Biostabilization of Gold Mine Tailings: Co-Inoculation of Cyanobacteria Under Sterile and Non-Sterile Conditions

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Abstract

Cyanobacterial crust formation has attracted attention for stabilizing erosion-susceptible soils in desert regions. However, limited information exists on its application in waste impoundments such as mine tailings. Identifying suitable inoculants with the ability to develop biocrusts in the more toxic conditions of mine tailings represents a challenge for exploiting this biotechnology for such applications. In this study, the performance of two nitrogen-fixing cyanobacteria (*Anabaena* sp. and *Nostoc muscorum*), individually and as a consortium, in creating biocrusts over gold mine tailings were investigated under sterile and non-sterile conditions. The results showed that *Anabaena* sp. and the co-inoculation of the species promoted higher synthesis of chlorophyll-a and total EPS compared to *N. muscorum*. The inoculated strains also exhibited different responses in the amount of the EPS fractions. The less soluble and more condensed tightly bound EPS represented a higher fraction of total EPS with co-inoculation and *N. muscorum*. With respect to wind erosion resistance and compressive strength of the biocrusts generated, co-inoculation showed better performance, followed by *N. muscorum*, while *Anabaena* sp. appeared to be less effective. The presence of indigenous microbial community within the tailings influenced the biostabilization performance of *Anabaena* sp., while the influence was insignificant under co-inoculation and *N. muscorum*. Overall, inoculating the cyanobacteria in a mixture with complementary traits (higher chlorophyll-a synthesis and total EPS secretion of *Anabaena* sp. vs. higher TB-EPS fraction and filamentous growth of *N. muscorum*) presented an effective strategy in the development of a resistant biocrust against wind erosion. With this inoculation strategy, the beneficial effects of the individual strains on biocrust formation could be combined, thus a comparatively stronger structure could be formed. Besides chlorophyll-a content, factors such as cyanobacteria morphology and EPS fractions would contribute to the biostabilization process. The results also suggested that sterilization of the tailings would influence the performance of cyanobacteria depending on the inoculant. Thus, the response of inoculants to other microbial communities should be considered prior to field-scale application.

Introduction

Mining is the largest waste-producing industry worldwide with the production of about 65 billion tons annually, of which 78% is waste rock (mine overburden) and 22% is fine-grained tailings (Jones and Boger 2012). Mine tailings are considered one of the leading anthropogenic sources of wind erosion (Blight 2008, Fernando and Claudio 2020, Punia 2021). Wind erosion and consequent dust generation from tailings facilities can lead to environmental, human health, and safety concerns at active mine sites and in surrounding communities (Duniway et al. 2019, Kossoff et al. 2014). Because of the adverse effects, appropriate treatment strategies for stabilizing mine tailings against erosion have received special attention in recent years. To date, a number of stabilization approaches have been tested in an effort to mitigate wind erosion, including the creation of a crust on the soil surface by adding chemical materials, the installation of wind barriers to minimize wind flow over the surface, and the establishment of a vegetative cover (Ding et al. 2021, Tordoff et al. 2000, Ye et al. 2002). The efficiency and success of these strategies have been limited due to their temporary effectiveness, instability, time-consuming application, high cost, and adverse environmental impacts onsite/off-site throughout their life cycle. The limitations associated with the currently available methods necessitate researchers to explore more sustainable management practices. Recently, innovations in the biotechnology sector have made the application of microorganisms for soil biostabilization possible (Zhou et al. 2020).

The creation/development of a biological crust over soil surface by inoculating cyanobacteria, an eco-friendly and energy-efficient technique, has been considered promising for stabilizing soil against wind-induced erosion (Rossi et al. 2017). Through the secretion of exopolysaccharides (EPS) and filamentous growth, cyanobacteria entangle loose particles and bind them together, favoring the formation of a resistant biocrust that protects the soil surface from erosion (Costa et al. 2018, Malam Issa et al. 2007). Nitrogen-fixing cyanobacteria species can grow using the atmosphere as the source of nitrogen/carbon and sunlight as their source of energy, providing these species with the ability to sustain growth in the extreme tailings environment with a limited amount of nutrients (García-Meza 2008, Nyenda et al. 2019). Despite these advantages, there is a limited number of studies that have explored the application of nitrogen-fixing cyanobacteria in artificial ecosystems such as tailings deposits for enhancing the biophysicochemical properties of these environments, as most studies have been conducted on inoculating cyanobacteria in natural soils in desert regions and moving dunes.

One of the challenges in exploiting this biological treatment approach for mine tailings is to identify appropriate inoculants capable of creating biocrusts in such harsh environments. Most studies have investigated cyanobacterial biocrust formation by inoculating individual species. However, co-inoculation of cyanobacteria could be a potential approach for improving their effectiveness at generating biocrusts. Synergistic interactions among cyanobacteria have been previously reported to enhance inoculation performance (Gheda and Ahmed 2015, Román et al. 2018). In a study by Xie et al. (2007) in sand dunes, the crusts formed by the co-inoculation of three cyanobacteria exhibited higher compressive strength than those formed by single species inoculation. Chamizo et al. (2018) inoculated two cyanobacterial species on different textured soils. The results showed higher amounts of photosynthetic biomass and nitrogen in the soil inoculated by *Scytonema javanicum*, while *Phormidium ambiguum* had a higher effect on the EPS quantity and soil physical structure. On the other hand, cyanobacterial species might have parasitic interactions that suppress their growth. Allelopathic competition between the two algal species has been investigated and the results showed that allelochemicals produced by *Chlorella vulgaris* had inhibitory effects on the growth of *Pseudokirchneriella subcapitata* (Fergola et al. 2007). In order to build an effective biostabilization process in mine tailings, it is beneficial to evaluate whether co-inoculation of cyanobacteria species would result in the formation of a more stabilized biocrust.

Nutrient deficiency, especially nitrogen deficiency, frequently constrains the growth of the microbial population in tailings environments (Sun et al. 2020). The inoculation of photosynthetic and nitrogen-fixing cyanobacteria could provide a significant input of nutrients and organic matter for a diverse microbial community inhabiting the tailings through biological CO_2 sequestration and N_2 fixation (Chamizo et al. 2019, Mager 2010). Increases in heterotrophic microbial biomass and enzymatic activities caused by photoautotrophic organisms have been reported in mine tailings, poorly structured agricultural soil, and high-elevation barren soils with low organic carbon and nitrogen contents (Maier et al. 2018, Rogers and Burns 1994, Schmidt et al. 2008). It has also been demonstrated that non-nitrogen-fixing cyanobacteria rely on the nitrogen fixation of diazotrophic species for their nitrogen needs in nitrogen-poor soils and there is a mutualistic relationship based on carbon for nitrogen exchange in these environments (Couradeau et al. 2019). The growth of the indigenous microbial communities would raise concerns regarding their potential interactions with the inoculated cyanobacteria. Previous studies reported that some heterotrophic bacteria could cause algal cell lysis by the action of glucosidases, chitinases, cellulases, and other enzymes, thereby utilizing intracellular algal compounds as a source of nutrient (Fuentes et al. 2016, Kim et al. 2007). The soil heterotrophic communities would also consume part of the existing nutrients during their activity. Thus, there could be competitive relationships between the inoculum and the indigenous community for the available nutrients, leading to a lower growth rate of the inoculated cells. On the other hand, some studies showed mutualism interactions between cyanobacteria and other microbial communities through the exchange of micro and macronutrients such as vitamin B12 and phosphorous, which facilitate their growth (Prasanna et al. 2012). Bacterial growth also results in the production of dissolved inorganic carbon that would be utilized by algae to sustain growth (Cho et al. 2015). Soil physicochemical properties such as nutrient resources and texture might affect the interactions among microorganisms, whether it is as a competition or facilitation (Bowker et al. 2014). In a study by Roncero-Ramos et al. (2019), the inoculated cyanobacteria showed positive interactions with the community inhabiting low abiotic stress zones. Conversely, there was a destructive symbiosis between the indigenous community and the inoculum in high abiotic stress soils, reducing the efficiency of the biocrust system. In most biocrust formation studies, cyanobacteria have been inoculated on non-sterile soil. In this scenario, the indigenous microbial community could grow simultaneously with the inoculant. In order to discern the effect of the inoculated cyanobacteria from the indigenous community existing in the environment or developing due to inoculation within the biocrust system, research needs to be conducted under both sterile and non-sterile conditions prior to field application.

To address some of the existing knowledge gaps, the objectives of the present study were to assess whether: (i) inoculation of nitrogen-fixing cyanobacteria (*Nostoc muscorum* and *Anabaena* sp.) on gold mine tailings improves the biophysicochemical properties of the tailings; (ii) co-inoculation of the cyanobacteria species leads to a better biostabilization performance; and, (iii) presence of the indigenous microbial population inhabiting the mine tailings can affect the performance of the inoculated cyanobacteria. The two nitrogen-fixing cyanobacterial species were selected for their high growth rate in culture medium, metal toxicity resistance, ability to fix nitrogen, and high amounts of EPS secretion. Chlorophyll-a, EPS fractions, degree of wind erosion, and compressive strength of the formed biocrusts under different treatment conditions were analyzed to assess the fertility properties and dust mitigation efficiency of the treated tailings.

Materials And Methods

Tailings materials, cyanobacterial species, and biomass preparation for inoculation

Tailings materials were collected from a gold mine site in Northern Ontario. The tailings textural analysis showed 45% sand, 42% silt, and 13% clay. The physicochemical characteristics of the gold mine tailings are presented in Table 1. The tailings were oven-dried, sieved, and homogenized for the biostabilization experiments.

Table 1 Physicochemical characteristics of the gold mine tailings (mean ± SD)

Parameter	Value
pH	7.6 ± 0.10
Sand (%)	45
Silt (%)	42
Clay (%)	13
TOC (mg g^{-1})	3.11 ± 0.21
TN (mg g^{-1})	0.09 ± 0.01
$CaCO_3$	18.91 ± 1.29
As (µg g^{-1})	726 ± 26.11
Cd (µg g^{-1})	70.90 ± 4.75
Cr (µg g^{-1})	81.05 ± 2.01
Co (µg g^{-1})	22.40 ± 0.61
Cu (µg g^{-1})	968.50 ± 21.18
Pb (µg g^{-1})	417 ± 18.75
Hg (µg g^{-1})	3.9 ± 0.25
Mo (µg g^{-1})	1.5 ± 0.13
Ni (µg g^{-1})	11.54 ± 0.95
Se (µg g^{-1})	3.32 ± 0.09
Zn (µg g^{-1})	13055.00 ± 741.59

Heterocystous cyanobacteria, *Anabaena* sp. UTEX 2576 and *Nostoc muscorum* UTEX 1037, were provided by the Culture Collection of Algae at the University of Texas at Austin (UTEX). These two species were selected in this study based on a number of factors. In preliminary experiments, growth and heavy metal tolerance of the two species were investigated by their cultivation in nutrient-poor liquid media enriched with heavy metals including lead, zinc, copper, and chromium (Rezasoltani et al., 2021). These experiments were conducted to simulate the conditions in the gold mine tailings that are nutrient-poor and metal-enriched (Table 1). Results showed that both cyanobacteria could sustain growth in the metal-enriched media. However, *Anabaena* sp. was more resistant to metal toxicity than *N. muscorum*, with better performance in biomass production and EPS secretion. On the other hand, previously reported studies showed good performance of *Nostoc* in biocrust development and soil biostabilization through its filamentous growth and EPS secretion (Román et al. 2018). Furthermore, the cyanobacteria were selected for their ability to fix atmospheric nitrogen. It was hypothesized that the co-inoculation of the two cyanobacteria could lead to a more stabilized biocrust over the surface.

Anabaena sp. and *N. muscorum* were cultivated diazotrophically, separately, in Erlenmeyer flasks containing nitrate-free BG11 medium. The cultures were grown in a temperature-controlled growth chamber at 26 ± 0.5 °C under light illumination of 75 μmol photons m^{-2} s^{-1}. Air pumps equipped with 0.22 μm pre-filtered membranes provided aeration and mixing from the bottom at 0.5 vvm.

For the tailings biostabilization experiments, the cyanobacterial biomass was harvested at the end of the exponential growth phase by centrifugation at 3800 rpm for 4 minutes, then the pellet was resuspended in sterile distilled water to a final biomass concentration of 1 g L^{-1}.

Experimental design of tailings biostabilization

Tailings biostabilization experiments were designed to evaluate the performance of the two nitrogen-fixing cyanobacteria (*Anabaena* sp. vs. *N. muscorum*) and different inoculation strategies (single inoculation vs. co-inoculation of the cyanobacteria) in enhancing the biophysicochemical properties of the tailings. Experiments were also carried out under sterile and non-sterile conditions with the objective of understanding the potential role the indigenous microbial population inhabiting the gold mine tailings could have on the cyanobacterial biocrust system. The tailings were well mixed and divided into two portions, one of which was sterilized by autoclaving at 121 °C for 20 min shortly before the experiment. The experiments were conducted in sterile Petri dishes with a diameter of 90 mm and depth of 15 mm, filled with oven-dried sterile and non-sterile tailings. Nine experimental conditions were considered with three replicates for each condition: non-sterile tailings without treatment (BT), non-sterile tailings samples either treated with distilled water (DW), single inoculation of *N. muscorum* (NI), single inoculation of *Anabaena* sp. (AI), or co-inoculation of the two cyanobacteria species (AI+NI), and sterile tailings samples either treated with distilled water (S-DW), single inoculation of *N. muscorum* (S-NI), single inoculation of *Anabaena* sp. (S-AI), or co-inoculation of the two cyanobacteria species (S-AI+NI).

In the single inoculation experiments, the inoculation was conducted by dispersing either *N. muscorum* or *Anabaena* sp. over the entire surface of the tailings samples with a biomass concentration of 4 g dry weight m^{-2}, the equivalent of 4 L of cyanobacterial suspension per m^2 of tailings surface. In the co-inoculation experiments, the two cyanobacterial suspensions were well mixed in equal amounts prior to the inoculation, leading to 2 g dry weight m^{-2} of each on the tailings samples. In the non-inoculated S-DW and DW samples, distilled water without the inoculum was added at an equivalent volume of the cyanobacterial suspension.

Samples were maintained in a growth chamber at a temperature of 26 ± 0.5 °C with light intensity of 75 μmol photons m^{-2} s^{-1} for 6 weeks. All treatments (inoculated and non-inoculated) were moistened 2 L m^{-2} every 3 days to compensate for daily evaporation.

Analytical methods

Chlorophyll-a content was quantified as an indicator of the cyanobacterial biomass in the biocrusts. Chlorophyll-a was extracted using the ethanol extraction method according to Castle et al. (2011).The extraction procedure consisted of adding 5 mL of ethanol to 1 g of tailings, following which the mixture was vortexed, heated at 80°C for 5 min, and then cooled at 4°C for 30 minutes. The resulting suspension was centrifuged, and light absorption of the supernatant was spectrophotometrically quantified at 665 nm. Chlorophyll-a concentration was then calculated by applying the ethanol solvent equation reported by Ritchie (2006). The EPS matrix is fractionated into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) in terms of their cell binding ability. The two operationally-defined fractions were extracted from the tailings samples following the method described by Chen et al. (2014). The procedure included recovering LB-EPS by water extraction and then recovering TB-EPS by extracting with 0.1 M Na_2EDTA. The extracted LB-EPS and TB-EPS were quantified by the phenol-sulfuric acid assay, using glucose as a standard (DuBois et al. 1956).

The degree of wind erosion on the tailings samples were analyzed to characterize the stability of the treated tailings against dust emission. A laboratory wind tunnel, capable of generating wind velocities of up to 25 m s^{-1}, was used to simulate wind force over the surface of the tailings samples. The tailings samples were subjected to five wind velocities of 10, 12.5, 15, 20, and 25 m s^{-1} for a 10-minute exposure time. The wind-induced mass loss was calculated as the difference in the initial weight of the tailings samples and the weight after exposure to the wind flow. The degree of wind erosion (%) was then expressed as a ratio of the mass loss to the initial weight of the tailings sample. The compressive strength of the samples was measured using an AMS 59032 pocket penetrometer. The penetrometer measures strength in kg cm^{-2} with a range of 0 to 4.5 kg cm^{-2} by pushing the loading piston into the samples to a depth of 6 mm until the calibration mark is level with the surface of the sample.

Statistical analysis

To determine the statistical relevance of the data, three independent biological replicates were conducted for the tailings biostabilization experiments, and three replicates were employed for the analytical methods. Statistical data analysis was conducted with SPSS version 27.0. One-way analysis of variance (one-way ANOVA) with Tukey's test was carried out to determine the significant differences for all possible comparisons among treatments.

Results And Discussion

Chlorophyll-a and EPS contents of the developed biocrusts under sterile and non-sterile conditions

Cyanobacterial biocrust development was evaluated by analyzing chlorophyll-a and EPS contents of the tailings samples after a 6-week incubation period (Fig. 1).

Under the non-sterile condition, the inoculated tailings (NI, AI, and NI+AI samples) showed significantly higher ($P \leq 0.01$) chlorophyll-a content compared to the tailings without the inoculants (BT and DW samples). The highest value of chlorophyll-a was found under the co-inoculation of the cyanobacteria (23.99 μg g^{-1} tailings), followed by the inoculation of *Anabaena* sp. (20.70 μg g^{-1} tailings), while the inoculation of *N. muscorum* resulted in the least amount of chlorophyll-a (15.89 μg g^{-1} tailings). The higher chlorophyll-a in the tailings inoculated with *Anabaena* sp. compared to those inoculated with *N. muscorum* could be attributed to the higher resistance of *Anabaena* sp. to the metal toxicity of the tailings. As mentioned previously, *Anabaena* sp. showed a better performance than *N. muscorum* to resist the inhibitory effects of the metal ions (lead, zinc, copper, and chromium) in the synthetic metal-enriched media. The higher chlorophyll-a in the co-inoculation of the cyanobacteria compared to the single inoculation suggests that there could be a mutually beneficial symbiosis between the cyanobacterial species, *N. muscorum* and *Anabaena* sp. The synergistic effect of the cyanobacterial mixture could be mainly attributed to the exchange of metabolites such as hormones, vitamins, and enzymes. It has been reported that *Anabaena* and *Nostoc* strains are highly capable of producing biologically active growth-stimulating phytohormones such as indole-3-acetic acid, and indole-3-propionic acid, brassinosteroid, and gibberellic acid, which would be a possible mechanism whereby the cyanobacteria exert beneficial stimulatory effects on their growth under the co-inoculation treatment strategy (Rezasoltani et al. 2019, Shariatmadari et al. 2015). The symbiotic interactions among cyanobacterial species have been previously reported to promote biocrust development. Gheda and Ahmed (2015) found that the soil inoculated with a cyanobacterial mixture (*N. kihlmani* and *A. cylindrical*) contained higher organic carbon and nitrogen compared to soil inoculated with single strains of the cyanobacteria. The results of their study showed that exopolysaccharides, indole acetic acid, and cytokinins were higher in *N. kihlmani*, whereas *A. cylindrical* had higher nitrogenase activity and gibberellin content. A study by Román et al. (2018) evaluated the effect of inoculating three nitrogen-fixing species (*N. commune*, *Scytonema hyalinum*, *and Tolypothrix distorta*), individually and as a consortium, on soil properties. Their results showed higher cyanobacterial coverage and biomass, as well as carbon and nitrogen contents in the soil inoculated with the mixture of cyanobacteria. The results of their study demonstrated that the high synthesis of exopolysaccharides sheath *by Nostoc* with their water-absorption characteristics played a primary role in establishing favorable growth conditions.

Under the non-sterile condition, the co-inoculation of the cyanobacteria and the single-inoculation of *Anabaena* sp. resulted in significantly higher secretion of total EPS compared to the inoculation of *N. muscorum* (P = 0.05), with the total EPS contents of 1.65, 1.56 and 1.36 mg g^{-1} tailings in AI+NI, AI, and NI samples, respectively. The inoculated strains also showed different effects in the amount of the EPS fractions. Under the co-inoculation and single inoculation of *N. muscorum*, TB-EPSs represented a higher fraction of total EPS.

Chlorophyll-a and EPS contents were significantly influenced by the sterilization of the tailings for the single inoculation of *Anabaena* sp. with higher values obtained in the sterile condition ($P \leq 0.01$). However, the effect of sterilization was insignificant for the single inoculation of *N. muscorum*, co-inoculation, and water treatment ($P > 0.05$). The difference in the EPS content of the tailings inoculated with *Anabaena* sp. under sterile and non-sterile conditions could be attributed to the high synthesis of the less condensed and more soluble LB-EPS by *Anabaena* sp. It has been reported that the two EPS fractions have different roles. The LB-EPS fraction is predominantly composed of low molecular weight molecules, likely having relevance as a carbon source readily available for microbial activity, thus more easily degraded by the soil microbial community in nutrient cross-feeding processes (Chen et al. 2014). While, the TB-EPS fraction is mainly composed of high molecular weight molecules that would be more resistant to microbial degradation and mainly play an important role in the improvement of tailings structure and cohesion. Consequently, the high synthesis of LB-EPS by *Anabaena* sp. might promote the availability of a carbon source for the indigenous microbial community inhabiting the tailings and could be partly utilized under the non-sterile condition. The high molecular weight sugars contained in the TB-EPS fraction, whose generation was significantly higher under the co-inoculation and single inoculation of *N. muscorum*, would be more resistant to microbial degradation, and thus did not exhibit a significant difference in the presence of the tailings microbial community.

Wind erodibility and surface strength of the developed biocrusts under sterile and non-sterile conditions

Table 2 presents the degree of wind erosion (%) of the non-sterile tailings samples subjected to five wind velocities of 10, 12.5, 15, 20, and 25 m s^{-1} for a 10-min exposure period. The degree of mass loss of the BT sample was relatively high even at the lowest wind velocity of 10 m s^{-1} and increased with the rise of velocity, with complete erosion at velocities higher than 12.5 m s^{-1}. For the non-inoculated DW sample, the loose crust created over the surface of the tailings with water addition was gradually eroded with the wind flow and the tailings particles were blown away at higher rates upon exposure to wind velocities above 15 m s^{-1}, resulting in a rmarked increase in erosion. The inoculated samples (NI, AI, and NI+AI) showed significant reductions in mass loss compared to the non-inoculated samples ($P \leq 0.01$). At the wind velocity of 15 m s^{-1}, the degree of mass loss of the inoculated samples was negligible. At the velocity of 20 m s^{-1}, there was no significant difference ($P > 0.05$) in the degree of erosion from the NI and NI+AI samples, while the degree of erosion was significantly higher in the AI sample compared to the NI and NI+AI ($P \leq 0.01$). The most significant differences between the degree of erosion were highlighted at the highest wind velocity of 25 m s^{-1} with 2.44 %, 8.51 % and 0.85 % mass loss for the NI, AI, and NI+AI samples, respectively. Sterilization of the tailings did not have a significant influence on the degree of erosion of the developed biocrusts.

Table 2 Degree of wind erosion (%) of the non-inoculated and inoculated tailings samples under non-sterile condition at different wind velocities after a 6-week incubation time. The data represent means and standard deviations of three biological replicates

Treatment	Wind velocity (m s^{-1})				
	10	12.5	15	20	25
BT	17.95 ± 0.42	38.42 ± 2.83	95.99 ± 1.41	100 ± 0.00	100 ± 0.00
DW	2.99 ± 0.14	4.01 ± 0.61	8.11 ± 1.82	25.17 ± 2.22	81.99 ± 3.54
NI	0.00	0.00	0.28 ± 0.04	0.75 ± 0.06	2.44 ± 0.19
AI	0.00	0.00	0.37 ± 0.09	3.11 ± 0.49	8.51 ± 0.78
NI+AI	0.00	0.00	0.23 ± 0.03	0.69 ± 0.02	0.85 ± 0.03

Fig. 2 illustrates the compressive strength of the samples resulting from the penetrometer test. As illustrated, the blank tailings had no compressive strength and the compressive strength of DW sample did not exceed 0.1 kg cm^{-2}. The compressive strength of NI+AI sample, which was the highest value, reached 1.90 kg cm^{-2}. The values for the NI and AI samples were 1.40 and 0.85 kg cm^{-2}, respectively. As can be seen, measurements of resistance to breaking pressure of the inoculated samples correlated well directly with their stability against continuous wind force, with higher compressive strength of NI+AI accompanied with higher resistance when subjected to wind force.

As shown in Fig. 2, sterilization of tailings did not affect the compressive strength of the developed biocrusts for either co-inoculation or single inoculation of *N. muscorum*. In contrast, sterilization appeared to affect compressive strength of the tailings treated by single inoculation of *Anabaena* sp., with a higher value obtained under non-stile condition. This improvement in strength of tailings suggested that there are positive symbiotic interactions between the rest of the tailings organisms and the inoculated *Anabaena* sp. in providing a resistant biocrust.

The higher synthesis of chlorophyll-a and total EPS by *Anabaena* sp. compared to *N. muscorum* did not lead to a reduction in the wind erodibility of tailings. A study by Belnap et al. (2007) suggested that a minimum value of 10 μg chlorophyll-a g^{-1} soil would be necessary for achieving sufficient erosion control. The results of the present study highlighted that chlorophyll-a is not the only parameter that should be monitored for the formation of a resistant biocrust against fugitive dust emission. Besides chlorophyll-a content of the developed biocrusts, other factors such as cyanobacteria morphology and EPS fractions could greatly contribute to the final success of the biostabilization process. The high synthesis of LB-EPS by *Anabaena* sp. would stimulate the growth of the indigenous microbial communities inhabiting the tailings and show less contribution to the improvement of tailings structure and stability. The co-inoculation of the cyanobacteria was better at reducing the erosion rate of the tailings and increasing their surface strength. *Anabaena* sp. yielded higher chlorophyll-a and total EPS contents. While *N. muscorum* led to a higher synthesis of TB-EPS and its filamentous growth would bond the loose tailings particles, favoring the formation of a more stabilized biocrust against the induced wind. Inoculating cyanobacteria in a mixture would enhance the beneficial effects of the individual strains on biocrust formation, thus a comparatively more resistant structure to wind erosion could be generated.

Conclusion

Co-inoculation of two nitrogen-fixing cyanobacteria, *Anabaena* sp. and *N. muscorum*, on gold mine tailings resulted in the development of a resistant biocrust against wind erosion with high compressive strength. *Anabaena* sp. promoted higher synthesis of chlorophyll-a and secretion of total EPS compared to *N. muscorum* presumably due to its high resistance to the metal toxicity of the tailings, and *N. muscorum* favored the formation of a more stabilized structure against wind erosion compared to *Anabaena* sp. possibly due to its higher secretion of the more condensed TB-EPS and filamentous growth. By inoculating the cyanobacteria in a mixture, the beneficial effects obtained with the use of single strains on biocrust formation could be combined and the synergistic effects of the species would lead to a comparatively stronger structure. Higher chlorophyll-a and total EPS of the biocrusts developed by *Anabaena* sp. did not lead to a reduction in the wind erodibility of tailings. These findings suggest that in addition to chlorophyll-a and total EPS, factors such as morphology of cyanobacteria and the EPS fractions can greatly contribute to the biostabilization process. The results also indicated that sterilization of the tailings influenced the performance of the cyanobacteria depending on the inoculant. Thus, the responses of inoculants to other microbial communities should be considered in site-specific biostabilization studies prior to field-scale application.

The above-mentioned factors would contribute to the higher performance of the co-inoculation treatment strategy compared to the single inoculation of the strains, although it would be beneficial to explore the specific contributions of each strain in future works. Research should also be focused on stimulate the interactions between the microbial communities to enhance the efficiency of the process.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions **S.R.** Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing- Original Draft; **P.C.** Resources, Supervision, Project administration, Funding acquisition, Validation, Writing-review & editing; **V.M.** Supervision, Validation, Writing–review & editing. All authors read and approved the final manuscript.

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Figures

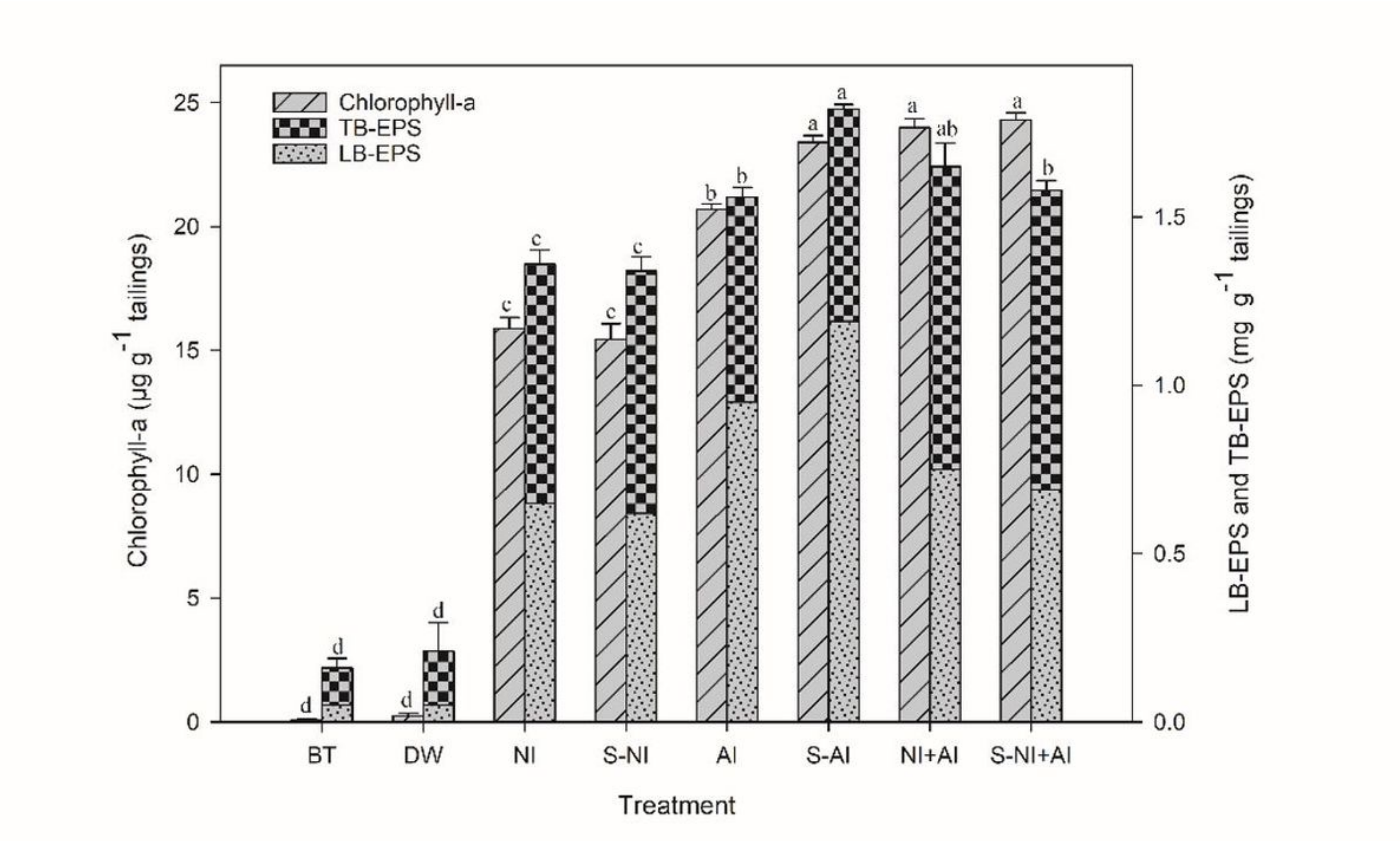

Figure 1

Chlorophyll-a, LB-EPS, and TB-EPS contents of the non-inoculated and inoculated tailings under sterile and non-sterile conditions after a 6-week incubation time. The data represent means and standard deviations of three biological replicates. Treatments that showed significant differences with each other at $P \leq 0.05$ are indicated by different letters

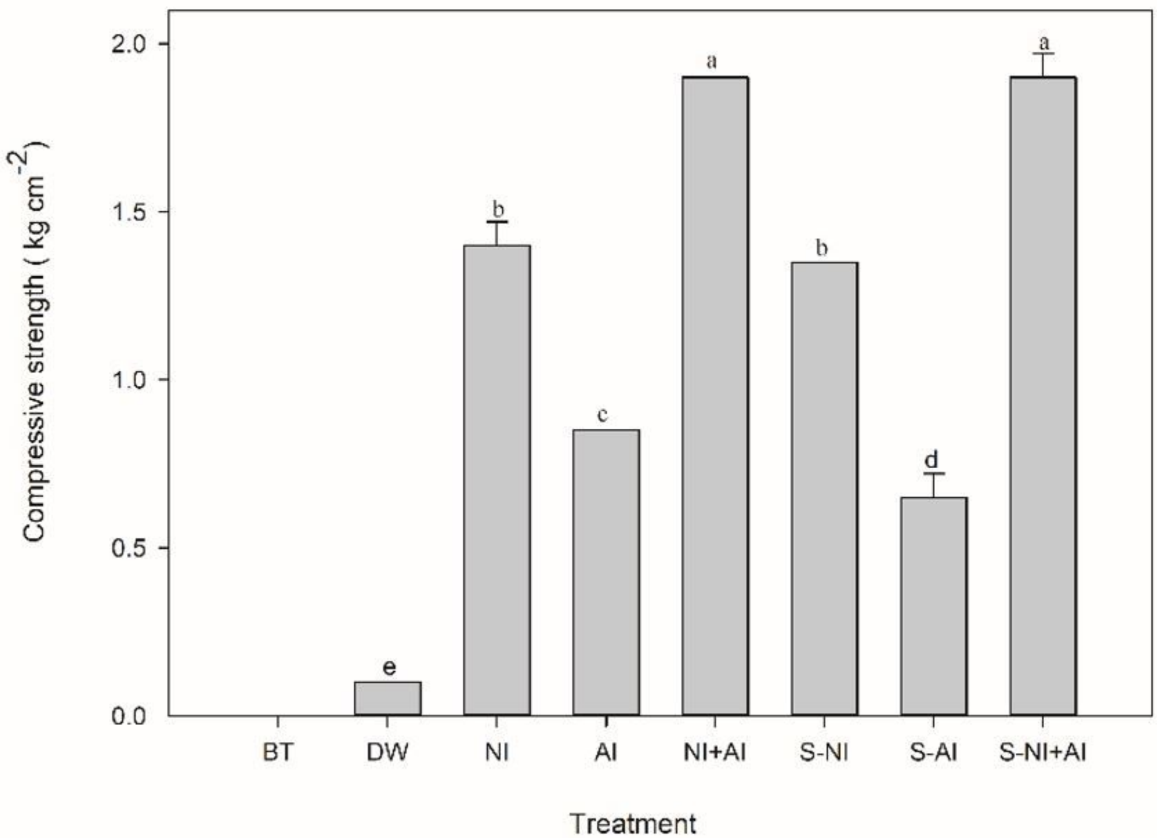

Figure 2

Compressive strength of the non-inoculated and inoculated tailings samples under sterile and non-sterile tailings after a 6-week incubation time. The data represent means and standard deviations of three biological replicates. Treatments that showed significant differences with each other at $P \leq 0.05$ are indicated by different letters