



Growth of soil algae and cyanobacteria on gold mine tailings material

AUTHORS:

Tanya Seiderer¹
Arthurita Venter¹
Fanus van Wyk¹
Anatoliy Levanets¹ 
Anine Jordaan¹

AFFILIATION:

¹Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

CORRESPONDENCE TO:

Anatoliy Levanets

EMAIL:

20868421@nwu.ac.za

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The goal of revegetation of gold mine tailings storage facilities is to reduce aeolian pollution, nutrient leaching and erosion caused by exposure to wind and water. The establishment of biological soil crusts may prove to be a more cost-effective way to reach the same goal and the aim of this study was therefore to determine if it is possible to establish algae and cyanobacteria on gold mine tailings. Different treatments of *Chlamydomonas*, *Microcoleus* and *Nostoc* were inoculated on gold mine tailings in controlled conditions and algal growth was measured on all of the treatments after 6 weeks. *Nostoc* treatments had the highest chlorophyll-*a* concentrations and produced a surface crust, while *Chlamydomonas* treatments penetrated the tailings material and provided the strongest crust. The results were promising but more research is necessary to determine the best organism, or combination of organisms, to colonise mine tailings and to eventually produce biological crusts.

Significance:

- Determination of the best organisms to colonise mine tailings and to produce biological crusts for the revegetation of gold mine tailings storage facilities.

Introduction

Tailings storage facilities (TSFs) are hydraulically deposited mine residue storage facilities for potential harmful waste products such as waste rock, cyanided sand, slimes, surplus mine water and discarded solutions¹, also known as tailings material or mill tailings². Uncovered slopes and sparse vegetation is a common sight on tailings material and these surfaces are exposed to the erosive forces of wind and water. Because of the often compacted nature of the material, decreased infiltration of water increases the possibility of run-off, erosion, sedimentation and air pollution through the generation of dust from the tailings material.³ Dust emissions from the tailings may have health effects on communities in the vicinity of TSFs^{1,4} and one of the goals of revegetation of these facilities is to reduce or eliminate human and wildlife exposure to contaminants that may be transported by airborne mine tailings⁵. The presence of acids and the high salt content of gold tailings material are likely synergistic in causing biotoxicity as low pH generally increases the bioavailability of metals.⁶ As a result, most gold mine TSFs are devoid of vegetation and have a stressed heterotrophic microbial community.^{5,7}

Rehabilitation methods implemented to reduce the environmental impact of erosion on TSFs include gravel mulching, rock cladding and vegetation establishment.^{3,8} The plant canopy reduces aeolian dispersion while the plant roots prevent water erosion and nutrient leaching.⁵ The establishment of vegetation for rehabilitation purposes is a very costly process because of the possible toxicity of heavy metals and immobilisation of some essential nutrients.² Therefore there is a great need for a cost-effective alternative to revegetate tailings material and provide reliable protection against erosion by wind and water.

Biological soil crusts (BSCs) consisting of bryophytes, lichens, algae and associated microorganisms are known to establish in extreme environments⁹⁻¹¹ such as mine tailings^{12,13} and can contribute significantly to the stabilisation of soils through soil aggregate formation¹⁴⁻¹⁷. BSCs could therefore potentially provide protection against the erosive forces of wind and water on tailings material. Improved stability of soil inoculated with cyanobacteria has been identified¹⁸ after only 6 weeks as a result of cyanobacteria binding and gluing aggregates and isolated mineral particles.

The aim of our study was to investigate establishment of algae and cyanobacteria on gold mine tailings in controlled conditions and to use scanning electron microscopy (SEM) to determine the success of colonisation. The first step was to establish a protocol for inoculation of algae and cyanobacteria on tailings material that can be used in the controlled study as well as in situ.

Materials and methods

Characterisation of the tailings material

Tailings material was collected from a gold mine TSF near Stilfontein (26.48° S, 26.47° E) in South Africa, and analysed in accordance with the standards set out by the Agricultural Laboratory Association of Southern Africa and the International Soil Analytical Exchange (Wageningen, the Netherlands).¹⁹ Exchangeable Ca, Mg, K and Na were estimated by 1 M ammonium acetate (pH=7), P was estimated by P-Bray 1 extraction, pH was estimated via 1:2.5 extraction and conductivity was determined with a saturated extraction as described in NSSSA.¹⁹

Algal and cyanobacterial species present in the tailings material before inoculation and for all treatments following the 6-week trial period were determined by the methods described by Orlekowsky et al.¹²

Test organisms

The chlorophycean *Chlamydomonas* sp. and the cyanobacteria *Microcoleus vaginatus* and *Nostoc* sp. were isolated from the collected tailings material. The isolations were made by incubating tailings material on 1.5%

agarised Bold's basal growth medium (BBM)^{20,21} in Petri plates at 20 °C in continuous light supplied from Philips Lifemax (TLD 58W/840) at 35 μmol photons/m²/s (standard conditions). Following 14 days incubation, discrete colonies were transferred to fresh agarised medium in Petri plates in order to obtain unialgal cultures. Each species was then transferred to 100 mL BBM²¹ and grown under standard conditions.

Cultures of *Chlamydomonas* sp., *M. vaginatus* and *Nostoc* sp. used for inoculation were grown in 200 mL BBM²¹ under standard conditions until they reached the exponential growth phase.

Inoculation of tailings

The effectiveness of three inoculation methods was compared using *Chlamydomonas* sp. The three methods were:

1. A volume of 200 mL of *Chlamydomonas* sp. suspended in BBM was evenly poured onto the surface of tailings.
2. A volume of 200 mL *Chlamydomonas* sp. suspended in 1% agarised BBM was evenly poured on the surface of tailings.
3. A volume of 200 mL of *Chlamydomonas* sp. suspended in BBM was sprayed over the surface of tailings using a 2-L adjustable Thema spray bottle (Thema Home Kitchen Essentials).

Glasshouse trials

Rectangular seed trays (300 x 275 x 100 mm) were filled to a depth of 80 mm with gold mine tailings material and placed in a glasshouse in which the air temperature varied between 26 °C during the day and 20 °C at night. The light intensity ranged from 50 to 300 μmol photons/m²/s depending on the time of day. Unsterilised tailings material was used because sterilisation by autoclaving resulted in excessive growth of fungi during the experiments.

Cultures grown in 200 mL BBM-agar suspension²¹ were carefully poured over the tailings material. Considering high cost as a factor in tailings revegetation projects, cultures of algae and cyanobacteria were grown in both full strength and half strength BBM. The different treatments are shown in Table 1. There were three replicates of each.

Table 1: Treatments tested in the glasshouse

Treatment†	Inoculum	Water	Agarised Bold's basal medium		
			Full strength	Half strength PO ₄ ³⁻	Half strength NO ₃ ⁻
1	-	+			
2	-		+		
3	-			+	
4	-				+
5	<i>Chlamydomonas</i>		+		
6	<i>Microcoleus</i>		+		
7	<i>Nostoc</i>		+		
6	<i>Chlamydomonas</i>			+	
9	<i>Microcoleus</i>			+	
10	<i>Nostoc</i>			+	
11	<i>Chlamydomonas</i>				+
12	<i>Microcoleus</i>				+
13	<i>Nostoc</i>				+

†Soils in all treatments contained the natural soil microflora.

The trays were placed at random positions and watered for short intervals 5 times a day. After 3 weeks, 200 mL BBM growth medium (without any organism) was added to Treatments 2–13. Treatments 3 and 4 as well as Treatments 8–13 received 200 mL BBM with the same phosphate and nitrate concentrations that were initially added (see Table 1).

Chlorophyll-a determination

After 6 weeks, 10 g soil was collected to determine the chlorophyll-a concentration using the method described by Castle²². The soil sample in 9 mL methanol solution (2% ammonium acetate in 100% methanol) was placed on a shaker for 4 h, after which the sample was filtered through a Whatman GF/C filter. The absorbance of the supernatant was determined at 652 nm, 665 nm and 750 nm. To calculate the chlorophyll-a concentrations the following equation from Porra et al.²³ was used:

$$\mu\text{g Chla/g soil} = [16.29 \cdot (A_{665} - A_{750}) - 8.54 \cdot (A_{652} - A_{750})] \cdot V / \text{g soil}$$

where V is the volume of solvent used (mL).

Penetration tests

Before inoculation, penetration tests were done on the dry tailings material with a handheld penetrometer that measures the force (in kg/cm²) needed to break a soil crust. The penetration tests were repeated 3 weeks after the trial on dry soil. Three penetration tests were performed on each replicate of each treatment.

Scanning electron microscopy

After the 6-week period, three trays of each treatment were left to dry out for SEM analyses. An area of 1 cm² of the soil surface was sampled using a scalpel blade. Following the method of Tiedt et al.²⁴, the samples were put in 4% osmium vapour; dried and mounted on aluminium stubs using carbon tape and Leit C plus (Analytical Laboratory Solution). The mounted samples were sputter coated with gold-palladium and observed in a FEI Quanta 250 FEG scanning electron microscope at 8 kV.

Data analysis

The differences between the data sets were determined with Statistica version 13 software. The Kruskal–Wallis ANOVA was used (non-parametric data) for comparing multiple independent samples to determine differences between the different treatments. Standard error bars were also added to graphs (Figures 1 and 2) to indicate the variability of the data.

Results

The chemistry of the tailings material

The chemical analysis of the tailings material used is given in Table 2.

Chlorophyll-a and penetration measurements

There was a statistically significant difference between the chlorophyll-a of the control treatment that received only water (Treatment 1) and treatments *M. vaginatus* + agar (6), *Nostoc* sp. + agar (7), *M. vaginatus* + PO₄ (9), *Nostoc* sp. + PO₄ (10), *M. vaginatus* + NO₃ (12) and *Nostoc* sp. + NO₃ (13) (Table 3). The chlorophyll-a of Treatment 2 with BBM and agar and no organism also differed significantly from treatments *M. vaginatus* + agar (6), *Nostoc* sp. + agar (7), *M. vaginatus* + PO₄ (9), *Nostoc* sp. + PO₄ (10), *M. vaginatus* + NO₃ (12) and *Nostoc* sp. + NO₃ (13). Treatments 3 and 4 (both with no organisms) as well as Treatment 11 (*Chlamydomonas* sp. + NO₃) differed significantly from treatments *Nostoc* sp. + agar (7), *M. vaginatus* + PO₄ (9) and *M. vaginatus* + NO₃ (12) while Treatment 8 (*Chlamydomonas* sp. + PO₄) differed significantly from treatments *Nostoc* sp. + agar (7) and *M. vaginatus* + NO₃ (12) (Table 3). There was no statistically significant difference between the controls and *Chlamydomonas* sp. treatments (5, 8 and 11).

Table 2: Physical and chemical analysis of the gold mine tailings soil

Soil analyses	Gold mine tailings (<5% pyrite)
pH (H ₂ O)	5.4
EC (mS/m)	193
SO ₄ (mg/kg)	1674
P (mg/kg)	1
K (mg/kg)	30
Ca (mg/kg)	1793
Mg (mg/kg)	94
Na (mg/kg)	9
CEC (cmol/kg)	1.3
Al (cmol/kg)	0.04
As (mg/kg)	0.05
Fe (mg/kg)	10.53
Mn (mg/kg)	14.90
Pb (mg/kg)	Not detected
Zn (mg/kg)	1.58

EC, electrical conductivity

All the *Nostoc* treatments grew well. Treatment 7 – the *Nostoc*–BBM–agar suspension (34.44 µg/g) – and Treatment 10 – the *Nostoc*–BBM–agar suspension with 8.1 g/L PO₄ (32.52 µg/g) – had the highest chlorophyll-*a* measurements (Figure 1). There was no statistical difference between these treatments and Treatment 13 in which *Nostoc* was grown in 9.12 g/L NO₃. Treatments with no test organism as well as treatments with *Chlamydomonas* sp. as test organism did not grow well.

The chlorophycean *Chlorococcum* sp. and species of the cyanobacteria *Nostoc*, *Phormidium* and *Scytonema* were present in the tailings material before inoculation. Table 4 shows that some organisms present in the tailings material before inoculation did not survive after the 6-week trial period. On the other hand, opportunistic genera such as *Arthrospira*, *Calothrix*, *Chlorobion*, *Chlorosarcinopsis*, *Klebsormidium*, *Lyngbya*, *Navicula*, *Stichococcus* and *Tetracystis* were found after the 6-week trial period in different treatments.

Penetration was measured after the treatments were allowed to dry out. Tailings material with *Chlamydomonas* sp. cultured in BBM–agar suspension (Treatment 5) needed the greatest force to break the soil crust (2.58 kg/cm²; Figure 2) but the force required did not differ significantly from that of the other treatments. The force required to penetrate the crust of the control differed significantly ($p=0.02$) from the force required to penetrate the crust of *Chlamydomonas* cultured in BBM–agar suspension with 8.1 g/L (Treatment 8).

Spraying delivered a more even coverage of the suspension but this method had a negative effect on the organisms and caused erratic growth. Pouring without agar showed a high initial growth but the chlorophyll-*a* concentrations (data not shown) declined from Day 6 onwards. The BBM–agar suspension that was poured on the surface proved to be the best method to inoculate algae and cyanobacteria into the soil.

Table 3: The *p*-values of chlorophyll-*a* measurements in comparisons of treatments showing a significant difference

Treatment	6	7	8	9	10	11	12	13
1	0.002	0.000001		0.000006	0.0001		0.00	0.003
2	0.009	0.000004		0.000031	0.0004		0.0000021	0.01
3		0.002		0.01			0.001	
4		0.003		0.01			0.002	
7			0.03			0.003		
8							0.02	
9						0.013		
11							0.0016	

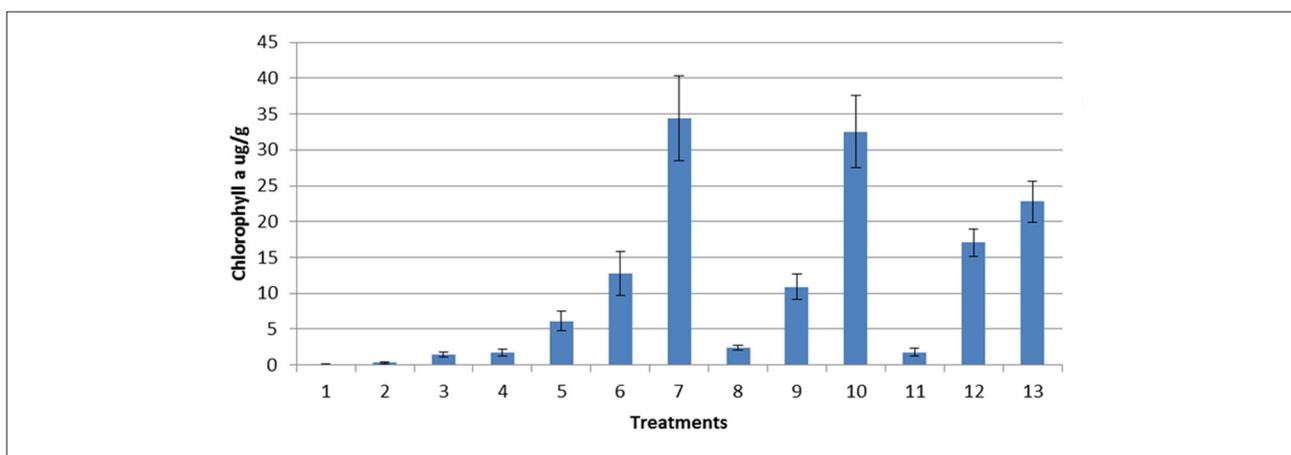


Figure 1: Mean chlorophyll-*a* concentrations in different treatments (1–13 listed in Table 1) following 6-week incubation.

Table 4: Genera present in the tailings material before inoculation and following 6-week treatment

Genus	Treatment													
	Before	1	2	3	4	5	6	7	8	9	10	11	12	13
Cyanobacteria														
<i>Arthrospira</i>							✓							
<i>Calothrix</i>		✓												
<i>Lyngbya</i>												✓		
<i>Microcoleus</i>							✓			✓			✓	
<i>Nostoc</i>	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓		✓
<i>Phormidium</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
<i>Scytonema</i>	✓	✓												
Chlorophyta														
<i>Chlamydomonas</i>		✓		✓	✓	✓		✓	✓			✓		✓
<i>Chlorella</i>														✓
<i>Chlorococcum</i>	✓	✓		✓			✓				✓			
<i>Chlorobion</i>											✓			
<i>Chlorosarcinopsis</i>					✓									
<i>Klebsormidium</i>			✓											
<i>Stichococcus</i>													✓	
<i>Tetracystis</i>				✓				✓	✓	✓				
Bacillariophyta														
<i>Navicula</i>												✓		
Total	4	5	3	5	4	3	5	4	4	3	3	5	3	4

See Table 1 for treatments.

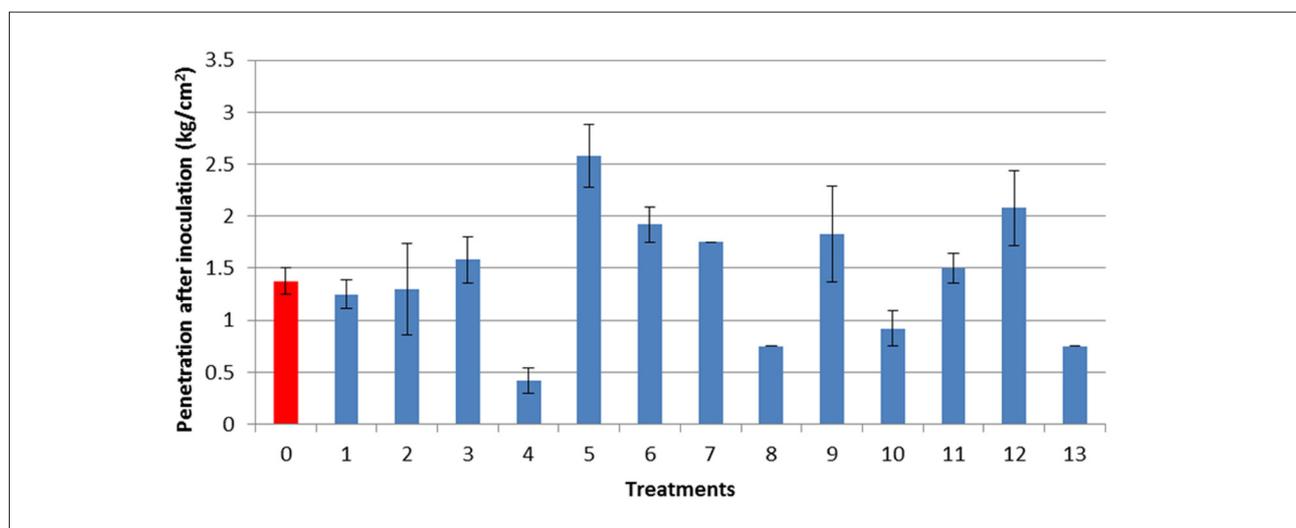


Figure 2: Results of soil penetration tests following treatment (see Table 1 for descriptions of treatments). The bar at 0 indicates penetration prior to inoculation.

This method showed a continuous increase in biomass, probably because the agar buffers the organism against the sudden change from a liquid medium to the harsher conditions of the soil. This method was therefore used in further trials.

Scanning electron microscopy

Figure 3 shows SEM images of the different treatments with the soil algae and cyanobacteria. *Phormidium* sp. grew on the surface of the tailings material – even in the case of the control treatment that received only water (Figure 3a). Crust development can be initiated and helped with input of water, nutrients and inoculum (Figure 3b).

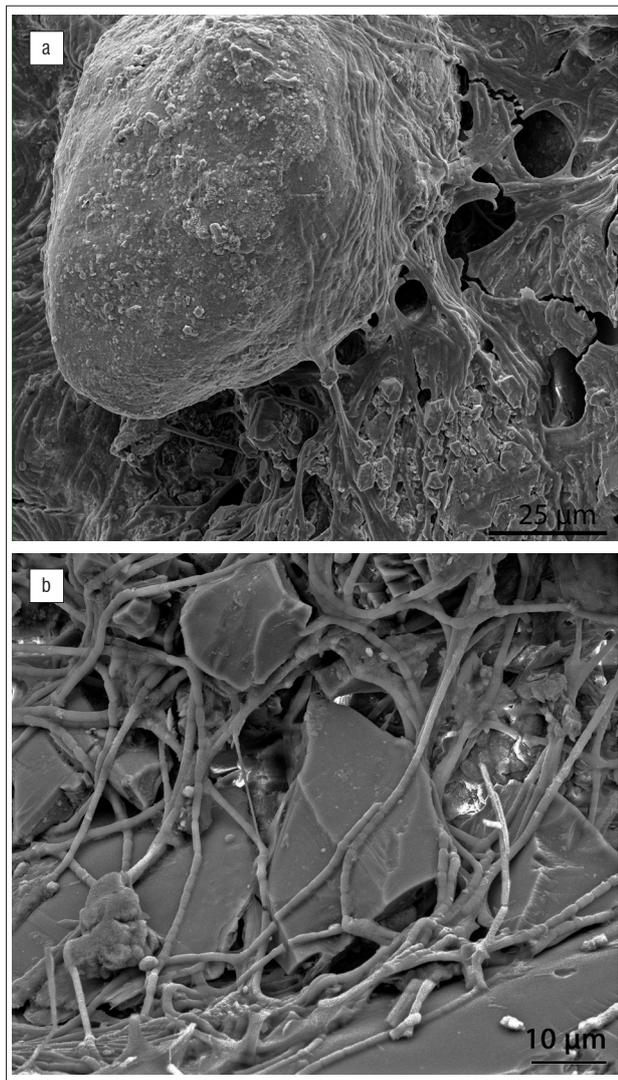


Figure 3: Scanning electron microscopy images showing the intimate relationship between algae, cyanobacteria and soil particles. (a) Control and (b) Treatment 9 after 6 weeks.

Discussion

The gold mine tailings material is acidic, has low cation exchange capabilities (CEC) and high electrical conductivity (EC; Table 2). According to Van Wyk²⁵, the ideal CEC for South African soil is about 5–20 cmol/kg and ideal EC between 60 and 100 mS/m. The CEC is the relative measure of the soil's ability to retain nutrients²⁶ and nutrients are less likely to leach out if CEC is high²⁷. Phosphate levels are very low and although calcium levels are high, calcium may not be available for utilisation by the organisms because of the low pH.²⁸ The availability of nutrients to plants is determined by the oxidative state and chemical properties of the element while the soil pH, interactions with soil colloids, microbial activity and soil physical conditions such as aeration, compaction, temperature and moisture also play key roles.²⁹

In spite of the harsh substrate conditions, this study shows that algae and cyanobacteria are able to colonise gold mine tailings and produce a viable crust within 6 weeks. There were significant differences between the treatments with *Nostoc* and *Microcoleus* and the treatments in which no organism was added (Table 3). The *Nostoc* treatments also had the highest chlorophyll-a concentrations and the lower phosphate and nitrate concentrations did not inhibit the growth of this organism (Figure 1; Treatments 10 and 13). *Nostoc* is a small and mostly immobile cyanobacterium that resides mostly on the soil surface and usually has a mucopolysaccharide sheath and UV-protective pigmentation to protect it from radiation exposure,³⁰ making it a good candidate to use as an inoculum on tailings material.

Better performance was expected from *Microcoleus* as BSC formation is often initiated by filamentous cyanobacteria such as *Microcoleus*³¹ followed by smaller cyanobacteria and chlorophytes³². *Microcoleus* is also a common soil cyanobacterium³³ that occurred in most of the revegetated gold mine TSFs investigated by Orlekowsky et al.¹² This organism has the ability to move around within the soil. When the soil is wet they can move towards the surface into the photosynthetic zone, while they retract deeper into the soil when the soil dries out.³² The continuous movement of the filaments in the soil ensures that sheath material is left behind in the soil layers thereby joining loose sand particles while forming a smooth biological soil crust on the soil surface. This type of crust may reduce water infiltration and can prevent the emergence of vascular plant seedlings³⁴ but may be a good cover to prevent dust emissions from mine tailings.

Of the three organisms chosen for this investigation, *Microcoleus* presented the most difficulties to work with as it produces films on the bottom of the culture flasks, making it difficult to spread the inoculum evenly on the tailings material, causing patchy growth patterns. However, treatments with *Microcoleus* (Treatments 6, 9 and 12) differed significantly from the control treatments (1, 2 in Table 3) and it is still a good candidate for inoculation on tailings material.

Chlorophytes such as *Chlamydomonas* are not pioneers and usually arrive later in³² the development of BSCs. This late arrival may explain the low chlorophyll-a concentrations measured for the *Chlamydomonas* treatments. However, treatments with *Chlamydomonas* penetrated the tailings material up to 641.5 μm and it may help to stabilise the soil. Results indicated that *Chlamydomonas* Treatment 5 needed a force of 2.58 kg/cm² to break the crust. There was, however, no significant difference between the control and any of the treatments, emphasising that the crusts in all treatments were in a state of development.

The glasshouse trials were duplicated in field trials on TSFs with *Nostoc* and *Microcoleus* species (data not shown) and in spite of an untimely thunderstorm, preliminary results show a higher diversity in all the treatment plots after 6 weeks compared with those of the glasshouse trials, presumably as a result of the high diaspora concentration in the atmosphere surrounding the storage facilities.¹² However, the chlorophyll-a concentrations measured were significantly lower (<2 μg/g) than those measured in the glasshouse trials.

Conclusion

This study was a preliminary one to determine if algae and cyanobacteria can grow on mine tailings; we have shown that with the inoculation of algae and cyanobacteria, as well as the input of moisture and nutrients, biological crusts can be established on tailings material within 6 weeks. *Nostoc* treatments had the highest chlorophyll-a concentrations and produced a surface crust, while *Chlamydomonas* treatments penetrated the tailings material and provided a stronger crust. *Microcoleus* species are more difficult to work with but this species also produced a viable crust, making it a good candidate to use as inoculum. By improving growth conditions, diaspora from algae and cyanobacteria in the environment also germinated on the tailings material, increasing the probability of forming viable biological crusts. One such organism is the filamentous cyanobacterium *Phormidium* that occurred on all the treatments after 6 weeks and may also be a good candidate to use as an inoculum.

The results were promising but more research is necessary to determine the best organism, or combination of organisms, to colonise mine tailings and to eventually produce biological crusts. Moisture is very important and irrigation must be available when attempting field trials. The application of inoculum on a TSF must also be investigated as the conditions within a glasshouse are different from those of a TSF exposed to the outside environment.

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Authors' contributions

F.v.W. conceptualised the project, acquired the funding and collected the data; T.S. undertook the data analysis and wrote the manuscript; T.S., A.V. and A.L. collected the data and analysed the samples; A.L. was responsible for student supervision; A.V. was the project leader, and was responsible for data curation and revisions; A.J. did the scanning electron microscopy.

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