurs because they become thicker



Fig. 4 Dry mass of two tree species grown with fertilization in different substrates (fertilized soil and fertilized tailings). (**a**), (**b**)=*Bowdichia virgilioides* and (**c**), (**d**)=*Dictyoloma vandellianum*. Bars represent means \pm SD of ten replicates (*n*=20). The symbols *, **, or

*** indicate significant differences between treatments by analysis of variance at P < 0.05, P < 0.01, and P < 0.001, respectively. The scale bar in the photos is 5 cm

in metal excess, which represents a great metal chelation surface and restrain of their transport to shoots (Gomes et al. 2011). However, this process of root thickening caused less absorption of magnesium by the roots grown in the iron tailing, considering that the root thickening process does not guarantee greater root absorption, which is done by the thin roots (Gomes et al. 2011). In the case of *D. vandellianum*, this species also had a lower Mg concentration in the shoots of the plants grown in the fertilized tailings, which explains the lower chlorophyll concentration (Guo et al. 2016). Magnesium acts as a cofactor for several enzymes and in the stabilization of nucleotides and nucleic acids, besides being the central element in the chlorophyll molecule (Maathuis 2009).

D. vandellianum transferred large amounts of Fe to the leaves as evidenced by the iron translocation factor. Translocation factor indicates the efficiency of plants to transfer metals from root to shoots. However, for both species, the iron TF (regardless of the substrate where plant grown) is considered low (<1; Nouri et al. 2011). Bowdichia virgilioides may have mechanisms that avoid the translocation of Fe to shoots with the aim of reducing the impairment of their physiological performance. In several species, the root system accumulated higher Fe concentrations in relation to shoots (Becker and Asch 2005; Nouri et al. 2009; de Araújo et al. 2014; Santana et al. 2014; Cruz et al. 2020). A greater transfer of calcium and consequent greater Ca accumulation in the shoot were observed in D. vandellianum plants grown in the fertilized tailings. This can evidence that Ca is involved in alleviation mechanism of iron-induced toxicity to plants. It has been suggested that calcium participates in mechanisms to decrease toxicity caused by arsenic (Rahman et al. 2015),

cadmium (Ahmad et al. 2015), nickel (Mozafari 2013), and zinc (Gai et al. 2017). Possible Ca mechanisms to protect plants against metal stress are as follows: controlling membrane permeability and movements of divalent cations across cell membrane, scavenging reactive oxygen species, promoting antioxidant system, competing for transporter sites, as well as participating in Ca-dependent signaling transduction under metal stress (Hirschi 2004; Farzadfar et al. 2013; Huang et al. 2017).

In summary, even assuring the adequate levels of important nutrients to plant growth (N, P, K, Ca, and Mg), the fertilization did not reverse the negative effect of tailing on B. virgilioides and D. vandellianum. We emphasize that fertilization techniques in areas affected by iron ore tailings must consider both the needs and tolerances of the species and the level of nutrients already present in the tailings. In addition, it is important to consider the age of the plants; here, recently germinated plants were exposed to the tailings; however, older plants might respond differently. For now, the high concentration of Fe in the tissues associated with less biomass production, lower plant height, smaller leaf area, bigger specific leaf area, and reduced chlorophyll content indicates a probable phytotoxic effect of iron present in the tailings for D. vandellianum. We observed, however, less sensitivity of B. virgilioides plants to Fe excess on tailings. The results of this study base further field evaluations and longer experiments, which will facilitate the understanding of the performance of tree species submitted to tailings with fertilization. So far, this study suggests that B. virgilioides are more tolerant to excess Fe from the tailings of Fundão dam than D. vandellianum.

Acknowledgements We thank Dr. Fabio Vieira for helping in the selection of areas and substrate sampling campaigns and Instituto Espinhaço–Biodiversidade, Cultura e Desenvolvimento Socioambiental for **Fig. 5** Macro- and micronutrients concentration in the roots of two tree species (*Bowdichia virgilioides* and *Dictyoloma vandellianum*) grown in different substrates (FS = fertilized soil and FT = fertilized tailings). Bars represent means \pm SD of three replicates (n=6). The symbols *, **, or *** indicate significant differences between treatments by analysis of variance at P < 0.05, P < 0.01, and P < 0.001, respectively



Fig. 6 Macro- and micronutrients concentration in the shoots of two tree species (*Bowdichia virgilioides* and *Dictyoloma vandellianum*) grown in different substrates (FS = fertilized soil and FT = fertilized tailings). Bars represent means \pm SD of three replicates (n=6). The symbols *, **, or *** indicate significant differences between treatments by analysis of variance at P < 0.05, P < 0.01, and P < 0.001, respectively



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Table 1 Metal translocation factor (TF) in *Bowdichia virgilioides* and*Dictyoloma vandellianum* after 10 weeks of treatment (fertilized soiland fertilized tailings). Values of averages \pm SD of three replicates.

*indicates significant differences (P < 0.05) by analysis of variance between treatments within the same species

Species	Treatment	Ca TF	Cu TF	Fe TF	K TF	Mg TF	Mn TF	Zn TF
Bowdichia virgilioides	Soil	2.81 ± 0.46	0.30 ± 0.05	0.07 ± 0.01	0.76 ± 0.09	0.50 ± 0.04	0.26 ± 0.12	0.25 ± 0.09
	Tailings	2.30 ± 0.18	0.35 ± 0.05	0.08 ± 0.03	0.74 ± 0.08	0.66 ± 0.10	0.73 ± 0.38	$0.55 \pm 0.07 *$
Dictyoloma vandellianum	Soil	1.48 ± 0.27	0.14 ± 0.03	0.06 ± 0.02	0.55 ± 0.03	0.77 ± 0.10	0.39 ± 0.08	0.31 ± 0.05
	Tailings	$2.40\pm0.20^*$	$0.35\pm0.15^*$	$0.16 \pm 0.02 *$	0.56 ± 0.03	0.83 ± 0.14	$0.72\pm0.21^*$	$0.64 \pm 0.21 *$

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Author contribution FVSC performed the study conception, formal analysis, investigation, and writing (original draft). MPG contributed with the conceptualization, supervision, and writing (review and editing). EMB contributed with the investigation and data collection. QSG was responsible for the acquisition of the financial support, conceptualization, and supervision. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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