

Biochemical attributes and establishment of tree seedlings in soil after *Urochloa decumbens* cultivation in soil with deposition of iron mining residues

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SILVICULTURE

ABSTRACT

Background: The recovery of areas impacted by mining activities is a major challenge and requires knowledge of how the biological and biochemical processes of the soil as well as the establishment of plant species are affected by mining residue. In this work, the influence of deposition of iron mining mud and tailings on soil biochemical attributes and the initial development of tree seedlings was studied in a simulated event in the greenhouse. Pots (n = 27) with natural soil (2.0 kg) received deposition (280 mL) of iron mining mud or tailings and by three-month was cultivated with *Urochloa decumbens*. At the end of this period, part of the pots was sampled and the soil biochemical activities were evaluated. Then tree seedlings (*Schinus terebinthifolius* and *Eremanthus incanus*) were planted and cultivated for a year, when then shoot (SDW) and roots (RDW) dry weight were evaluated.

Results: Deposition of iron mining mud and tailings did not affect microbial biomass carbon or basal soil respiration, however both conditions reduced (p < 0.05) β -glucosidase activities by up to 30%, and the easy extractable glomalin by about 19%. In addition, the mud deposition reduced (p < 0.05) the SDW and RDW of *S. terebinthifolius* by about 21% and 24%, respectively. Nevertheless, *Urochloa decumbens* and *Eremanthus incanus* had no variation in dry weight production between treatments, so they have good ability to grow settle in soil affected by mining residue.

Conclusion: This study shows that the sequential planting of *U. decumbens* and *E. incanus* can be an alternative for use in environments with deposition of iron mining tailings.

Keywords: Degraded Areas, Soil Bioindicators, Revegetation, Dam collapse, Enzymatic activity.

HIGHLIGHTS

Iron mining residues (mud and tailings) reduced β -glucosidase activity and glomalin levels. *U. decumbens* and *E. incanus* developed well in the presence of tailings or iron mining mud. *S. terebinthifolius* had reductions in SDW and RDW when it was grown in the presence of iron mining mud.

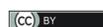
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INTRODUCTION

The collapse of the Fundão dam in Mariana-Minas Gerais spilled 35-45 million m³ of iron mining residues over approximately 800 km in the Doce River Basin. Consequently, ~1500 hectares of riparian forests and agricultural areas were impacted by deposition or displacement of the tailings mud. The most impacted section corresponds to the first 80 km of the Basin, on the banks of the Gualaxo do Norte River, which had partial or total changes in its physical, chemical, and biological soil properties (Davila *et al.*, 2020; Segura *et al.*, 2016; Garcia *et al.*, 2017; Hatje *et al.*, 2017; Pires *et al.*, 2017; Zago *et al.*, 2019; Batista *et al.*, 2020).

The rehabilitation of extensive areas impacted by spills of tailings resulting from dam collapses, such as occurred at the Fundão dam (Mariana) and Córrego do Feijão (Brumadinho), in the State of Minas Gerais - Brazil, is a great challenge. This process requires the recomposition of landscapes and the restoration of ecosystems at large scale levels. For this, the use of grasses, characterized by fast growth and good shoot production, such as those of the *Urochloa* genus, has shown positive results in the initial stages of the rehabilitation of environments related to mining activities (Stumpf *et al.*, 2014; Santos *et al.*, 2016; Pedroso *et al.*, 2018). In addition to grasses, the use of native tree species capable of adapting to the new limiting soil conditions is essential, and some characteristics presented by tree species can be determinant for the success or failure of rehabilitation, such as: being resistant to pests and diseases, ease in acquiring propagules, adaptability, high growth rate, efficiency in absorption and use of nutrients, and tolerance to heavy metals (Accioly *et al.*, 2000; Araújo *et al.*, 2018). Moreover, it has been reported the benefits of the associated soil microbiota with plants to achieve success in revegetation processes (Carneiro *et al.*, 2008; Herzberger *et al.*, 2014; Franchi *et al.*, 2016; Santos *et al.*, 2016; Rangel *et al.*, 2017; Pedroso *et al.*, 2018). Soil microorganisms play an essential role in the availability of nutrients for plants (Van der Heijden *et al.*, 2008; Morgan and Connolly, 2013; Tak *et al.*, 2013; Lenart-Boroń and Boroń, 2014; Santoyo *et al.*, 2016; Jacoby *et al.*, 2017), besides participating in the immobilization, chemical transformation, and detoxification of chemical elements potentially toxic to plants (Tak *et al.*, 2013; Lenart-Boroń and Boroń, 2014; Ma *et al.*, 2015; Dzionek *et al.*, 2016). Soil microorganisms indirectly also favor the establishment of plants in the soil by acting on the stabilization of soil aggregates, for example by the physical effects of fungal hyphae, as well as by the production of cementing agents such as glomalin (Wright and Upadhyaya, 1998; Rillig *et al.*, 2004; Pedroso *et al.*, 2018; Lehmann *et al.*, 2017; Barbosa *et al.*, 2019) or due to the release of biopolymers (exopolysaccharides) synthesized by certain bacterial groups (Deng *et al.*, 2015; Lehmann *et al.*, 2017; Costa *et al.*, 2018). All these mechanisms depend heavily on biochemical reactions mediated by enzymes, which are part of the carbon, nitrogen, phosphorus, and sulfur cycle through the processes of decomposition and mineralization (Hojati and Nourbakhsh, 2006; Bowles *et al.*, 2014).

The success of revegetation programs in impacted areas, therefore, will depend on the presence of an active

soil microbiota associated with plants established on substrates with physical and chemical impediments. In this context, knowing how the deposition of iron mining mud and tailings affects the microbial activity and the establishment of plants may favor decision making aimed at accelerating the process of rehabilitation of areas impacted by iron mining.

Therefore, this study aimed to evaluate the impact of iron mining mud and tailings deposition on soil microbial activity and on the establishment of *U. decumbens* and tree species with potential for use in rehabilitation processes. We hypothesized that the deposition of iron mining mud or tailings on the soil reduces the soil microbial activity and consequently affects the development of pre-existing vegetation (*U. decumbens*), as well as the initial growth of native plants used in the revegetation process.

MATERIAL AND METHODS

The study was conducted in a greenhouse at the Department of Soil Sciences at the Federal University of Lavras, in two stages (Fig 1). I - simulation of iron mining mud and tailings deposition on a non-impacted soil, cultivated with *Urochloa decumbens*. This species of grass was chosen for this study because it has the ability to grow in soils with low fertility and physical limitations and produce abundant vegetation cover in a short time. Three months later, the effects of this deposition on different soil biological attributes and development of *U. decumbens* were evaluated. II - began with the planting of seedlings of two tree species and, after one year, evaluation of the development of these native tree species in the affected soils. The experiment was conducted in a completely randomized design with 27 replications. Each pots received 2.0 kg of Dystrophic Red Latosol (non-impacted soil by tailings or mining mud, collected in a native forest at the Federal University of Lavras, 21°13'42" S, 44°58'02" W). Chemical characteristics of the soil are shown in Tab. 1. At the time of sowing, each pot received 15 seeds of *U. decumbens* and 50 ml of nutrient solution (g.L⁻¹) 26.42 g NH₄H₂PO₄, 3.5 g K₂SO₄, 0.20 g H₃BO₃, 0.54 g MnSO₄·H₂O, 0.14 g CuSO₄·5H₂O, 0.47 g ZnSO₄·7H₂O, 0.37 g KCl, and 26.19 g NH₄H₂PO₄. 26 and 37 days after sowing (DAS), cover fertilization was carried out with N and K in the doses of 0.189 g and 0.197g of K in both seasons. The iron mining mud and tailings were provided by the company Samarco S/A. The mud was collected from a mining dam (20°12'58"S, 43°28'20"O). The tailings were obtained from a stretch affected by the mining dam burst, one year after the accident. The stretch affected is located on the banks of the Gualaxo do Norte river (Pedras District, municipality of Mariana – MG; 20°16'22" S, 43°12'04" O). The mud and the tailings presented silt and loam sandy. Compositional analysis of some potentially toxic elements in the tailing and mud, performed using portable X-ray fluorescence (XRF), are shown in Tab. 2.

First stage - Mud and tailings deposition and impacts on plant development and soil biochemical attributes.

To simulate the impact of iron mining mud and tailing deposition on the soil, the following treatments

Tab. 1 Chemical characteristics of iron mining mud and tailings and of a Dystrophic Red Latosol used in the experiment.

Treatments	pH (H ₂ O)	P mg dm ⁻³	K ----- cmol _c .dm ⁻³	Ca -----	Mg -----	Zn -----	Fe -----	Mn -----	Cu -----	MO g kg ⁻¹	Clay -----	Silt -----	Sand -----
Mud	8.7	17.46	0.04	2.24	0.1	1.17	199.85	321.67	0.80	3.30	4.00	95.00	1.00
Tailing	8.4	10.24	0.19	1.21	0.1	1.26	221.08	168.41	0.95	7.00	8.00	36.60	55.40
Not-impacted soil	5.8	0.28	0.04	0.10	0.1	0.90	19.00	5.60	1.00	6.50	68.00	14.00	18.00

Tab. 2 Compositional analysis of elements of iron mining mud and tailings performed using portable X-ray fluorescence (XRF).

Elements (mg.kg ⁻¹)	Mud	Tailing
As	<DL	<DL
Cd	<DL	<DL
Cr	350.00	235.00
Cu	20.00	28.00
Fe	43.557.00	178050.00
Hg	<DL	<DL
Mn	2090.00	1008.00
Ni	0.00	6.20
Pb	570.00	52.7
Si	109309.00	1811285.00
Zn	10.00	17.2

<DL - Values below the detection limit

were established one month after planting *U. decumbens*: nine pots received 280 mL of mining mud or tailing. For comparison, nine other pots had no material deposition (control treatment, not-impacted soil) (Fig. 1).

After three months, measurements of gas exchange were made in leaves of *U. decumbens*. The measurements

were carried out in the period between 9 - 11 am and recorded using an infrared gas analyzer (IRGA) model - Li-Cor - 6400XT). LED camera of equipment was programmed to Photosynthetic photon flux density (PPFD) (DFFFA) of 600 μmol m⁻².s⁻¹. The liquid photosynthesis (A - μmol CO₂ m⁻².s⁻¹), stomatal conductance (gs - mol H₂O m⁻².s⁻¹) and transpiration (E-mmol H₂O m⁻².s⁻¹) values were determined. After these assessments, the shoots of the plants were collected and dried in a forced-air circulation (60 °C) until they reached a constant weight to determine the shoot dry weight of the aerial part (SDW).

Besides, one third (nine pots) of the treatments were disassembled, and the entire volume of material was homogenized, after which soil samples were collected. Then, the following biochemical attributes were analyzed: basal soil respiration (RBS) by capturing CO₂ evolved by NaOH solution (0.05M), after 3 days of incubation according to Alef (1995); microbial biomass carbon (MBC) by the fumigation-extraction method described by Vance et al (1987); the qCO₂ of each treatment was determined from the ratio between BR and MBC, (Anderson and Domsch, 1993); the activity of the β -glucosidase enzyme according to the method of Eivazi and Tabatabai (1988) and the

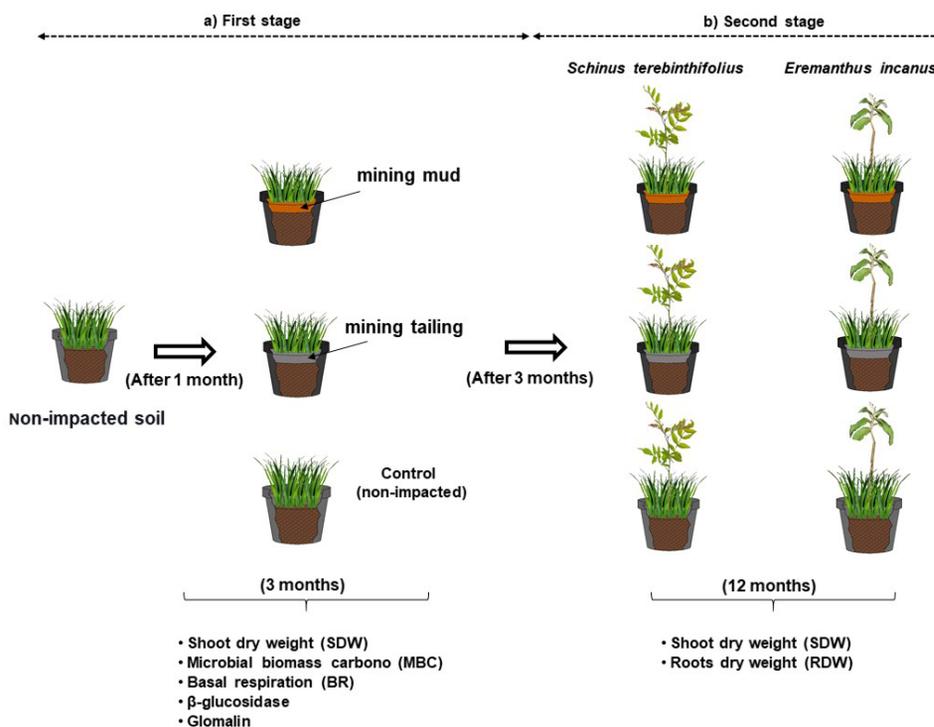


Fig. 1 Description of the experiment in a greenhouse to assess the impact of deposition of mud and tailings from iron mining on the soil biological and biochemical attributes and the establishment of *Urochloa decumbens*, *Schinus terebinthifolius* and *Eremanthus incanus*.

extraction of easily extractable glomalin from the soil was carried out with sodium citrate solution according to the methodology described by Wright and Upadhyaya (1998) and its levels were quantified according to the method of described by Bradford (1976).

Second stage - Development of seedlings of tree species on impacted soils

In the second stage of the experiment, after cutting the shoots of *U. decumbens*, two tree species (*Schinus terebinthifolius* Raddi (Aroeirinha) and *Eremanthus incanus* (Less.) (Candeia)) were planted in the remaining eighteen pots (Fig. 1). The seedlings of tree species were supplied by the UFLA seedling nursery. For one year the pots with the plants were kept in a greenhouse and irrigated to maintain field capacity. For this, the pots were weighed daily once a day and the volume of water needed to maintain the field capacity was added. After 12 months of cultivation, all plants were collected and their aerial parts and root systems were subjected to drying (60°C) until reaching constant weight, to determine the shoot (SDW) and roots (RDW) dry weight.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and Tukey test at 5% probability, using the SISVAR statistical program (Ferreira, 2014). Previous to normal and homoscedasticity were examined with the Shapiro-Wilk and Levene tests using software R (R Core Team, 2020), respectively, and were not violated.

RESULTS

Microbial biomass carbon (MBC) and basal respiration (BR) and $q\text{CO}_2$ were not affected ($p < 0.05$) by the deposition of mud or tailings. On the other hand, the activity of the β -glucosidase enzyme decreased ($p < 0.05$) by 46% and 16% due to the deposition of mud and mining tailings, respectively. The Glomalin was also affected ($p < 0.05$) by the deposition of mud and mining tailings, in both cases leading to a reduction of about 19% ($p < 0.05$) in its contents when compared to the non-impacted soil, after three months of cultivation of *U. decumbens* (Tab. 3).

The SDW of *U. decumbens* was not significantly affected after three months of cultivation in soil impacted by mud or iron mining tailings (Tab. 4). A similar effect was observed for SDW and RDW of *E. incanus*, even after one year of cultivation on soils impacted by mud and tailings. On the other hand, the mud negatively affected the values of SDW and RDW of *S. terebinthifolius*, with a reduction

of 21 and 24%, respectively, when compared to the values observed in plants grown in non-impacted soil (Tab. 4).

Regarding gas exchange, there were no significant differences in rates and liquid photosynthesis (A), transpiration (E), and stomatal conductance (Gs) in plants of *U. decumbens* cultivated in non-impacted soil and soils impacted by mud or iron mining tailings (Tab. 5).

DISCUSSION

The high values for MBC indicate that the original soil microbiota was resistant to deposition of mud and mining tailings, keeping the statistically the same values to those observed for the non-impacted soil (Tab. 3). Although several studies have shown a reduction in microbial biomass in affected soils due to mining activities (Wang *et al.*, 2007; Carneiro *et al.*, 2008; Santos *et al.*, 2013), it has also been reported that when adaptations of the remaining microbiota occur, in affected areas, it can maintain itself or even to re-colonize the soil, and so, it can raise MBC values (Carneiro *et al.*, 2008; Santos *et al.*, 2016), even if this increase in microbial biomass does not mean maintenance microbial diversity (Wang *et al.*, 2007; Santos *et al.*, 2016).

The BR rate also showed a balance between treatments, but the high values indicate intense microbiota activity (Tab. 3). High respiratory rate is related to consumption of organic compounds readily assimilated by soil microorganisms, directing microbial metabolism more to maintenance than to increasing microbial biomass, which commonly occurs in stressful conditions for soil microbiota (Carneiro *et al.*, 2008; Santos *et al.*, 2013; Silva *et al.*, 2018). This aspect is relevant because the increase in CO_2 emissions implies a reduction in the carbon stock of the soil over time.

The activity of β -glucosidase, an enzyme that is involved with carbon metabolism and obtaining energy by the microbial cell, was negatively affected by the deposition of the iron mining mud and tailings (Tab. 3). The lower activity this enzyme can be explained by the presence of excess metals such as manganese and iron itself, besides to others potentially toxic elements, which are commonly present in composition of the iron mining mud and tailings, as reported in this work (Tab. 1) and also verified by other authors (Segura *et al.*, 2016; Santos *et al.*, 2019). Another important aspect is the physical condition of both the mud and tailings, due to the high levels of silt (95%) and sand (55%) respectively (Tab. 1), also reported in other studies (Carmo *et al.*, 2017; Batista *et al.*, 2020), and that reduces the total porosity of the soil, especially the macropores, limiting the aeration of the soil and consequently the

Tab. 3 Biochemical attributes of soils, cultivated with *Urochloa decumbens*, impacted by the deposition of iron mining mud and tailings, and a non-impacted soil, in a greenhouse experiment.

Treatments	MBC* ($\mu\text{g C/g dry soil}$)	BR ($\text{mg C-CO}_2 \text{ g}^{-1}\text{h}^{-1}$)	$q\text{CO}_2$ ($\text{g C-CO}_2 \text{ g}^{-1}\text{MBC h}^{-1}$)	β -Glucosidase ($\mu\text{g PNP}\cdot\text{g}^{-1}\text{dry soil}\cdot\text{h}^{-1}$)	Glomalin ($\text{mg}\cdot\text{g}^{-1}$)
Mud	633.28 a**	18.79 a	41.20 a	456.82 c	2.88 b
Tailing	698.46 a	20.96 a	32.12 a	712.46 b	2.91 b
Not-impacted soil	790.02 a	17.44 a	23.19 a	842.53 a	3.57 a

*MBC – Microbial biomass carbon; BR – Basal respiration; $q\text{CO}_2$ – Metabolic quotient. **Means followed by the same letter in the column are not significantly different based on the Tukey test at 5% probability.

Tab. 4 Shoot (SDW) and Root (RDW) dry weight of plants grown in soils impacted by deposition of iron mining mud and tailing, and in non-impacted control soil, in a greenhouse experiment *Urochloa. decumbens* was evaluated after three months of cultivation; *Schinus terebinthifolius* and *Eremanthus incanus* - evaluated after 12 months of cultivation.

Treatments	SDW			RDW	
	<i>U. decumbens</i>	<i>S. terebinthifolius</i>	<i>E. incanus</i>	<i>S. terebinthifolius</i>	<i>E. incanus</i>
	----- g.pot ⁻¹ -----				
Mud	53.71 a*	4.04 b	2.51 a	3.89 b	2.24 a
Tailing	53.20 a	5.47 a	3.15 a	4.36 a	2.63 a
Not-impacted soil	55.57 a	5.11 a	2.75 a	5.12 a	2.12 a

*Means followed by the same letter in the column are not significantly different (P < 0.05) based on the Tukey test.

Tab. 5 Photosynthesis (A), stomatal conductance (Gs), and transpiration (E) of *Urochloa decumbens* cultivated in soils impacted by deposition of iron mining mud and tailings, and in non-impacted soil, in a greenhouse experiment.

Treatments	A	Gs	E
	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	$\text{gs} - \text{mol H}_2\text{O m}_2 \text{ s}^{-1}$	$\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$
Mud	2.05 a*	0.006 a	0.35 a
Tailing	2.53 a	0.003 a	0.30 a
Not-impacted soil	2.49 a	0.005 a	0.40 a

*Means followed by the same letter in the column are not significantly different (P < 0.05) based on the Tukey test

microbial activities performed by aerobic microorganisms (Pupin *et al.*, 2009; Nawaz *et al.*, 2013). Limitations in the enzymatic activities of the soil directly affect the cycling and the availability of carbon and nutrients for the plants and consequently can compromise the long-term restoration of impacted ecosystems (Batista *et al.*, 2020).

The mining mud negatively affected glomalin concentrations (Tab. 3). This effect is related to the characteristic of the mud, which is a biologically inert material, and for this reason, it had a diluting effect on the glomalin concentrations due to its spillage on the natural soil used as substrate, compared to the not-impacted soil (Tab. 3).

A similar effect occurred with the tailings. However, unlike mud, tailings are a mixed material, since as a result of the rupture of the dam, the mud was originally revolved and, consequently, mixed with the natural soil along the bank of the Gualaxo do Norte River. Thus, the tailings changed the chemical and physical characteristics, as well as biological components of the natural soils of the places where it passed (Segura *et al.*, 2016; Carmo *et al.*, 2017; Prado *et al.*, 2019; Santos *et al.*, 2019). Thus, the tailings incorporated part of the chemical, physical and biological characteristics of the soil in the affected areas. Therefore, unlike mud, the tailings, collected on the banks of the Gualaxo do Norte river, one year after the accident, presented some microbial load and this must have contributed to the intermediate values to mud and not-impacted soil. Glomalin is a glycoprotein considered to be persistent in the soil (6 - 92 years) and takes part in the cementation of soil particles playing a key role in its aggregation (Wright and Upadhyaya, 1998; Rillig *et al.*, 2001; Preger *et al.*, 2007; Zhang *et al.*, 2017). Therefore, this biological attribute plays a key role in improving the physical properties of the soil in areas impacted by mining, and for this reason, it can be an important indicator of the evolution of soil rehabilitation processes.

U. decumbens is known for being a very aggressive plant in terms of competition with other species, and

the ability to grow in soils with low fertility and physical limitations (Flávio Neto *et al.*, 2015; Monteiro *et al.*, 2016; Silva *et al.*, 2019). These characteristics explain the absence of significant differences in the plant's SDW in soil affected with mud and tailings from iron ore mining, compared with non-impacted treatment (Tab. 4). One of the characteristics that make the *U. decumbens* such competitive advantages possible in different environments is the high photosynthetic performance due to C4 metabolism. Plants with this metabolism need a lower concentration of CO₂, are more efficient in the use of water and more effective in the production of biomass.

In the presence of mining tailings, the species *S. terebinthifolius* and *E. incanus* had SDW and RDW like those of cultivated plants in non-impacted soil by deposition of mining residues (Tab. 4). On the other hand, in the presence of iron mining mud only *E. incanus* was not affected. These results indicate that plant species respond differently to the type of material deposited. Mining mud and tailings have high pH values, which generally decreases the availability of potentially toxic elements for plants (table 1). However, even in these conditions, it is observed that the total and available levels of Mn in the mud was double those observed in the tailings (Tab. 1 and 2). In addition, total Cr and Pb contents were higher in the mud. Even high levels of silt in the mining mud favor the formation of a sealing layer, leading to limitations to root development. Combined effects of these characteristics for mining mud were more evident in affecting the development for *S. terebinthifolius* than for *U. decumbens* and *E. incanus*. Therefore, depending on the area that will be rehabilitated, one species or another may be more suitable. However, both species can be considered in new stages of studies, including in the field, since the material deposited along the Gualaxo do Norte River, the main area physically affected by the collapse of the Fundão dam is predominantly of iron mining tailings, which is characterized by being a material more diluted (with natural soil) than the iron mining mud stored inside the dam.

CONCLUSIONS

Mud and tailings from iron mining did not affect the biological indicators: microbial biomass and basal respiration, nor the plant species *U. decumbens* and *E. incanus*. On the other hand, both conditions had negative effects on the activity of β -glucosidase, glomalin and development of *S. terebinthifolius* seedlings. Therefore, the recovery of areas impacted by iron mining residues

must consider both improvements in the biological and biochemical activities of the soil, as well as the reintroduction of plant species more adapted to the new soil conditions.

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