Chemosphere 262 (2021) 127803

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Synergistic effect of organo-mineral amendments and plant growth-promoting rhizobacteria (PGPR) on the establishment of vegetation cover and amelioration of mine tailings

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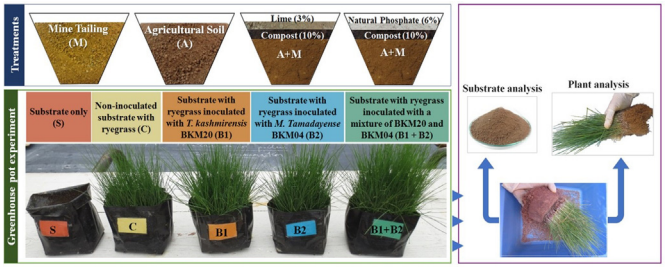
HIGHLIGHTS

- Organo-mineral amendments play a key role in plant establishment in mine tailings.
- Combined use of organo-mineral amendments and PGPR enhanced ryegrass growth.
- Co-inoculation with metal resistant PGPR further improved plant growth.
- Bacterial inoculation improved plant resistance to metals.
- Bioinoculation stimulated ryegrass antioxidant defense system.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 April 2020
Received in revised form
28 June 2020
Accepted 22 July 2020
Available online 26 July 2020

Handling Editor: T Cutright

Keywords:
Bioinoculation
Metals
Amendments
Phytoremediation
Soil enzymes
Oxidative stress

ABSTRACT

Mine tailings pose a huge hazard for environmental and human health, and the establishment of vegetation cover is crucial to reduce pollutant dispersion for the surroundings. However, their hostile physicochemical conditions hamper plant growth, compromising phytoremediation strategies. This study aims to investigate the role of organo-mineral amendments and plant growth-promoting rhizobacteria (PGPR) on the improvement of mine tailings properties and *Lolium perenne* L. (ryegrass) growth. Plants were grown in mine tailings mixed with an agricultural soil (1:1), 10% compost, and supplied with two different inorganic amendments – rock phosphate (6%) or lime (3%), and inoculated with the rhizobacterial strains *Advenella kashmirensis* BKM20 (B1) and *Mesorhizobium tamadayense* BKM04 (B2). The application of organo-mineral amendments ameliorated tailings characteristics, which fostered plant growth and further enhanced soil fertility and microbial activity. These findings were consistent with the increase of total organic carbon levels, with the higher numbers of heterotrophic and phosphate solubilizing bacteria, and higher dehydrogenase and urease activities, found in these substrates after plant establishment. Plant growth was further boosted by PGPR inoculation, most noticeable by co-inoculation of both strains. Moreover, inoculated plants showed increased activities for several antioxidant enzymes (catalase, peroxidase, polyphenoloxidase, and glutathione reductase) which indicate a reinforced antioxidant system.

The application of agricultural soil, compost and lime associated with the inoculation of a mixture of PGPR proved to enhance the establishment of vegetation cover, thus promoting the stabilization of

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https://doi.org/10.1016/j.chemosphere.2020.127803

Kettara mine tailings. Nonetheless, further studies are needed in order to confirm its effectiveness under field conditions.

1. Introduction

Metal soil contamination constitutes a permanent threat for ecosystems and human health, being recognized as one of the biggest environmental concerns worldwide (Science Communication Unit, 2013; Li et al., 2019). Intensive urbanization and rapid industrial development, particularly of the mining sector, are responsible for the prominent increase of the spread of metals in the environment (Li et al., 2014; Mokhtari et al., 2018). Mining activity is a very important sector in the Moroccan economy. In particular, the southern region of the country concentrates a large number of metalliferous sites, where some mines have been abandoned, while others are still under exploitation (Boularbah et al., 2006a; El Khalil et al., 2008). As a by-product of mine processing, high amounts of metal-contaminated wastes are produced and deposited in open-air tailing ponds without any kind of treatment (Wong, 2003; Mendez and Maier, 2008). These wastes cause many environmental problems, such as pollution and loss of soil function, and contamination of surface and groundwater (Wang et al., 2017; Mokhtari et al., 2018). The 30 years of miming operations in the Kettara mine resulted in more than 3 million tons of residues, that have been deposited over an area of approximately 16 ha (Hakkou et al., 2008).

During the past decade, several remediation techniques have emerged to address the environmental problems caused by mining operations. The most common remediation processes used in mine tailings include physical treatments, such as excavation and storage or stabilization and containment, and chemical stabilization (Tordoff et al., 2000; Wong, 2003; Wang et al., 2017). Nevertheless, such treatments are often temporary solutions in natural environments (Mendez and Maier, 2008), and may cause other negative impacts including the impoverishment of soil properties. Indeed, conventional physicochemical techniques may lead to partial or total disruption of soil biota and structure, contributing to the deterioration of soil ecosystems (Burges et al., 2018; Lacalle et al., 2020; Pérez-de-Mora, 2020).

The implementation of vegetation cover to stabilize mine wastes is widely accepted as a more desirable long-term solution (Tordoff et al., 2000; Mendez and Maier, 2008), since it constitutes an environmentally friendly, sustainable and relatively less expensive technique (Mani and Kumar, 2014; Mani et al., 2015). The establishment of vegetation cover on tailings will prevent downward leaching, dispersion of contaminated particles by wind and lateral runoff (Berti and Cunningham, 2000; Tordoff et al., 2000; Gajić et al., 2018; Buta et al., 2019). Despite these well-known advantages, phytostabilization is often compromised since plant survival may be affected by the inhospitable physicochemical conditions of tailings, including high levels of toxic metals, extreme acidity, low levels of nutrients, and poor substrate structure and water-holding capacity. The adverse climatic conditions of arid and semi-arid areas further intensify this problem (Tordoff et al., 2000; Mendez and Maier, 2008).

Since tailings are a highly hostile substrate for the growth and development of plant and soil microorganisms, improving their physicochemical properties is crucial before carrying out large-scale rehabilitation programs (Santibañez et al., 2012; Pardo et al., 2014; Saleem et al., 2020a). Plant colonization and soil quality of mine-affected areas may be ameliorated through the application of amendments, by the use of tolerant plant varieties, and/or by the application of microbial inoculants (Wang et al., 2017). The addition of organic and/or inorganic amendments to mine wastes has been a strategy widely used to promote adequate conditions for plant growth (Acosta et al., 2018; Al-Lami et al., 2019; Saleem et al., 2020a). These amendments not only diminish metal availability, but also provide nutrients for plant growth and improve soil microbial activity (Lwin et al., 2018). Nonetheless, in highly contaminated areas, high amounts of organic matter may be required to support plant growth, which can greatly increase the costs of remediation (Grandlic et al., 2009).

Combining the inoculation of plant growth-promoting rhizobacteria (PGPR) with amendments is a promising tool to reduce the use of organo-mineral treatments, while improving plant survival (Grandlic et al., 2008; Mengual et al., 2014) and soil quality and health (Solís-Domínguez et al., 2012; Mengual et al., 2014). PGPR isolated from the rhizosphere of metallophytes growing on mining areas, as well as their inherent resistance to metals, may also possess exceptional plant growth-promoting (PGP) traits, including the production of phytohormones and siderophores, and P solubilization (Navarro-Noya et al., 2010; Pereira et al., 2015; Benidire et al., 2016). Several studies suggested that root-associated microorganisms may enhance revegetation of mining areas, by improving plant survival and growth (Grandlic et al., 2008, 2009; Mengual et al., 2014; Moreira et al., 2016a,b; Ohsowski et al., 2018; Al-Lami et al., 2019).

High concentrations of metals may induce phytotoxicity, the most common symptoms being chlorosis and necrosis, decreased biomass above ground, and poor root development (Rizvi and Khan, 2018; Saleem et al., 2020b). Moreover, the high accumulation of metals in plant tissues induces the formation of reactive oxygen species (ROS), namely H_2O_2, $O_2^{\bullet -}$ and OH^-, which may cause irreversible cellular damage (Hossain et al., 2012). To tackle oxidative stress, plants possess a complex net of enzymatic and non-enzymatic ROS-scavenging mechanisms (Hossain et al., 2012; Saleem et al., 2020b,c).

Besides their beneficial effect on plant growth under metal stress conditions, PGPR are known to improve plant tolerance by inducing the activity of several antioxidant enzymes, such as catalase (CAT), peroxidases (POD) and polyphenol oxidases (PPO) among others, in order to combat the deleterious effects of ROS (Thakur and Kapila, 2017; Jian et al., 2019).

Phytoremediation has been widely used as a strategy to remediate metal contaminated soils (Acosta et al., 2018; Aboudrar et al., 2013; Burges et al., 2018; Mendez and Maier, 2008). However, in Morocco only a few studies have specifically investigated the effects of a dual application of amendments and bacterial inoculants on plant growth in mine tailings (El Faiz et al., 2015. Midhat et al., 2018). To the best of our knowledge, this is one of the first studies, aiming to evaluate the synergistic and beneficial effect of organo-mineral amendments and indigenous metal-tolerant PGPR on mine tailings' physicochemical, biochemical and biological parameters, and on the growth and antioxidative response of *Lolium perenne* L. (ryegrass) plants. Results from this study will provide guidelines for the selection of suitable PGPR/amendment combinations to assist phytostabilization of Kettara mine tailings, which

can serve as a basis for other sites with similar contamination profiles.

2. Materials and methods

2.1. Site description

The Kettara pyrrhotite ore mine is located in the northwest of Morocco, 30 km away from the center of Marrakech (31° 52′ 15″ N 8°10' 31" W, about 400 m above sea level). The mine operated from 1962 to 1982 and was periodically active for the extraction of Cu, Zn, Fe and S. After closing the mine was abandoned, leaving behind about 3 million tons of tailings spread over an area of approximately 16 ha leading to the contamination of surrounding soils and vegetation with metals (Boularbah et al., 2006a,b; El Hamiani et al., 2015; Benidire et al., 2016).

2.2. Mine tailings and organo-mineral amendments

Mine tailings were collected from the tailings pond at the Kettara mine. Previous studies showed that these tailings were characterized by highly acidic pH, low levels of essential nutrients, significant levels of sulfur and high concentrations of several metals including Fe, Cu and Zn (Boularbah et al., 2006 a; b; Benidire et al., 2016). In order to obtain a substrate able to support plant growth, mine tailings were first mixed and homogenized in equal proportions (w/w) with an agricultural soil. The agricultural soil was collected from the agricultural zone of the Targa region located in a peri-urban area of Marrakech city. The physicochemical properties were as follows: pH 7.36 ± 0.40; electrical conductivity (mS cm^{-1}) 0.47 ± 0.01; total organic carbon (%) 1.00 ± 0.09; available P (mg g^{-1}) 0.24 ± 0.01. The agricultural soil and tailings were initially air dried for 2 weeks and sieved to a particle size of <2 mm. In order to improve soil structure, decrease the availability of metals and increase soil organic matter and nutrients, different amendments were added to mine tailings mixed in a 1:1 ratio with agricultural soil (AM). These amendemts were an organic one (compost) with one of two minerals (rock phosphate or lime). The compost was obtained from green waste and was provided by the Laboratory of Ecology and Environment of the Science Faculty of Université Cadi-Ayyad, while rock phosphate was obtained from the mining waste of a phosphate mine located in Youssoufia, Morocco. The compost was used to increase organic matter, whereas lime ($CaCO_3$) and rock phosphate were used to increase the tailings' pH (Holland et al., 2018; Xiao et al., 2017). Rock phosphate was also used as a source of phosphorus (P). Different proportions of these amendments were added to the AM substrate as follows: T1 (treatment 1) - AM + 10% compost + 6% rock phosphate; T2 (treatment 2) - AM + 10% compost + 3% lime. In order to stabilize the microbial activity and soil physicochemical properties, the final substrates were incubated for 30 days at room temperature before the start of the greenhouse experiment. Humidity was maintained at 50% of water holding capacity with deionized water. Substrates used in this study were not subjected to sterilization by autoclaving or any other means.

2.3. Bacterial inoculants

The rhizobacterial strains *Advenella kashmirensis* BKM20 (formely named *Tetrathiobacter kashmirensis*) (B1) and *Mesorhizobium tamadayense* BKM04 (B2) were selected for the pot experiments based on their ability to tolerate metals and on their PGP traits, including good indole acetic acid (IAA) and siderophore production, P solubilization and 1-aminocyclopropane-1-carboxylic (ACC)-deaminase activity (Table 1; Benidire et al., 2016). Both strains were previously isolated from the rhizosphere of metallophytes growing in the vicinity of the Kettara mine, being well-adapted to the environmental conditions (Benidire et al., 2016).

For bacterial inoculation, strains were grown in tryptic soybean broth (TSB) for 48 h at 28 °C. Cells were recovered by centrifugation (12000 g, 10 min), washed twice with 0.9% NaCl sterile solution, and adjusted to 10^9 CFU mL^{-1}.

2.4. Greenhouse pot experimental design

The greenhouse pot experiments were performed with ryegrass. *L. perenne* is a perennial grass that supports high concentrations of metals, and has been reported as a good candidate for the revegetation of mining areas (Carvalho et al., 2013; Santibáñez et al., 2008). The experimental design consisted of five inoculation treatments: substrate only (S); non-inoculated substrate with *L. perenne* (C); substrate with *L. perenne* inoculated with *A. kashmirensis* BKM20 (B1); substrate with *L. perenne* inoculated with *M. tamadayense* BKM04 (B2); substrate with *L. perenne* inoculated with a mixture of *A. kashmirensis* BKM20 and *M. tamadayense* BKM04 (B1 + B2), and four substrate treatments: A – agricultural soil; M – untreated mine tailings; T1 - AM + 10% compost + 6% rock phosphate; T2 - AM + 10% compost + 3% lime, all replicated 3 times. *L. perenne* seeds were provided by Turfline Co. (Marrakech, Morocco).

Seeds were surface sterilized in a 1% NaOCl for 5 min and subsequently rinsed thoroughly with sterile deionized water. Seeds (3 g) were placed in sterile petri dishes, one for each pot, containing two layers of Whatman No.1 filter paper moistened with sterile deionized water. Seeds were incubated in darkness at 25 °C until the first true leaf was fully developed. All sprouted seedlings were then transferred to plastic pots containing 400 g of agricultural soil, or untreated and amended mine tailings (T1 and T2). Then, 10 mL of bacterial suspension was sprayed onto the substrate surface. A re-inoculation was performed four weeks after seedling transference.

The experiment was carried out for 90 days, from March 2016 at the Faculty of Sciences and Techniques, Marrakech-Morocco, in a greenhouse under natural light (15 h of photoperiod) and temperature ranging between 15 and 30 °C. Pots were watered daily with deionized water to maintain moisture content at 70% water holding capacity.

2.5. Substrate analysis

Prior to filling the pots with substrates (t_0), their physicochemical properties were analyzed. At the end of the pot experiment (t_f - 90 days) substrate samples were collected and divided into two subsamples. One subsample was stored at 2 °C for biological and biochemical analysis, while the other was air-dried at room temperature and sieved (2 mm) for physicochemical analysis.

2.5.1. Physicochemical analysis

Electrical conductivity (EC) and pH were measured in water suspensions (1:2.5 and 1:5 substrate/water, respectively) using a conductivity and pH meters (ORION 4 STAR pH-Conductivity portable, Thermo Scientific; CRISON micro pH 2000 pH-meter) after shaking for 1 h. Total organic carbon (TOC) was estimated by Anne's method based on the oxidation of organic matter by potassium dichromate as described by Aubert (1978). The molybdenum blue method was used to determine the available P content (Olsen and Sommers, 1982). For all methods blank samples were used. All measurements per biological replicate were carried out in triplicate.

Table 1
Plant growth promoting (PGP) traits of rhizobacterial strains selected for pot experiments (retrieved from Benidire et al., 2016).

PGP traits	*Advenella kashmirensis* BKM20 (B1)	*Mesorhizobium tamadayense* BKM04 (B2)
MIC Zn (mM)	15 mM	5
MIC Cu (mM)	10 mM	5
MIC Cd (mM)	5 mM	1
NH_3 production	++	+++
P solubilization	+	−
Siderophore production	+	+++
IAA (mg L^{-1})	24.1	156.3
ACC-deaminase activity (μmoles α-ketobutyrate g^{-1} h^{-1})	0.125 ± 0.021	0.654 ± 0.027
HCN	+++	+
Cellulase	−	++
Pectinase	−	−
Protease	+	−
Lipase	−	−

MIC: minimal inhibitory concentration; HCN: hydrogen cyanide; NH_3: ammonia; IAA: indole acetic acid.

2.5.2. Enumeration of culturable bacteria

The total heterotrophic bacteria and phosphate solubilizing bacteria (PSB) were counted in trypticase soy agar (TSA, Fluka) and National Botanical Research Institute's phosphate growth medium (NBRIP), respectively. Briefly, 10 g of fresh soil/substrate was suspended in 90 mL of sterile saline solution (9 g l^{-1}, NaCl) and shaken for 30 min at room temperature. Serial dilutions were prepared and 0.1 mL of each dilution was plated in triplicate onto the selected media (TSA and NBRIP) supplemented with cycloheximide at 50 mg L^{-1} to avoid fungi contamination (Alef, 1995a,b). Heterotrophic bacteria and PSB were counted after incubation at 30 °C for 3 and 7 days, respectively, and expressed as colony-forming units (CFU) per gram of fresh substrate.

2.5.3. Enzymatic activity

The dehydrogenase (EC 1.1.1.) activity was determined in 1 M Tris-HCl buffer (pH 7.5) by the method of Alef (1995a,b), using 2,3,5-triphenyltetrazoliumchloride (TTC) as an electron acceptor. The triphenyl formazan (TPF) produced was measured spectrophotometrically at 490 nm. Urease (EC 3.5.1.5) activity, which catalyzes the hydrolysis of urea to CO_2 and NH_3, was determined according to Kandeler and Gerber (1988). Briefly, 2.5 mL of urea (79.9 mM) was added to 5 g of fresh soil. After 2 h of incubation at 37 °C, the reaction was stopped by adding 50 mL of 1 N KCl (in 0.01 N HCl). Then the tubes were centrifuged for 5 min at 3000 g. The concentration of NH_3–N formed was determined by the following colorimetric procedure: 1 mL of the supernatant was diluted (1:10) with deionized water, and then 5 mL of sodium salicylate and 2 mL of sodium dichlorisocyanurate were added. The optical density was determined at 690 nm after incubation for 30 min at room temperature. The blank solutions were prepared as above using 2.5 mL of deionized water instead of urea. For the determination of phosphomonoesterase (EC 3.1.3.2) activity, 1 g of fresh soil was incubated for 1 h at 37 °C in the presence of 1 mL p-nitrophenyl phosphate (15 mM) and 4 mL of Universal Modified buffer (pH 4.5). The reaction was stopped by adding 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH. Then tubes were centrifuged for 3 min at 3000 g. For each soil sample, a blank was prepared under the same conditions by adding $CaCl_2$ and NaOH solutions to the reaction mixture before incubation. The amount of p-nitrophenol (PNP) produced was measured at 400 nm (Tabatabai and Bremner, 1969).

2.6. Plant analysis

2.6.1. Biometric parameters

After 90 days, plants were harvested and washed thoroughly with tap and deionized water. Shoot height was registered. Dry biomass was determined after shoots and roots were oven dried at 70 °C for 72 h. From each pot, 1 g of fresh shoots was stored at −80 °C for enzymatic assays.

2.6.2. Antioxidant enzymatic activities

For the determination of enzymatic activities, about 500 mg of frozen shoots were ground in liquid nitrogen and homogenized in an extraction buffer (1:6 w/v) containing 50 mM potassium phosphate buffer (pH 7.5), 1 mM phenylmethylsulphonyl fluoride (PMSF), 1 mM polyethylene glycol (PEG), 5% (w/v) polyvinylpolypyrrolidone (PVP) and 0.1% (v/v) Triton X-100 (Chakraborty et al., 2015). The homogenates were then centrifuged at 10000 g and at 4 °C for 30 min, and the supernatant recovered and stored at −80 °C.

Protein content in shoot extracts was determined according to Bradford (1976) using bovine serum albumin (BSA) as standard.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the rate of decomposition of H_2O_2 at 240 nm for 3 min as described by Aebi (1984). The reaction mixture consisted of 200 μL of extract and 2 mL of 10 mM H_2O_2 prepared in potassium phosphate buffer (100 mM, pH 7). The reaction was initiated by the addition of the extract. CAT activity was expressed in mmol H_2O_2 min^{-1} mg $protein^{-1}$ using the molar absorption coefficient of 39.6 mM^{-1} cm^{-1}. The polyphenoloxidase (PPO) (EC 1.14.18.1) activity was determined by the method described by Hori et al. (1997). The reaction mixture contained 500 μL of 1.6% (v/v) catechol prepared in potassium phosphate buffer (100 mM, pH 6) was incubated for 3 min at room temperature after the addition of 100 μL of extract. The optical density was measured at 410 nm. PPO activity was expressed as the amount of enzyme used to increase one unit of absorbance (475 nm) min^{-1} mg^{-1} protein.

The peroxidase (POD; EC 1.11.1.7) activity was measured according to Hori et al. (1997) using guaiacol as a hydrogen donor. The reaction mixture consisted of 100 μL of the enzymatic extract, 300 μL of 20 mM guaiacol and 2 mL of potassium phosphate buffer (100 mM, pH 6). The reaction was triggered by the addition of 200 μl of 0.3% (v/v) hydrogen peroxide. The formation of tetraguaiacol was determined spectrophotometrically at 470 nm. POD activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} and expressed as mmol tetraguaiacol min^{-1} mg^{-1} protein.

The glutathione reductase (GR; EC 1.6.4.2) activity was measured at 25 °C according to the method described by Esterbauer and Grill (1978). The reaction mixture consisted of 200 mM Tris-HCl (pH 7.8), 3 mM EDTA, 0.2 mM NADPH, and 0.5 mM GSSG. The reaction was initiated by the addition of 50 μL of extract.

The reduction of GSSG was determined indirectly by measuring the consumption of NADPH at 340 nm. Results were expressed as units of GR min^{-1} mg^{-1} protein, using the molar absorption coefficient of 6.22 mM^{-1} cm^{-1} of NADPH.

All enzymatic assays were repeated at least three times per biological replicate.

2.7. Data analysis

All the results were analyzed by one-way ANOVA and Student-Newman-Keuls as post hoc test using the SPSS statistical program (IBM, Armonk, NY, USA, version 25.0). Significance was set at $P < 0.05$. Pearson's correlations ($P < 0.01$ and $P < 0.05$) between soil physicochemical, microbiological, and biochemical parameters were also determined.

3. Results

3.1. Synergistic effect of organo-mineral amendments and PGPR inoculation on mine tailings' properties after plant growth

3.1.1. Physicochemical properties

Physicochemical properties of untreated mine tailings (M), agricultural soil (A) and organo-mineral amended tailings (T1 and T2) after 30 days of amendments' application and after 90 days of pot experiments are presented in Table 2. In general, the addition of organo-mineral amendments significantly improved the physicochemical properties of mine tailings, in inoculated and non-inoculated treatments (S and C). Indeed, a very pronounced increase in pH and a decrease in EC (2 times) was observed in treated tailings (T1 and T2) if compared to untreated ones (T). The TOC levels also increased substantially, after supplementing tailings with rock phosphate or lime in combination with compost and the agricultural soil. Nonetheless values remained low compared to the average levels detected in the agricultural soil (A).

After 90 days (t_f), non-planted (S) amended tailings (T1 and T2) showed a pH higher than that recorded at 30 days (t_0) after mixing the amendments, indicating the need for these compounds to be matured in substrates.

Plant growth led to a significant increase in TOC (from 1.018 to 1.132%, and from 0.98 to 1.977%, in T1 and T2, respectively) content in amended tailings. Moreover, it also induced a reduction of EC by 2 times (from 2.890 to 1.652 mS cm^{-1}, and from 2.190 to 1.428 mS cm^{-1}, in T1 and T2, respectively) when compared to non-planted amended tailings. However, plant colonization did not induce any significant effect on soil pH, whereas a decrease of available P level was observed in planted treated tailings (from 0.31 to 0.16 mg g^{-1}, and from 0.25 to 0.20 mg g^{-1}, for T1 and T2, respectively).

Bacteria inoculation generally significantly enhanced ($P < 0.05$) the amount of available P and TOC. Nonetheless, in the case of the lime containing substrate (T2), plants alone had a much more positive influence on TOC content than when the substrate was inoculated with bacterial strains. The available P increased from 0.21 to 0.30 mg g^{-1}, and from 0.16 to 0.35 mg g^{-1}, in the agricultural soil (A) and in rock phosphate amended tailings (T1), respectively, when co-inoculated with both bacterial strains (B1 + B2), while in the lime containing substrate (T2) available P increased from 0.20 to 0.27 mg g^{-1} with single inoculation with the strain *A. kashmrensis* BKM20 (B1). Combined inoculation of both PGPRs seems to have a greater effect on P availability than single inoculation.

3.1.2. Biological and biochemical parameters

Culturable heterotrophic bacteria and PSB were not detected in untreated mine tailings (Fig. 1). After the treatments, mean values ranging from 3.77×10^6 to 1.14×10^7 CFU g^{-1} for heterotrophic bacteria, and from 1.24×10^4 to 3.41×10^4 CFU g^{-1} for PSB, were determined.

Overall, the application of organo-mineral amendments significantly increased ($P < 0.05$) numbers of heterotrophic and phosphate solubilizing bacterial populations when compared to counts detected in agricultural soil, which was particularly evident in non-planted and non-inoculated treated tailings (S). The cultivation of *L. perenne* reinforced the bacterial numbers of both populations if compared to non-planted treatments. In fact, in the control assay (C) bacterial counts of heterotrophic bacteria and PSB were 2–6 times and 1–2 times greater than those in non-planted ones, respectively. This increment was particularly evident in amended tailings (T1 and T2). However, the number of total heterotrophic bacteria declined with the addition of bioinoculants, especially in

Table 2
– Physicochemical properties of substrates 30 days after the addition of organo-mineral amendments (t_0) and at the end of pot experiments (t_f).

	Substrates	t_0	Bacterial treatments (t_f)				
			S	C	B1	B2	B1 + B2
pH	**A**	$7.36 \pm 0.40^{a,A}$	$7.83 \pm 0.06^{a,B}$	$8.14 \pm 0.07^{a,BC}$	$8.15 \pm 0.03^{a,BC}$	$8.31 \pm 0.02^{a,C}$	$8.30 \pm 0.03^{a,C}$
	M	$2.13 \pm 0.04^{b,A}$	$1.72 \pm 0.04^{b,B}$	$1.94 \pm 0.08^{b,C}$	$1.80 \pm 0.03^{b,B}$	$1.84 \pm 0.01^{b,B}$	$1.81 \pm 0.02^{b,B}$
	T1	$6.33 \pm 0.03^{c,A}$	$7.03 \pm 0.02^{c,B}$	$7.12 \pm 0.05^{c,B}$	$7.10 \pm 0.02^{c,B}$	$7.11 \pm 0.03^{c,B}$	$7.13 \pm 0.03^{c,B}$
	T2	$6.42 \pm 0.02^{c,B}$	$7.16 \pm 0.01^{c,B}$	$7.28 \pm 0.05^{c,AB}$	$7.19 \pm 0.04^{c,B}$	$7.14 \pm 0.08^{c,B}$	$7.19 \pm 0.09^{c,B}$
EC (mS cm^{-1})	**A**	$0.47 \pm 0.01^{a,A}$	$0.86 \pm 0.024^{a,B}$	$0.16 \pm 0.018^{a,C}$	$0.23 \pm 0.008^{a,C}$	$0.21 \pm 0.014^{a,C}$	$0.18 \pm 0.048^{a,C}$
	M	$6.00 \pm 0.20^{b,A}$	$4.91 \pm 0.336^{b,B}$	$4.09 \pm 0.033^{b,C}$	$5.33 \pm 0.069^{b,D}$	$4.55 \pm 0.024^{b,B}$	$4.67 \pm 0.129^{b,B}$
	T1	$2.88 \pm 0.02^{c,A}$	$2.89 \pm 0.050^{c,A}$	$1.65 \pm 0.132^{c,B}$	$1.79 \pm 0.237^{c,B}$	$1.78 \pm 0.140^{c,B}$	$1.52 \pm 0.046^{c,B}$
	T2	$3.01 \pm 0.02^{c,A}$	$2.19 \pm 0.015^{d,B}$	$1.43 \pm 0.026^{d,C}$	$1.50 \pm 0.205^{d,C}$	$1.56 \pm 0.064^{d,C}$	$1.63 \pm 0.153^{c,C}$
TOC (%)	**A**	$1.00 \pm 0.09^{a,A}$	$1.28 \pm 0.05^{a,B}$	$1.16 \pm 0.05^{a,B}$	$1.66 \pm 0.03^{a,C}$	$1.64 \pm 0.11^{a,C}$	$1.68 \pm 0.02^{a,C}$
	M	$0.18 \pm 0.06^{b,A}$	$0.18 \pm 0.05^{b,A}$	$0.13 \pm 0.02^{b,A}$	$0.17 \pm 0.00^{b,A}$	$0.19 \pm 0.00^{b,A}$	$0.18 \pm 0.01^{b,A}$
	T1	$1.10 \pm 0.10^{a,B}$	$1.02 \pm 0.02^{c,A}$	$1.13 \pm 0.03^{a,B}$	$1.56 \pm 0.15^{a,D}$	$1.38 \pm 0.08^{c,D}$	$1.40 \pm 0.03^{c,D}$
	T2	$1.09 \pm 0.10^{a,A}$	$0.98 \pm 0.04^{c,A}$	$1.98 \pm 0.00^{c,B}$	$1.29 \pm 0.04^{c,C}$	$1.27 \pm 0.01^{c,C}$	$1.106 \pm 0.05^{d,D}$
P Olsen (mg g^{-1})	**A**	$0.24 \pm 0.0100^{a,A}$	$0.20 \pm 0.0030^{a,A}$	$0.21 \pm 0.0020^{a,A}$	$0.28 \pm 0.0004^{a,A}$	$0.28 \pm 0.0015^{a,A}$	$0.30 \pm 0.0010^{a,B}$
	M	n.d	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	$0.32 \pm 0.0700^{b,A}$	$0.31 \pm 0.001^{b,B}$	$0.16 \pm 0.0004^{b,C}$	$0.27 \pm 0.0017^{c,D}$	$0.24 \pm 0.001^{c,E}$	$0.35 \pm 0.0021^{b,F}$
	T2	$0.31 \pm 0.0600^{b,B}$	$0.25 \pm 0.0008^{c,A}$	$0.20 \pm 0.0006^{c,C}$	$0.27 \pm 0.0006^{c,D}$	$0.22 \pm 0.0025^{c,E}$	$0.23 \pm 0.0031^{c,E}$

n.d: not detected; EC: Electrical conductivity; TOC: Total organic carbon; P Olsen: available phosphorus. Results are expressed as mean ± SD (n = 3). A - 100% agricultural soil; T1 - AM (mixture of agricultural soil + mine tailings (M) 1:1 w/w) + 10% compost + 6% rock phosphate; T2 – AM + 10% compost + 3% $CaCO_3$). C – control – non inoculated; B1 - inoculated with strain *TA. kashmirensis* BKM20; B2 - inoculated with strain *M. tamadayense* BKM04; B1+B2 - inoculated with a mixture of strains BKM20 and BKM04. Lower case letters indicate significant differences ($P < 0.05$) between substrate treatments (A, M, T1, T2). Upper case letters indicate significant differences ($P < 0.05$) between inoculation treatments (S, C, B1, B2, B1+B2) in the same substrate.

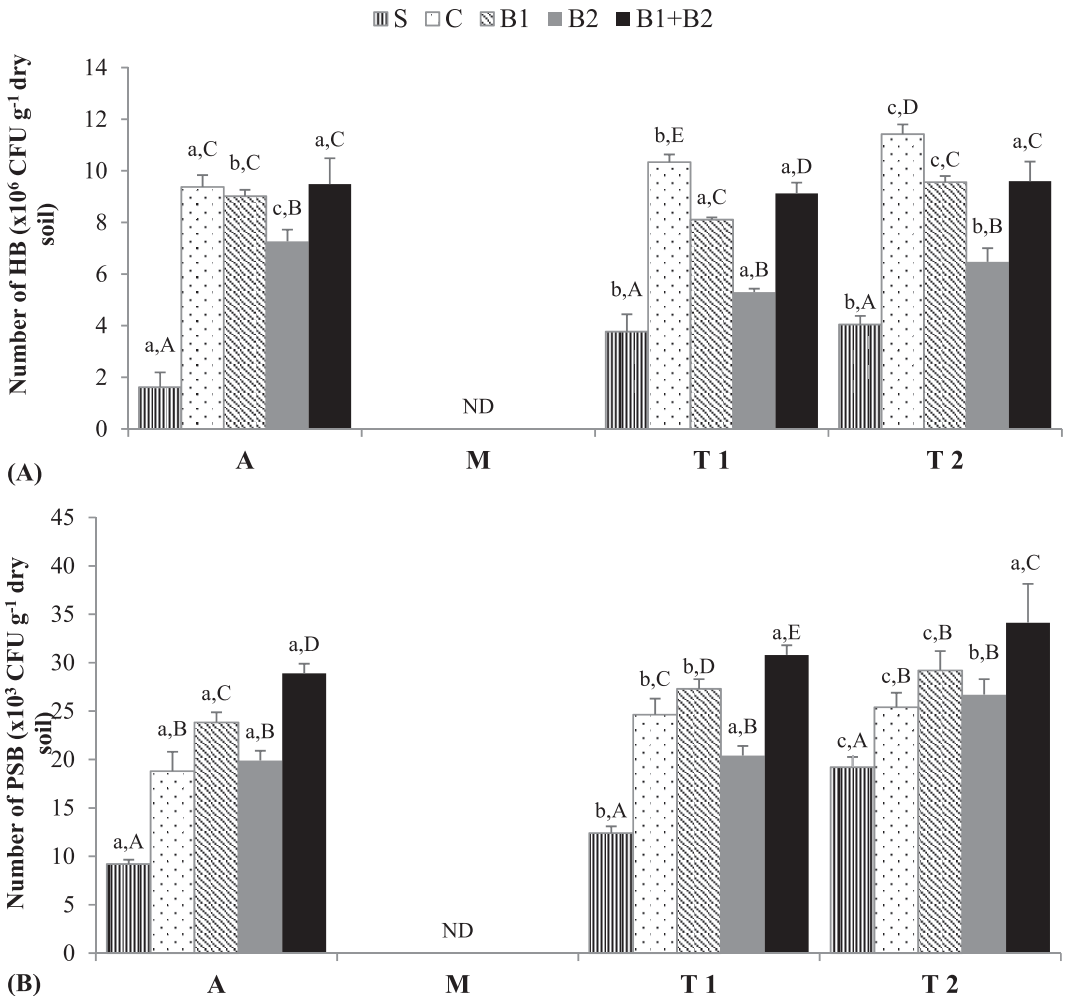

Fig. 1. Effect of organo-mineral amendments and bacterial inoculation on the number of culturable heterotrophic bacteria - HB (A) and phosphorus solubilizing bacteria – PSB (B) in the different substrates. Results are expressed as mean ± SD (n = 3). A - 100% agricultural soil; T1 - AM (mixture of agricultural soil + mine tailings (M) 1:1 w/w) + 10% compost + 6% rock phosphate; T2 – AM + 10% compost + 3% $CaCO_3$). S – substrate only; C – control – non inoculated; B1 - inoculated with strain *A. kashmirensis* BKM20; B2 - inoculated with strain *M. tamadayense* BKM04; B1+B2 - inoculated with a mixture of strains BKM20 and BKM04. ND – Not Detected. Lower case letters indicate significant differences ($P < 0.05$) between susbtrate treatments (A, M, T1, T2); upper case letters indicate significant differences ($P < 0.05$) between inoculation treatments (S, C, B1, B2, B1+B2) in the same susbtrate.

substrate T1 (AM + compost + rock phosphate). A higher decrease was observed when the bacterial strain *M. tamadayense* BKM04 (B2) was inoculated. Nevertheless, inoculation with bacterial strains generally increased PSB numbers, especially when co-inoculated (B1+B2).

The effect of organo-mineral amendments and bacterial inoculation on substrate enzymatic activity are shown in Fig. 2. Dehydrogenase and urease activities were in general very low in untreated mine tailings (M), unlike in the agricultural soil (A), where their activities (42.20 μg TPF g^{-1} dry soil h^{-1} and 19.61 μg NH_4–N g^{-1} dry soil h^{-1}) were high (Fig. 2A and B). The addition of organo-mineral amendments increased by an average of 39 and 5 times the activity of dehydrogenase and urease, respectively, in amended tailings, if compared to those untreated. The presence of plants (C) greatly enhanced dehydrogenase activity in agricultural soil and in amended tailings, particularly in the lime containing substrate (T2) (Fig. 2A). Similar results were observed for urease activity in treated tailings (Fig. 2B). Overall, PGPR inoculation decreased dehydrogenase activity in rock-phosphate (T1) and lime containing substrates (T2), while B2 and B1+B2 treatments increased its activity in agricultural soil. On the other hand, bio-inoculation stimulated urease activity in untreated mine tailings (M) and treated mine tailings (T1 and T2), exceeding, in the latter, the values found in the agricultural soil. Co-inoculation of both bacterial strains remarkably increased urease activity (69.42 μg NH_4–N g^{-1} dry soil h^{-1}) in the lime containing substrate (T2).

A different trend was observed for acid phosphomonoesterase activity (Fig. 2C), since its activity was significantly higher in untreated mine tailings (180.7–217.2 μg PNP g^{-1} dry soil h^{-1}) than either in agricultural soil or supplemented tailings. Overall, plant growth and bacterial inoculation significantly promoted acid phosphomonoesterase activity in substrate T1; however, the enzymatic activity was lower than that observed in untreated mine tailings (M).

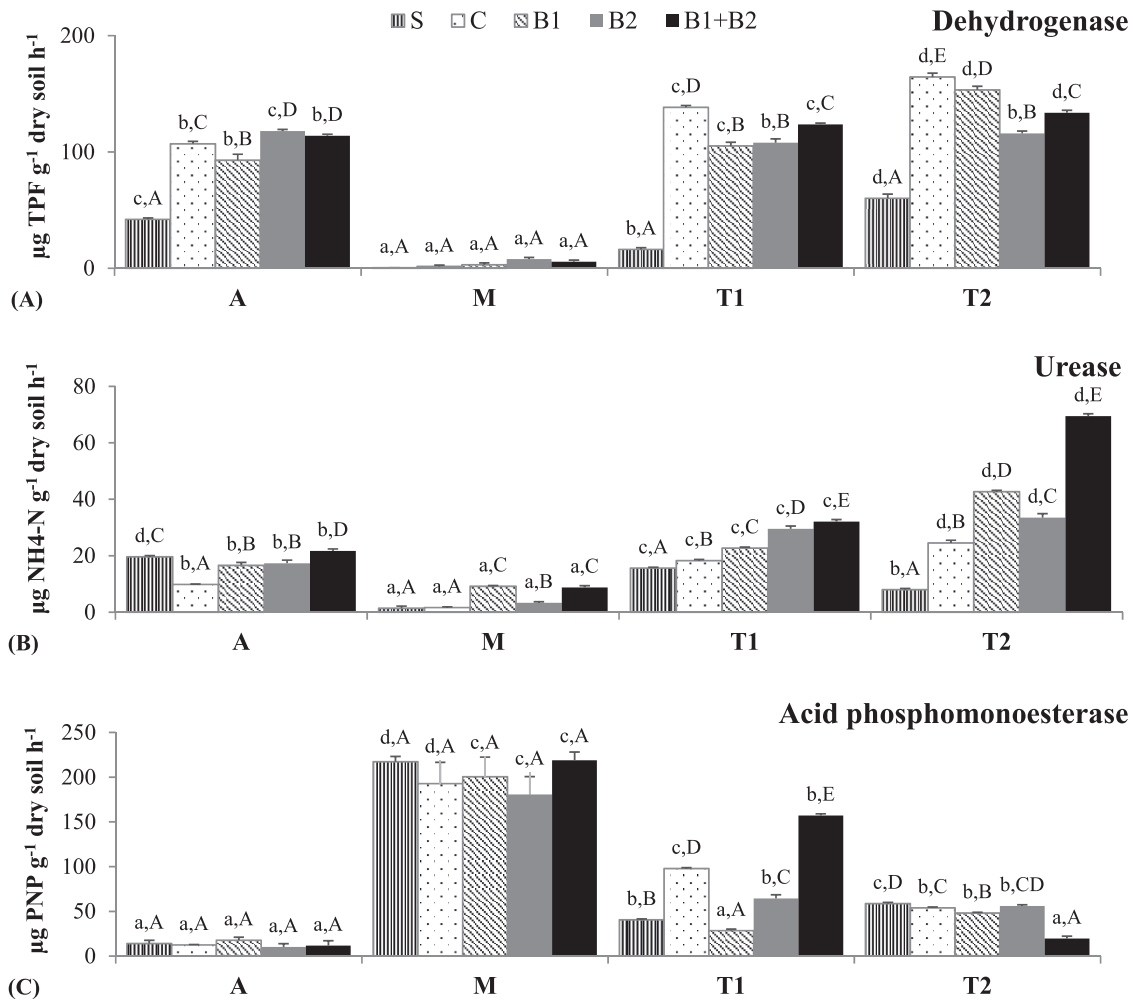

Fig. 2. Effect of organo-mineral amendments and bacterial inoculation on dehydrogenase (A), urease (B), and acid phosphomonoesterase (C) activities. Results are expressed as mean ± SD (n = 3). A - 100% agricultural soil; T1 - AM (mixture of agricultural soil + mine tailings (M) 1:1 w/w) + 10% compost + 6% rock phosphate; T2 – AM + 10% compost + 3% $CaCO_3$). S – substrate only; C – control – non inoculated; B1 - inoculated with strain *A. kashmirensis* BKM20; B2 - inoculated with strain *M. tamadayense* BKM04; B1+B2 - inoculated with a mixture of strains BKM20 and BKM04. Lower case letters indicate significant differences ($P < 0.05$) between susbtrate treatments (A, M, T1, T2); upper case letters indicate significant differences ($P < 0.05$) between inoculation treatments (S, C, B1, B2, B1+B2) in the same susbtrate.

3.2. Synergistic effect of organo-mineral amendments and PGPR inoculation on plant growth and antioxidant response

3.2.1. Plant growth parameters

During the greenhouse experiment period, *L. perenne* plants developed well and without visible symptoms of toxicity. However, in untreated mine tailings (M) a complete inhibition of plant growth was observed (Fig. 3). In general, the addition of organo-mineral amendments significantly increased ($P < 0.05$) shoot height and dry biomass of *L. perenne* plants when compared to agricultural soil (Fig. 3). In fact, shoot height and root dry biomass of *L. perenne* plants grown in amended tailings (T1 and T2) were 1.4 and 1.7 times higher, respectively, than those observed in control pots. Nonetheless, dry shoot biomass was not significantly ($P < 0.05$) affected by organo-mineral amendments.

Generally, the positive effect of amendments on plant growth was promoted by bacterial inoculation (Fig. 3), which significantly enhanced shoot height and biomass production of *L. perenne* plants grown in amended tailings (T1 and T2). Indeed, the positive effects of bacterial inoculation on shoot height were only observed in plants grown in the lime containing substrate (T2). On the other hand, the increases in below- and aboveground biomass were an average of 1.3 and 1.6 times, respectively that of treated mine tailings. The largest increases were observed for treatments

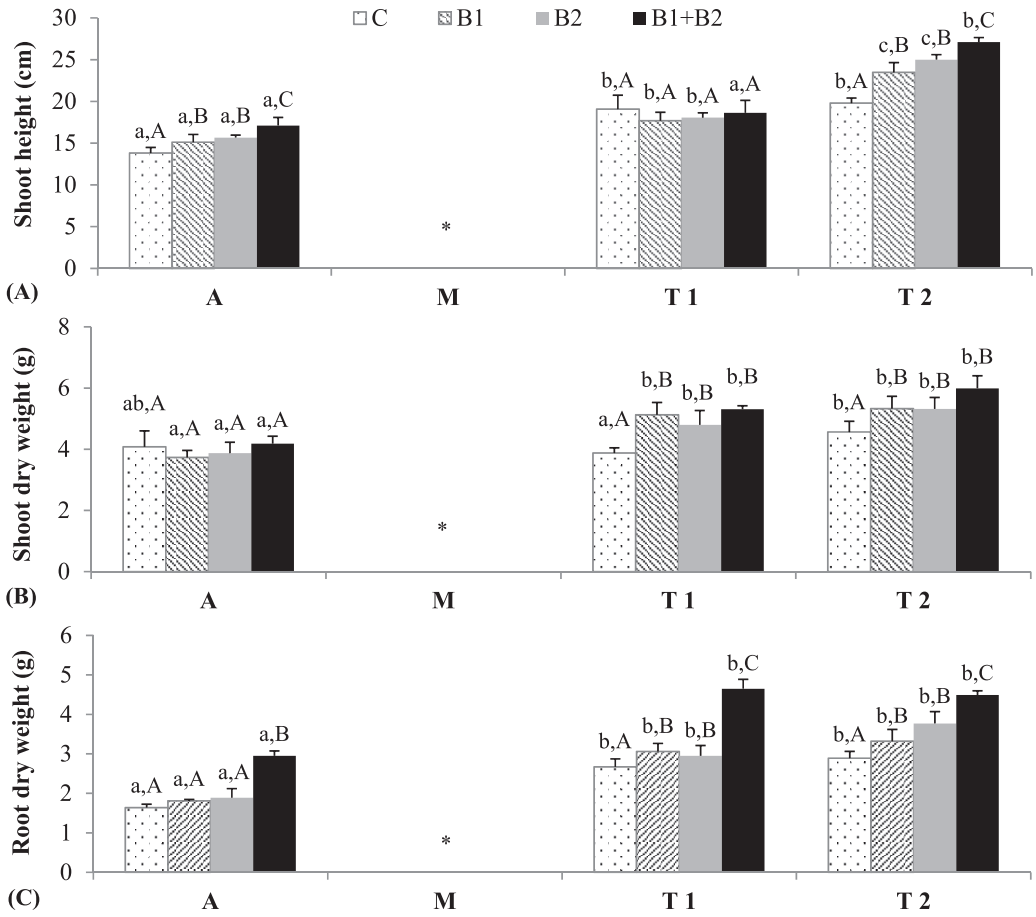

Fig. 3. - Effect of organo-mineral amendments and bacterial inoculation on shoot height (A) and shoot (B) and root (C) dry biomass of *L. perenne* plants. Results are expressed as mean ± SD (n = 3). A - 100% agricultural soil; T1 - AM (mixture of agricultural soil + mine tailings (M) 1:1 w/w) + 10% compost + 6% rock phosphate; T2 − AM + 10% compost + 3% $CaCO_3$). C − control − non inoculated; B1 - inoculated with strain *A. kashmirensis* BKM20; B2 - inoculated with strain *M. tamadayense* BKM04; B1+B2 - inoculated with a mixture of strains BKM20 and BKM04. * − Absence of plants. Lower case letters indicate significant differences (P < 0.05) between susbtrate treatments (A, M, T1, T2); upper case letters indicate significant differences (P < 0.05) between inoculation treatments (C, B1, B2, B1+B2) in the same susbtrate.

inoculated with a mixture of both bacterial strains.

3.2.2. Antioxidant enzymes

The activity of several antioxidant enzymes in shoots of *L. perenne* plants grown for 90 days in different substrates and suffering the influence of several inoculation treatments are shown in Fig. 4. Non-inoculated plants grown in agricultural soil (A) showed higher enzymatic activity (CAT, PPO, and POD) than those grown in treated tailings, indicating that the addition of organo-mineral amendments reduced the plant's antioxidant activity. Furthermore, CAT, PPO, and POD activities in shoots of plants grown in agricultural soils (A) were significantly reduced by the application of PGPR. Nonetheless, bioinoculation promoted the activity of the same antioxidant enzymes in plants grown in amended tailings (T1 and T2), particularly in plants inoculated with both rhizobacterial strains.

Contrary to what was observed for the other plant enzymes, no significant differences were observed for GR activity in plants grown in agricultural soil and in treated tailings. GR activity was also induced by the inoculation of PGPR, being the highest activity found in plants grown in substrates inoculated with a mixture of both strains.

4. Discussion

The present work showed that organo-mineral amendments and bioinoculation can promote the establishment of a plant cover in mine tailings and restore soil functions. The combined application of organo-mineral amendments and bacterial inoculants, especially when inoculated as mixture of both PGPR, proved to ameliorate physicochemical, biological, and biochemical properties of the mine tailings, rendering them able to support and further enhance plant growth and its antioxidative response.

4.1. Physicochemical properties of substrates

In the present study, results showed that 30 days after the addition of organo-mineral amendments the pH increased, reaching values close to neutrality (6.33−6.42) in amended tailings. Moreover, after 90 days (experimental time) the pH of non-planted and non-inoculated substrates further increased (7.03−7.16), suggesting that the effect of organo-mineral amendments can become

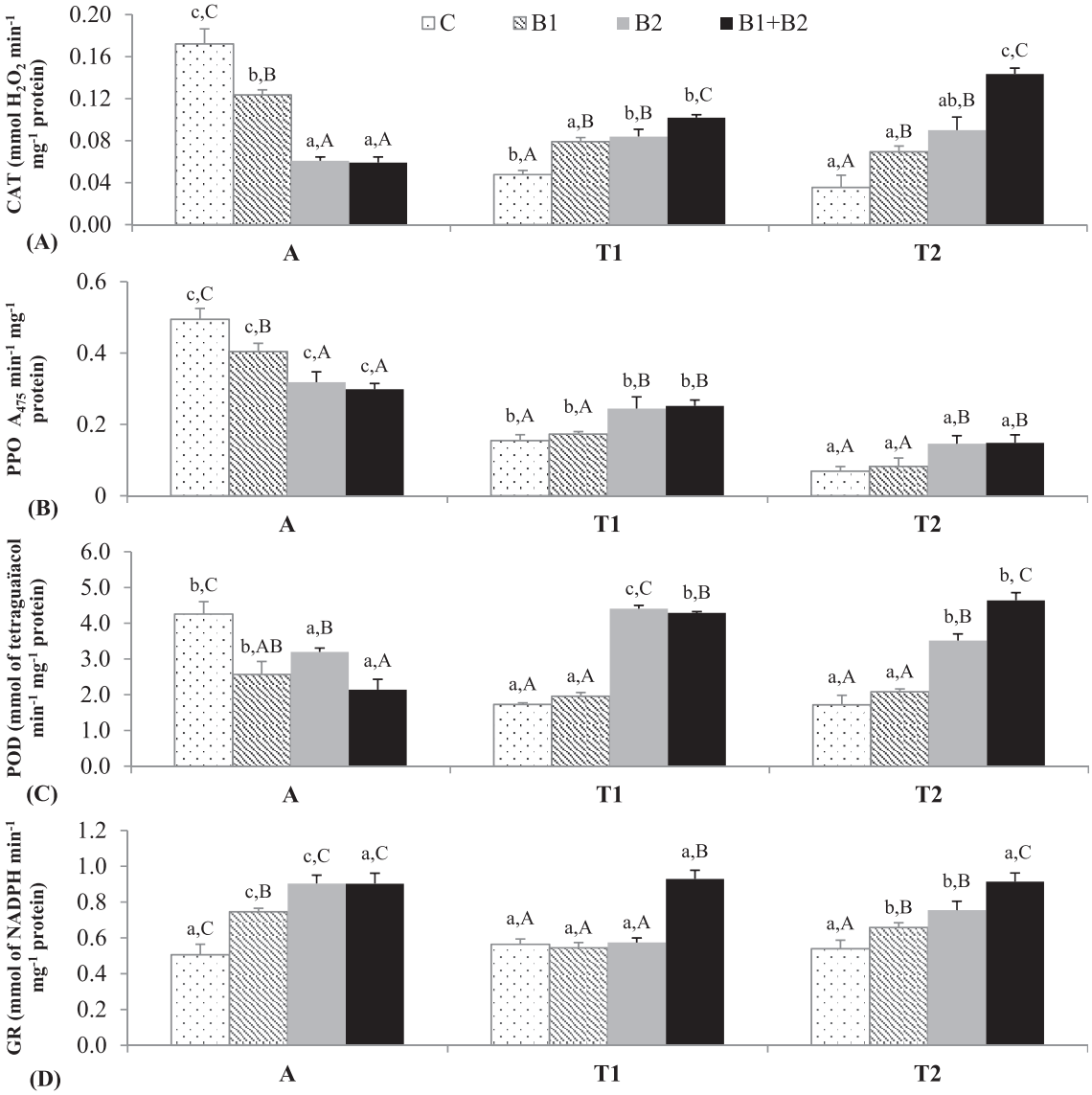

Fig. 4. Effect of organo-mineral amendments and bacterial inoculation on the activity of catalase – CAT (A), polyphenoloxidases - POP (B), peroxidases - POD (C) and glutathione reductase – GR (D) in the shoots of *L. perenne* plants grown in the different substrates. Results are expressed as mean ± SD (n = 3). A - 100% agricultural soil; T1 - AM (mixture of agricultural soil + mine tailings (M) 1:1 w/w) + 10% compost + 6% rock phosphate; T2 – AM + 10% compost + 3% $CaCO_3$). C – control – non inoculated; B1 - inoculated with strain *A. kashmirensis* BKM20; B2 - inoculated with strain *M. tamadayense* BKM04; B1+B2 - inoculated with a mixture of strains BKM20 and BKM04. Lower case letters indicate significant differences ($P < 0.05$) between susbtrate treatments (A, M, T1, T2); upper case letters indicate significant differences ($P < 0.05$) between inoculation treatments (C, B1, B2, B1+B2) in the same susbtrate.

more visible over time. In agreement with our results, previous studies showed that the addition of compost, lime and/or rock phosphate to highly acidic substrates, including mine tailings, reduced extreme acidity and maintained pH close to neutrality for long periods of time (Gil-Loaiza et al., 2016; Xiao et al., 2017; Holland et al., 2018). In this study, the supplementation of mine tailings with 10% of green waste derived-compost was enough to increase TOC levels to values similar to those found in agricultural soil. In fact, the addition of organic matter as polysaccharides, fulvic and humic acids, as well as of P in labile form, have been described as a booster of soil fertility in metal degraded areas by several authors (Clemente et al., 2012; Pardo et al., 2014; Gao et al., 2020). Furthermore, as a result of organo-mineral supplementation, bioavailability of metals tends to decrease as the soil pH becomes more alkaline, and its organic matter content increases (Spark et al., 1997; Bernal et al., 2007). Several authors reported that the application of green waste derived-compost and alkaline amendments such as phosphate minerals and lime reduced metal availability in mine tailings (van Herwijnen et al., 2007; Beesley et al., 2010; Bade et al., 2012).

L. perenne plants were completely absent in untreated mining tailings. However, the improvement of the tailings' physicochemical properties, resulting from the application of amendments, allowed the growth of healthy plants, which further increased TOC

levels and decreased EC in the soil. Epelde et al. (2009a) also related improvements in soil properties with the establishment of vegetation cover in mine soils. PGPR inoculation contributed to enhance TOC and available P levels in agricultural soil and in tailings supplemented with compost and rock phosphate (T1). These increases might be related to the better development of root systems of inoculated plants, which can contribute to soil organic matter stabilization and to C storage (Dignac et al., 2017), and/or to the direct effect of bacterial inoculants on root exudate production (Kudoyarova et al., 2014) and on solubilization of P (Pereira and Castro, 2014). The latter may explain the higher levels of available P in the inoculated rock phosphate containing substrate, as bacterial inoculation stimulated PSB proliferation, which may have led to a higher solubilization of P present in the added rock phosphate.

4.2. Microbiological and biochemical evaluation

Soil physicochemical parameters have been widely used to evaluate the success of phytoremediation strategies (Gil-Loaiza et al., 2016). However, biological indicators of soil health (Norris and Congreves, 2018; Williams et al., 2020), namely those related to soil microbial activity and diversity, also constitute an important tool for evaluating the effectiveness of remediation techniques in metal-degraded sites (Epelde et al., 2009a,b). In this study, bacterial enumeration and enzymatic activities showed a clear deterioration of microbial communities in the mine tailings, since no viable bacterial cells were detected, and the activity of dehydrogenase was extremely low. Nevertheless, the addition of organo-mineral amendments and the concomitant improvement of physicochemical properties promoted the numbers of bacterial populations, which is consistent with the positive and significant correlations between heterotrophic and PSB counts and pH ($r = 0.81^{**}$ and $r = 0.84^{**}$), TOC ($r = 0.85^{**}$ and $r = 0.85^{**}$) and available P ($r = 0.75^{**}$ and $r = 0.85^{**}$), as well as with the negative correlation ($r = -0.80^{**}$ and $r = -0.81^{**}$) with EC (Table A - see Appendix). The increase of organic matter is often described as beneficial for the development of heterotrophic rhizospheric microorganisms in metal polluted soils (Benidire et al., 2020), since it will improve soil nutrient status, aeration and water retention capacity (Lal, 2006). This is fundamental to diminish autotrophic populations of iron- and sulfur-oxidizing bacteria and, consequently, to the reduce mine tailings' acidification (Mendez and Maier, 2008). As expected, heterotrophic bacteria and PSB counts are also strongly correlated ($r = 0.95^{**}$ and $r = 0.92^{**}$) to dehydrogenase activity (Table A - see Appendix), since the latter constitutes an indicator of total microbial activity (Wolińska and Stępniewska, 2012).

Plant growth seems to have improved soil functions by increasing microbial abundance and activity. Similarly, Mendez et al. (2007) showed that quailbush plants increased heterotrophic bacterial counts in compost-amended mine tailings, which in turn promoted plant growth. Clemente et al. (2012) also showed that, although the application of compost considerably favored the development of soil microbial biomass, its effect was further enhanced by the presence of *Atriplex halimus*. Furthermore, several authors reported that soil microbial communities can be heavily influenced by root exudates and by readily biodegradable compounds that can be used as source of nutrients by rhizospheric microorganisms (Berg and Smalla, 2009; Chaparro et al., 2014). In this investigation, bioinoculation influenced differently the numbers of heterotrophic bacteria and PSB in substrates. A decrease in the number of heterotrophic bacteria was observed after PGPR inoculation, which can be attributed to the effect of several compounds with antimicrobial activity, such as antibiotics, hydrogen cyanide or siderophores, often produced by PGPR (Saharan and Nehra, 2011). This antagonistic activity may give them a competitive advantage, allowing their proliferation and at same time leading to the exclusion of less competitive native microorganisms. Similarly, the activity of dehydrogenase was also negatively affected by bioinoculants, corroborating their antagonistic effect on indigenous microbial communities. On the other hand, PSB counts in inoculated treatments were significantly higher than those found in non-inoculated ones, especially when co-inoculated with both strains.

The activity of acid phosphomonoesterase was higher in untreated mining tailings than in the other treatments, contrary to what was observed for dehydrogenase and urease, suggesting that the latter are more sensitive to the inhospitable environmental conditions of mine wastes. The low enzymatic activities in mine tailings can be explained by the high amounts of metals and the very acidic pH. Martínez-Toledo et al. (2017) also showed strong negative correlations between soil enzyme activities and metals in soils with long-term contamination from mine tailings. Nonetheless, the application of organo-mineral amendments enhanced the activities of dehydrogenase and urease. Indeed, the addition of compost not only supplies nutrients for plants and living microorganisms, but also serves as a source of microorganisms that can play an important role in biogeochemical cycles (Walker et al., 2004; Mendez and Maier, 2008).

A decrease in acid phosphomonoesterase activity was already reported in metal polluted soils after application of organo-mineral amendments (Pardo et al., 2014). This inhibition seems to be either related to an increase of soil pH (Alvarenga et al., 2008, 2009) or to its available P levels (Epelde et al., 2009b) in soils. Pérez-de-Mora et al. (2006) reported a negative correlation between available P and acid phosphomonoesterase activity, confirming the inhibition of its synthesis by the high levels of available P in soils. Similarly, in this work, acid phosphomonoesterase activity decreased significantly in tailings supplemented with compost + rock phosphate (T1) or compost + lime (T2), indicating that the rise in pH and in available P was the main factor contributing to the decline of acid phosphomonoesterase activity in treated tailings, which was consistent with the negative correlations obtained between this enzyme and available P levels ($r = -0.81^{**}$) and pH ($r = -0.93^{**}$) (Table A - see Appendix).

4.3. L. perenne growth

The untreated mining tailings could not support *L. perenne* growth; however, the addition of organo-mineral amendments overcame this problem, presumably due to the improvement of the substrate's pH and fertility, and to the reduction of metal phytotoxicity. The biometric parameters (shoot height and dry biomass) of *L. perenne* plants grown in amended tailings were comparable to, or even higher than, those observed in plants grown in the

agricultural non-contaminated soil. These results might be related to the immobilization of metals by organic matter present in compost, and to the higher supply of nutrients. Similar results were obtained by van Herwijnen et al. (2007), which reported that after the addition of compost mixed with inorganic amendments (clinoptilolite or bentonite) to a heavily metal-contaminated soil, there was a significant increase in biomass production of *L. perenne* plants. Likewise, Karamia et al. (2011) and Smolinska (2015) reported that the addition of green waste derived-compost to metal polluted soils had positive effects on growth of *Lepidium sativum* and *L. perenne*.

L. perenne growth was heightened by the combined effect of organo-mineral amendments and PGPR inoculation. Several studies showed the beneficial effects of PGPR augmentation on plant development in metal-contaminated soils (Moreira et al., 2016a; b, 2019), including on the phytostabilization of mine tailings (Grandlic et al., 2008; de-Bashan et al., 2010). However, the synergistic effects of organo-mineral amendments and PGPR inoculation on plant establishment and growth in mine tailings have not yet been fully explored. The results obtained in this study prove the beneficial effects of such association. The plants inoculated with a mixture of both bacterial strains (B1 + B2) had, undoubtedly, the best performance, showing larger increases in shoot height and in root biomass for most substrates. These results are probably related to the PGP traits of inoculated metal-tolerant bacterial strains (Benidire et al., 2016). It is important to highlight that bacterial strains showed complementary PGP traits, the strain B1 proved to be more resistant to metals and acted as a P solubilizer, while strain B2 showed higher production of IAA and siderophores and higher ACC-deaminase activity. The combined effect of these traits may have contributed to improve their performance as PGP agents. Shilev et al. (2019) also showed that PGPR inoculated as consortia induced a higher increase in shoot biomass than the same bacterial strains single-inoculated.

4.4. *L. perenne* antioxidative response

The antioxidative defense system in plants plays an important role in reducing the harmful effects of ROS formed in response to environmental stresses (Wang et al., 2008), including those mentioned in the present investigation. In general, the activity of antioxidant enzymes in *L. perenne* plants grown in amended tailings (T1 and T2) were lower if compared to plants grown in non-inoculated agricultural soil, which suggest that the application of organo-mineral amendments attenuated the oxidative stress in those plants. These results are probably related to the effect of amendments on pH neutralization, nutrient enrichment and decrease of metal bioavailability, contributing at same time to improve plant growth. Our results are consistent with previous reports, Moradi et al. (2019) also demonstrated a reduction in the activities of antioxidant enzymes of plants grown in Cd-contaminated soils amended with biochar. Nevertheless, other studies have shown that the application of amendments stimulated the activity of several antioxidant enzymes, allowing plant protection against metallic stress by considerably reducing ROS levels (Singh and Prasad, 2014; Arshad et al., 2016).

PGPR inoculation significantly increased growth of plants grown in amended tailings. This rise may be accompanied by a higher accumulation of metals in plant tissues, as often reported by several authors (Saleem et al., 2018; Ren et al., 2019). However, there were no negative effects on plants, since no symptoms of phytotoxicity were observed. These findings showed that the antioxidant system was effective, as corroborated by the general increase in activity of all antioxidant enzymes (CAT, PPO, POD, and GR), preventing oxidative damages in those plants. Indeed, several studies have also found increased activity of antioxidant enzymes in plants inoculated with PGPR and grown in metal-contaminated soils (Islam et al., 2014; Sarathambal et al., 2017).

5. Conclusions

This study provides new insights into the efficient management of phytoremediation of mine tailings. The joint application of organo-mineral amendments and bioinoculants significantly improved physicochemical properties of tailings, while new microorganisms were added through the addition of compost and agricultural soil. These changes favored *L. perenne* growth and development, which further stimulated microbial abundance and activity as proven by the increased populations of heterotrophic and PSB and dehydrogenase and urease activities in amended mine tailings. The more pronounced effects were observed in the lime containing substrate (T2) inoculated with the bacterial consortium, constituting a promising combination to assist stabilization of tailings in the Kettara mine. Further study on the effect of amendments and PGPR combination on metal mobility in mine tailings, and on their uptake in plants, will be examined in our next paper (Benidire et al., manuscript in preparation). Nevertheless, additional *in situ* studies would be necessary to confirm the long-term effectiveness of co-application of organo-mineral amendments and bioinoculants in the settlement of stable vegetation cover in mining areas in arid to semiarid regions.

Credit author statement

Benidire L: Methodology, Software, Writing- Original draft preparation, Data curation. Madline A.: Methodology, greenhouse experiment. Pereira, S.I.A: Writing- Reviewing and Editing. Castro, P.M.L: Reviewing and Editing. Boularbah A. : Conceptualization, Writing- Reviewing and Editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was financially supported by the Convention de coopération CNRST-Morocco/FCT-Portugal, Centre National de Recherche Scientifique et Techniques [grant no. PPR 22/2015] and by the project PhytoSudoe (SOE1/P5/E0189) - Demostração de melhorias na biodiversidade do solo, funcionalidade e serviços ambientais de locais contaminados e/ou degradados sob intervenção de fitotecnologias dentro da região Interreg Sudoe, funded by FEDER-Fundo Europeu de Desenvolvimento Regional under Programa INTERREG. The authors would like to thank the scientific collaboration of Fundação Ciência e Tecnologia (FCT) project UIDB/50016/2020. Thanks to Dr R.C. Pullar for editing the English of this paper.

Appendix

Table A
Pearson correlation coefficients between soil physicochemical and microbiological and biochemical parameters.

	DHA	URE	AP	CFU_{HB}	CFU_{PSB}	pH	EC	TOC	P Olsen
DHA	1								
URE	0.68**	1							
AP	−0.65**	−0.48*	1						
CFU_{HB}	0.95**	0.60**	−0.69**	1					
CFU_{PSB}	0.92**	0.76**	−0.72**	0.93**	1				
pH	0.78**	0.51*	−0.93**	0.81**	0.84**	1			
EC	−0.78**	−0.44*	0.89**	−0.81**	−0.80**	−0.95**	1		
TOC	0.84**	0.49*	−0.84**	0.85**	0.85**	0.93**	−0.91**	1	
P Olsen	0.70**	0.53*	−0.81**	0.75**	0.85**	0.93**	−0.84**	0.87**	1

DHA: Dehydrogenase; URE: Urease; AP: Acid phosphatase; CFU: number of culturable cells of heterotrophic bacteria (HB) and phosphate solubilizing bacteria (PSB); EC: Electrical conductivity; TOC: Total organic carbon; P Olsen: Available phosphorus.
Correlations are significant at: *$p < 0.05$; **$p < 0.01$.

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