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Micron-size metal-binding hydrogel particles improve germination and radicle elongation of Australian metallophyte grasses in mine waste rock and tailings

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Abstract

Metal contamination of landscapes as a result of mining and other industrial activities is a pervasive problem worldwide. Metal contaminated soils often lack effective vegetation cover and are prone to contaminant leaching and dispersion through erosion, leading to contamination of the general environment. Hydrogels and similar hydrophilic and negatively-charged compounds are well-established ameliorants for wastes and tailings, but their application is constrained by transport (bulk), attributes, and cost. In this paper we demonstrate that metal-binding hydrogel particle amendments could be used to ameliorate substrates prior to planting, in order to enhance seedling emergence. In this study, micron-size thiol functional cross-linked acrylamide polymer hydrogel particles (X3) were synthesised and tested in laboratory-scale experiments on extremely saline and metal contaminated mine waste rock and tailings to determine: (i) their capacity to increase substrate water holding capacity (WHC); (ii) their metal-binding efficiency and capacity to reduce metal availability to plants to below the phytotoxicity threshold; and (iii) their effect on the germination characteristics and early radicle development of two Australian metallophyte grasses, Astrebla lappacea *and* Austrostipa scabra*, under limiting and non-limiting water conditions.*

Addition of X3 to waste rock (18.4% dry weight) and tailings (3.2% dry weight) increased the WHC of both substrates, by more than 536% and 174% respectively, over the tested pH range from 2.0 to 6.3. X3 also significantly ($P<0.05$) lowered soluble concentrations of Al and Cu in leachate from amended waste rock, by up to 55 and 59% respectively between pH 3.2 and 6.3. Below pH 3.2, metal-binding efficiency declined to almost zero, suggesting a loss of particle functionality in extremely acidic conditions.

X3 was not toxic to seed germination and significantly ($P<0.05$) increased germination percentages of A. lappacea *and* A. scabra *in both waste rock and mine tailings in Petri dishes under controlled conditions. The highest germination percentages were recorded under a restricted water regime (substrates were watered to field capacity on the first day of the experiment but received no additional water thereafter). In* A. lappacea*, germination percentages increased to 43 and 10% in amended waste rock and tailings respectively compared to 9 and 0% in unamended treatments. In* A. scabra*, germination percentages increased from 0.5 to 24%, and from 6 to 21% in waste rock and tailings respectively. X3 also significantly ($P<0.05$) enhanced the radicle elongation of both species in contaminated waste rock and tailings. Under restricted water regime, radicle length of* A. lappacea *was increased to up to 19 and 13.5 mm in amended waste rock and tailings respectively as compared with 2 and 3 mm in unamended treatments. In* A. scabra*, radicle length was increased to up to 5.5 and 4.5 mm in amended waste rock and tailings respectively versus 0 and 1 mm in unamended treatments.*

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Overall, greater radicle growth in X3 amended waste rock and tailings in water limited environments suggests that X3 was able to ameliorate metal toxicity to radicles, and provide moisture in water restricted conditions, which improved the imbibition and consequent germination of the seeds. X3 appears to have potential for the establishment of vegetation on contaminated land and wastes through a combination of reduced metal toxicity and increased soil-water availability. Together, these factors can potentially stabilise surfaces and reduce leaching or runoff of contaminants.

1 Introduction

Heavy metal contaminated soils resulting from mining and other industrial activities are major environmental problems because they create unfavourable conditions for plant growth due to the lack of soil structure, low nutrient content, high salinity, high or low soil pH and elevated concentrations of toxic elements such as aluminium (Al), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn) and arsenic (As) (de Varennes et al., 2011). As a consequence, contaminated soils, tailings and waste rock may lack protective layers of vegetation that prevent dispersion of toxic contaminants to adjacent aquifers and other areas (Alvarez et al., 2003; Antonijevic et al., 2012; Mendez and Maier, 2008). The contaminants may be leached, absorbed by vegetation or retained by the soil (Alvarez et al., 2003) so they may enter the food chain and put human and wildlife health at risk.

A vegetative cover on mine wastes reduces dispersion of toxic elements to surrounding ecosystems (de Varennes et al., 2011). Phytoremediation is the use of metal tolerant plants (i.e. metallophytes) to remove or stabilise toxic elements in soils and is a cost-effective and ecologically advantageous approach compared to other remediation techniques such as excavation and disposal to landfill (Hegazy et al., 2011; Nedunuri et al., 2010; Salt et al., 1995). Native plant species are often preferred for phytoremediation as they are likely to be resilient to local stresses such as low soil nutrient and organic matter contents and climate (for instance drought in arid and semi arid environments) (Mendez and Maier, 2008). Moreover, they are generally not invasive, and thereby maintain and possibly increase local plant biodiversity (Keeling and Werren, 2005; Mendez and Maier, 2008). Grasses can often achieve rapid ground cover and limit short term erosion (Williams and Currey, 2002) and shrubs and trees offer a broad canopy cover and establish deep root systems that prevent erosion over the long term (Belsky et al., 1989; Tiedemann and Klemmedson, 2004). However, successful plant establishment of even metallophytes may be limited or impossible when metal contaminations in soils exceed plant toxicity thresholds. So, enhancing the quality of heavily impacted soils prior to revegetation is often a prerequisite for effective phytoremediation.

Nanotechnology offers a further approach to in situ remediation of metal contaminated soils which could complement existing techniques (Sun et al., 2006). The use of nanoparticles (surface-functionalised polymer and nanoscale zero-valent iron) to remove metals such as mercury (Hg), Pb, cadmium (Cd) and chromium (Cr) from contaminated water and soils has been reported previously (Bell et al., 2006; Singh et al., 2012). Plant establishment in polluted sites could be enhanced through the targeted design and application of micron- or nano-sized particles that have the capacity to bind toxic soluble metals in soils and improve plant-water relations (Rossato et al., 2011). For example, application of insoluble hydrophilic polyacrylate polymers in mine soil contaminated with Pb^{2+} has been shown to increase soil water holding capacity (Guiwei et al., 2008), reduce bioavailability of toxic trace elements such as Pb^{2+} and Cu^{2+}, and enhanced plant growth (de Varennes and Queda, 2005; Guiwei et al., 2008; Torres and de Varennes, 1998). Application of hydrophilic polymers from diapers at 0.3% (m/m) to mine soil has also been found to induce faster establishment of plant cover (Qu and de Varennes, 2010). Nano-sized vivianite was effective in reducing the leachability and bioaccessibility of Cu(II) in calcareous, neutral and acidic soils (USEPA, 2005). Rossato et al. (2011) showed that micron-size thiol functional cross-linked acrylamide polymer particles (called X3) were able to reduce the available solution concentrations of Pb^{2+} (9.65 mM), Cu^{2+} (4 mM) and Zn^{2+} (10 mM) by 86.5, 75.5 and 63.84%, respectively. They also reported that X3 was not toxic to seed germination as it allowed normal germination and root elongation of the native metallophyte curly Mitchell grass (*Astrebla lappacea*) at phytotoxic available concentrations of Pb^{2+} (9.65 mM) and Zn^{2+} (10 mM).

The objectives of this study were i) to assess the potential of X3 to bind toxic soluble metals in extremely saline and metal contaminated mine waste rock and tailings, and to increase substrate WHC, and ii) to investigate X3 effect on the germination characteristics and early radicle development of two Australian metallophyte grasses, *Astrebla lappacea* and *Austrostipa scabra*, under water limiting and non-limiting conditions.

2 Materials and methods

2.1 Substrate collection and characterisation

Substrates were collected from two mine sites where revegetation attempts had been unsuccessful for over 30 years. Waste rock (top 10 cm only) was collected from an abandoned gold mine in Queensland. Tailings (representative sample from the top 7 m) was collected from an active base metal mine in Queensland, Australia. River sand with no metal contamination was used as a control and was collected from Mt. Tamborine (Australia) and acid washed and thoroughly rinsed with deionised water (DIW) prior to experiments.

All substrates were air dried, crushed, mixed and sieved (<2 mm) to produce a fine, homogenous medium. Substrates were then analysed for pH and EC in a soil water suspension (4 g of soil to 20 ml distilled water) after shaking for 1 h by inversion at ~40 rpm in a Heidolph ReAx shaker and standing for 1 h (Rayment and Higginson, 1992). Plant available metals were extracted using 0.01 M $CaCl_2$ (4 g of soil to 40 mL 0.01 M $CaCl_2$) (Menzies et al., 2007). Determination of total metals was achieved by reducing the substrate to fine particles using a ball mill (Planetary Ball Mills PM 200, RETSCH, Germany). Acids (5 mL nitric acid 70% from Labscan Asia Co. Ltd., Bangkok, 2 ml hydrochloric acid 32% and 2.5 mL hydrofluoric acid 50% from Ajax Finechem, Australia) were added to 150–200 mg of sample which was then heated in a microwave digester (MDS-200, CEM Corporation) at 120 atm (12.16 MPa) and 185°C for 30 minutes. Samples were then analysed for a range of metals using inductively coupled plasma optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS). A National Institute Standard and Technology (NIST) Standard Reference Material (SRM) (2709 San Joaquin soil) which has certified values for most of the metals was used to verify the measurements.

2.2 Determination of metal-binding and water holding capacities of X3 in mine substrates under various pH conditions

Mixing experiments were carried out to test the efficiency of functional X3 in reducing available concentrations of metals and increasing the WHC of the waste rock and tailings over a large pH range (6.3 to 2) in DIW and 0.01 M $CaCl_2$. Two different percentages of X3 were added to each substrate based on their plant available metal contents so as to bind all or half of the soluble metals as described by Rossato et al. (2011). For DIW treatments (pH 6.3), percentages of X3 added to substrates were 18.4 and 9.2% dry weight (DW) for waste rock and 3.2 and 1.6% DW for tailings. For 0.01 M $CaCl_2$ treatments (pH 6.3 to 2), 9.2% DW and 1.6% DW of X3 were added to waste rock and tailings respectively. Required amounts of X3 were thoroughly mixed with substrates for 12 h using a shaker (Gerhardt Rotoshake RS12 Elution Shaker) at 5 rpm.

Ten mL of DIW (pH 6.3) or 0.01 M $CaCl_2$ at various pH were added to one gram of waste rock or tailings unamended or amended with X3 in 50 mL polypropylene conical tubes. Samples were shaken overnight (12h) at 5 rpm and then centrifuged (3,000 rpm for 30 min at 22°C). The supernatant (mine substrate leachate), which contained the fraction of free soluble metals not bound to the particles, was pipetted into 10 mL PPTR tubes and 25 µL of nitric acid (70%) was added per 1 mL of supernatant with stirring to digest the samples prior to metal analysis via ICP-OES/MS. Three replicates per treatment were used.

The metal-binding efficiencies of the particles in mine substrates were calculated using the formula by Rossato et al. (2011):

$$\%\text{metal adsorption} = \frac{\left[\begin{array}{c}\text{Metal concentration in}\\ \text{supernatant of unamended mine substrate}\end{array}\right] - \left[\begin{array}{c}\text{Metal concentration in}\\ \text{supernatant of X3 amended mine substrate}\end{array}\right]}{\text{Metal concentration supernatant of unamended mine substrate}} \times 100 \quad (1)$$

The water holding capacities (WHC) of waste rock and tailings under saturated conditions were calculated using the formula below (Rossato et al., 2011):

$$\text{WHC (\%DW)} = \frac{W_h - W_d}{W_d} \times 100 \quad (2)$$

Where:

W_h = hydrated weight of unamended or X3 amended waste rock or tailings.

W_d = dry weight of unamended or X3 amended waste rock or tailings.

2.3 Measurement of germination characteristics and radicle elongation of *A. lappacea* and *A. scabra* in mine substrates amended with X3 under different water regimes

Viable seeds of *A. lappacea* and *A. scabra* were obtained from Native Seeds (New South Wales, Australia). They were not dormant as the seeds had been collected and stored dry at 3–5°C for more than 12 months prior to the germination experiments.

The amounts of X3 particles applied to waste rock and tailings were calculated so as to bind all toxic soluble metal as described by Rossato et al. (2011) and were 18.4 and 3.2% DW for waste rock and tailings respectively. Particles were mixed with the waste rock and tailings overnight using a shaker (Gerhardt Rotoshake RS12 Elution Shaker) at 5 rpm. Uncontaminated acid washed river sand was used for the control.

Seeds were surface-sterilised in 20% sodium hypochlorite for 10 min and then rinsed three times in sterilised deionised water (SDIW) for one min. Thirteen grams of waste rock or tailings or 20 g of sand (control) with or without particles were placed in each Petri dish (disposable dish 90 x 25 mm) and a predetermined volume of SDIW was added to each dish to bring it to field capacity. Forty seeds of each species were placed on top of the substrates in each Petri dish, with five replicates for each treatment. Two different water regimes (field capacity and water-limited) were tested to investigate the potential of the X3 particles to supply water during a normal germination period.

The Petri dish treatments maintained at field capacity were sealed with parafilm and placed in a transparent plastic zip resealable bag to minimise water loss. In the water-limited treatments, substrates were watered to field capacity on the first day of the experiment but received no additional water thereafter. Water-limited Petri dishes were not sealed with parafilm nor placed in plastic bags. All the Petri dishes were placed in a germination cabinet under controlled conditions (30°C day, 25°C night, 12 hours light per day).

Germinated seeds were counted and removed daily, within a laminar flow cabinet until the maximum germination percentage was reached i.e. for 15 and 21 days for *A. scabra* and *A. lappacea* respectively. Seeds were considered germinated when 1 mm of radicle had emerged.

Mean germination times (MGT) were calculated using the following formula of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n} \quad (3)$$

Where:

D = number of days counted since the beginning of the experiment.

n = number of germinated seeds on day D.

Radicle length of germinated seeds was measured from the radicle-shoot junction to the tip of the longest radicles after three days for *A. scabra* and four days for *A. lappacea*.

The radicle tolerance index (RTI) was used as sensitive indicator of the alleviation of the substrate toxicity by X3 addition (Rossato et al., 2011) and was calculated using the following formula (Ellis and Roberts, 1981):

$$RTI = \frac{\text{Length of the longest radicle in X3 amended substrate}}{\text{Length of the longest radicle in control substrate}} \times 100 \qquad (4)$$

Where:

Control substrate = sand treatment alone or with X3 particles as appropriate.

2.4 Determination of pH, plant available metal concentrations and cumulative evaporation in germination substrates

Substrates used for germination experiments were collected and air dried at the end of the experiment for pH and plant available metal analysis following the methods described in the Section 2.1.

Cumulative evaporation in germination substrates was measured by weighing germination Petri dishes on every second day until Day 14 for *A. scabra* and Day 20 for *A. lappacea*. For field capacity treatment, weighing of the Petri dishes was done before addition of SDIW.

3 Data analysis

Data were presented as means and standard errors of either three (mixing experiment) or five (germination experiment) replicates, and they were tested for significant differences between treatment means using Student's t-tests in Microsoft Office Excel 2007. Analyses were conducted using a 95% confidence interval and statistical significance was indicated by P-values $P < 0.05$.

4 Results

4.1 Characteristics of substrates

Characterisations of the waste rock and tailings are presented in Table 1. Aluminium and copper were the major contaminants of waste rock and total concentrations of these metals (Al = 29,000 mg kg^{-1} and Cu = 1,289 mg kg^{-1}) reached recognised soil plant toxicity levels (Kopittke et al., 2010; Mendez and Maier, 2008). The plant available concentrations of Al (308 mg kg^{-1}) and Cu (34.6 mg kg^{-1}) were also high compared to toxicity threshold limits for plants of 0.5 mg kg^{-1} of Al or Cu (Mulvey and Elliott, 2000). Other metals, manganese (Mn) and Zn were also present in the waste rock although their plant available concentrations were lower than plant toxicity levels for these metals. Concentrations of total sodium (Na) and sulphur (S) in waste rock exceeded plant toxicity limits (Mulvey and Elliott, 2000). Unlike waste rock, the main contaminants in tailings were Mn and Zn with total and plant available concentrations of these metals exceeding plant toxicity limits (Kopittke et al., 2010; Mendez and Maier, 2008). Total Al and Cu were also high but the bioavailabilities of these metals were lower than the plant toxicity levels. The tailings also contained total Na and S with concentrations above toxicity limits for plants (Mulvey and Elliott, 2000). Sand showed no trace of metal contamination (Table 1) and was an appropriate control for the germination experiment.

The waste rock was also very acidic (pH 3.03 ± 0.003). With very low pH, the plant availabilities of metals in waste rock, such as Al, Cu and Zn are likely to increase as these metals are more available at low pH. In contrast, pH of the tailings was neutral (7.09 ± 0.006) which would reduce metal availability to plants. Both substrates were extremely saline (EC = 2.79 ± 0.003 and 3.63 ± 0.006 mS.cm^{-1} in waste rock and tailings, respectively; The State of Queensland, 2011). These ECs are toxic to plants, reducing growth, early maturation and plant yield (50% yield reduction was observed in grasses) (Mulvey and Elliott, 2000).

Table 1 Characterisation of waste rock, tailings and sand (control). Results are given as the mean ± standard error (SE) (n = 3)

Elements	Waste Rock		Tailings		Sand (Control)	
	Total	Plant Available	Total	Plant Available	Total	Plant Available
		(0.01 M $CaCl_2$)		(0.01 M $CaCl_2$)		(0.01 M $CaCl_2$)
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Al	29,000 ± 703	308 ± 4.5	14,566 ± 388	0.9 ± 0.4	15,049 ± 294	0.0 ± 0.0
Cu	1,389 ± 11	34.6 ± 0.6	842 ± 16	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Na	8,279 ± 109	–	602 ± 25	–	4,051 ± 97	–
S	23,856 ± 454	–	44,070 ± 792	–	193 ± 14	–
Mn	452 ± 52	8.0 ± 0.2	2,706 ± 51	76.7 ± 1.7	166 ± 1.6	0.2 ± 0.0
Zn	368 ± 6.4	4.6 ± 0.1	3,647 ± 75	18.5 ± 0.4	8 ± 0.7	0.0 ± 0.0

4.2 Effect of X3 particles on soluble metal concentrations in mine waste leachates

X3 particles significantly reduced soluble concentrations of Al and Cu in leachate of amended waste rock (Table 2). Concentrations of soluble metals in waste rock amended with 9.2% DW of X3 were decreased by about 23% for Al (from 26.5 to 20.3 mg L^{-1}) and 27% for Cu (from 3.6 to 2.7 mg L^{-1}) compared to unamended waste rock. The concentrations of Al and Cu in amended waste rock were further reduced by 40% (to 12 mg L^{-1}) and 43% (to 1.5 mg L^{-1}) respectively when the percentages of X3 was increased to 18.4%.

The pH did not affect the functionality of X3 in reducing soluble concentrations of Al and Cu in leachate of X3-amended waste rock (9.2% DW) between pH 6.3 and 3.2 (Table 2). The average reduction of Al and Cu soluble concentrations was almost constant at 23.5 ± 1.5% and 26.7 ± 1.6% between pH 6.3 and 3.2. Below pH 3.2, percentage metal reduction decreased to 5.6 ± 1.0% for Al and 0% for Cu, which suggests a loss of particle functionality in extremely acidic conditions.

Table 2 Soluble concentrations of metals in leachate of waste rock at different percentages of X3 and under various pH conditions in DIW and 0.01 M $CaCl_2$. Data are presented as the mean ± SE (n = 3); nt stands for 'not tested'

Extractant		Solution Metal Concentration (mg L^{-1}) in Waste Rock Leachates					
		Al			Cu		
		Percentage of X3 to Waste Rock (% DW)			Percentage of X3 to Waste Rock (% DW)		
	pH	0	9.2	18.4	0	9.2	18.4
DIW	6.3	26.5 ± 0	20.3 ± 0.6	12 ± 0.1	3.6 ± 0	2.7 ± 0.1	1.5 ± 0
0.01 M $CaCl_2$	6.3	31 ± 0.3	21.6 ± 2	nt	3.7 ± 0	2.4 ± 0.2	nt
0.01 M $CaCl_2$	5.2	30.4 ± 0.8	23.5 ± 0.1	nt	3.6 ± 0.1	2.7 ± 0	nt
0.01 M $CaCl_2$	4.5	31.2 ± 0.2	23.6 ± 0.8	nt	3.7 ± 0	2.7 ± 0.1	nt
0.01 M $CaCl_2$	3.2	33.3 ± 0.7	25.9 ± 0.3	nt	4 ± 0.1	3 ± 0	nt
0.01 M $CaCl_2$	2	47 ± 0.8	45.3 ± 0.3	nt	5 ± 0.1	5.1 ± 0	nt

As compared with waste rock, tailings had relatively low soluble metal concentrations in leachates and addition of X3 did not significantly ($P>0.05$) decrease concentrations of soluble metals (data not shown).

4.3 Water holding capacities of mine substrates amended with X3

The WHCs of unamended and X3 amended waste rock and tailings were tested to examine the potential of X3 to provide water for imbibition and germination of seeds. X3 particles greatly improved WHCs of both the waste rock (at 9.2 and 18.4% DW of X3) and tailings (at 1.6 and 3.2% DW of X3) (Table 3). The addition of 9.2% of X3 to waste rock and 1.6% of X3 to tailings increased the WHCs of the substrates approximately 4.5 fold (42 to 190%) and 2 fold (29 to 60%) respectively. As the percentages of X3 increased to 18.4% for waste rock and 3.2% for tailings, the WHCs of the substrates increased significantly, to around 266 and 79%, respectively.

There was no effect of pH on the capacity of X3 particles to increase the WHCs of waste rock and tailings (Table 3). Compared with unamended waste rock and tailings, the WHCs of X3-amended waste rock (9.2% DW of X3) and tailings (1.6% DW of X3) in 0.01 M $CaCl_2$ solution increased by nearly five times from 44 to 210% and by two times from 29 to 59%, respectively and were constant over the range of pH tested (6.3 to 2).

Table 3 Water holding capacities of waste rock and tailings amended with different percentages of X3 and under various pH conditions in DIW and 0.01 M $CaCl_2$. Data are presented as the mean ± SE (n = 3); nt stands for 'not tested'

			Substrate Water Holding Capacities (% DW)		
			Percentage of X3 to Substrates (% DW)		
Treatment	**Extractant**	**pH**	**0%**	**9.2%**	**18.4%**
Waste rock	DIW	6.3	42.2 ± 0.3	189.8 ± 11	265.8 ± 4.9
	0.01 M $CaCl_2$	6.3	36.3 ± 1.3	211.9 ± 18.9	nt
	0.01 M $CaCl_2$	5.2	43.4 ± 3.7	201.9 ± 14.1	nt
	0.01 M $CaCl_2$	4.5	47.4 ± 1	199.3 ± 13.6	nt
	0.01 M $CaCl_2$	3.2	47.1 ± 1	187.7 ± 5.6	nt
	0.01 M $CaCl_2$	2	43.7 ± 2.1	243.0 ± 26.7	nt
			0%	**1.6%**	**3.2%**
Tailings	DIW	6.3	28.8 ± 0.3	59.6 ± 5.3	78.8 ± 4.4
	0.01 M $CaCl_2$	6.3	28.5 ± 1.2	58.3 ± 1.3	nt
	0.01 M $CaCl_2$	5.2	33.0 ± 1.7	62.2 ± 1.7	nt
	0.01 M $CaCl_2$	4.5	29.6 ± 1.1	59.7 ± 1.4	nt
	0.01 M $CaCl_2$	3.2	27.6 ± 0.8	59.4 ± 0.2	nt
	0.01 M $CaCl_2$	2	27.8 ± 0.4	57.0 ± 3.1	nt

4.4 Effect of X3 on seed germination

4.4.1 Effect of X3 on pH, plant available metal concentrations and cumulative evaporation of germination substrates

Waste rock used for the germination experiment was analysed for pH and plant available metal concentrations after the experiment concluded (Table 4Table). Regardless of the water treatment and the grass species, the addition of X3 increased the pH significantly ($P<0.05$), from around 3.2 to up to 3.9.

Plant available concentrations of Al and Cu in waste rock decreased remarkably and significantly ($P<0.05$) with the addition of X3 in the substrates of both grass species (Table 4). Metal concentrations were lowered by between 78 and 85% and between 70 and 81% for water limited and field capacity treatments respectively, and maintained below the phytotoxicity thresholds for *Astrebla lappacea* and *Austrostipa scabra*.

Table 4 pH and plant available metal concentrations (0.01 M $CaCl_2$) in unamended and X3 amended (18.4% DW) waste rock used for the germination of *A. lappacea* and *A. scabra* under two different water regimes. Data are presented as the mean ± SE (n = 3)

Treatment	pH	Plant Available Metal Concentration (mg kg^{-1}) Al	Cu
a) *A. lappacea*			
Waste rock + water limited	3.3 ± 0	239 ± 1	27.6 ± 0.2
Waste rock + field capacity	3.3 ± 0	212.6 ± 3.4	23.1 ± 0.4
Waste rock + X3 + water limited	3.9 ± 0	38.2 ± 0.8	6.0 ± 0.3
Waste rock + X3 + field capacity	3.8 ± 0	46.3 ± 1	6.8 ± 0.2
b) *A. scabra*			
Waste rock + water limited	3.3 ± 0	281.2 ± 2.3	31.8 ± 0.2
Waste rock + field capacity	3.2 ± 0	290 ± 1.7	32.2 ± 0.3
Waste rock + X3 + water limited	3.9 ± 0	42.6 ± 2.1	6.4 ± 0.2
Waste rock + X3 + field capacity	3.9 ± 0	54.0 ± 1.6	7.3 ± 0.1

In tailings, regardless of the water treatment and the grass species, addition of X3 (3.2% DW) did not modify pH and plant available metal concentrations, which were not significantly different ($p>0.05$) from those in unamended tailings (Data not shown).

Cumulative evaporation from the substrates for both species was also measured during the germination experiment (Figure 1). Unamended waste rock and tailings maintained uniform evaporation for 10 days, by which time they appeared dry. For unamended sand (control), evaporation ceased after 14 days.

In the presence of X3, cumulative evaporation increased linearly for at least 20 days in waste rock and control sand (Figure 1a) and for 18 days in tailings under restricted water regime (Figure 1b) for *A. lappacea*. This shows that X3 increased the capacities of both substrates to hold water and release it progressively during the germination period under restricted water conditions. The cumulative water loss from the waste rock was greater than from the tailings as the percentage of X3 added to waste rock was 18.4% DW against only 3.2% DW for tailings (Figures 1a and 1b). In field capacity treatments, the evaporation rates were low in both substrates amended with X3 because the Petri dishes were placed in plastic zip resealable bags to reduce water loss (Figures 1a and 1b). Similar trends were observed for *A. scabra* (Figures 1c and 1d).

4.4.2 *Effect of X3 on germination percentages and mean germination time of* **A. lappacea** *and* **A. scabra** *in germination substrates*

The capacity of X3 particles to bind metals and provide water to plants in contaminated soils was tested in a germination experiment using Australian native metallophyte grasses, *A. lappacea* and *A. scabra*. X3 particles were not toxic to seed germination and significantly ($P<0.05$) enhanced germination percentages of *A. lappacea* and *A. scabra* in both waste rock and mine tailings (Table 5). Increases in germination percentages under restricted water regime were significantly ($P<0.05$) higher than in field capacity treatments except for *A. lappacea* on tailings.

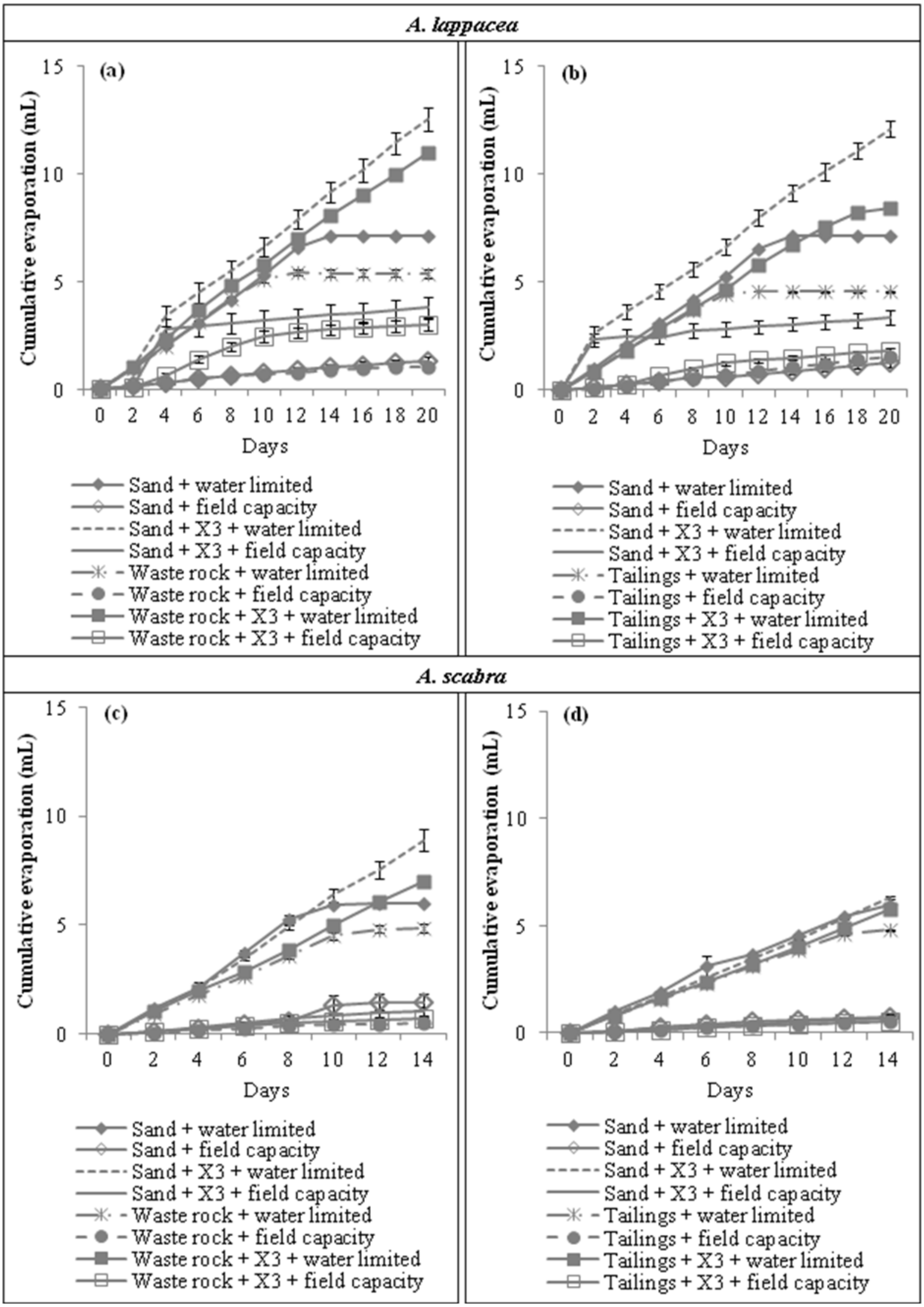

Figure 1 Cumulative water loss from sand, waste rock and tailings for *A. lappacea* (a and b) and *A. scabra* (c and d) in Petri dishes maintained at field capacity or water limited conditions. Vertical bars when larger than the symbol indicate ± SE of the mean (n = 5)

In *A. lappacea*, maximum germination percentages at field capacity increased significantly (P<0.05) with X3 amendment, from 0.5 to 29.6% and 12.9 to 23% for waste rock and tailings respectively. The increase was higher for waste rock under the water limited regime (43.5% in X3 amended compared to 9.4% in unamended waste rock). The maximum germination percentage of *A. lappacea* in tailings was about 10.4% in the water limited treatment versus only 0.9% in the unamended water limited control. In *A. scabra*, maximum germination percentages increased significantly (P<0.05) on X3 amended substrates (from 0 to 12% and 0.5 to 24.5% in waste rock; from 8 to 13.5% and 6.5 to 21.5% in tailings) under field capacity and water limited regimes respectively. Overall, maximum germination percentages of both species in X3 amended waste rock and tailings at field capacity were not significantly different (P>0.05) from the relevant sand controls. Under water restricted conditions and with the exception of *A. scabra* in amended waste rock (significantly higher, P<0.05) and in amended tailings (not significantly different, P>0.05), maximum germination percentages were lower (P<0.05) than the amended sand controls but significantly higher (P<0.05) than the unamended waste controls.

Addition of X3 particles to waste rock and tailings did not significantly (P>0.05) affect the MGT of either *A. lappacea* or *A. scabra*, with the exception of *A. lappacea* in tailings at field capacity, where germination was accelerated by almost five days in the presence of X3 (Table 5). MGTs of both grasses in both substrates was not affected by the addition of X3 to limited water treatments and were not significantly different (p>0.05) from those at field capacity, with the exception of *A. lappacea,* which germinated nearly 3.5 days faster in tailings under water limited conditions. Regardless of the water treatment, MGTs of both species in X3 amended mine substrates were not significantly different (P>0.05) from the appropriate sand control, except for *A. scabra* in amended tailings under limited water regime which was slightly higher (P<0.05) than the amended sand control.

Table 5 Maximum germination percentage (MaxGerm %) and MGT of *A. lappacea* and *A. scabra* on unamended and X3 amended waste rock (18.4% DW of X3) and tailings (3.2% DW of X3) under different water regimes. Results are given as the mean ± SE (n = 5). Treatments with the same letter do not differ significantly (P>0.05); asterisks indicate values based on one replicate only

	A. Lappacea		*A. Scabra*	
Treatment	**MaxGerm (%)**	**MGT (Days)**	**MaxGerm (%)**	**MGT (Days)**
a) Waste rock				
Sand + water limited	20.5 ± 2.9[a]	5.3 ± 0.3[a]	15.5 ± 3.0[ac]	3.1 ± 0.1[a]
Sand + field capacity	29.7 ± 6.5[aef]	4.1 ± 0.1[b]	18 ± 2.4[ac]	3.0 ± 0.2[a]
Sand + X3 + water limited	78.4 ± 3.9[b]	5.4 ± 0.3[a]	14.0 ± 3.0[a]	5.2 ± 0.8[b]
Sand + X3 + field capacity	18.4 ± 6.5[acf]	4.5 ± 1.1[ab]	15.0 ± 3.5[ac]	3.1 ± 0.4[a]
Waste rock + water limited	9.4 ± 3.2[c]	4.6 ± 0.3[ab]	0.5 ± 0.5[b]	6*
Waste rock + field capacity	0.5 ± 0.5[d]	4*	0.0 ± 0.0[b]	–
Waste rock + X3 + water limited	43.5 ± 4.0[e]	4.7 ± 0.4[ab]	24.5 ± 2.7[c]	4.7 ± 0.4[b]
Waste rock + X3 + field capacity	29.6 ± 3.0[f]	5.2 ± 0.3[a]	12.0 ± 2.0[a]	3.9 ± 0.7[ab]
b) Tailings				
Sand + water limited	20.5 ± 2.9[ade]	5.3 ± 0.3[a]	20.5 ± 3.0[ac]	2.7 ± 0.1[a]
Sand + field capacity	29.7 ± 6.5[a]	4.1 ± 0.1[b]	13.5 ± 0.6[bd]	2.9 ± 0.2[a]
Sand + X3 + water limited	80.1 ± 2.9[b]	4.1 ± 0.1[b]	22.0 ± 2.3[a]	5.1 ± 0.6[b]
Sand + X3 + field capacity	18.4 ± 1.9[ade]	5.3 ± 0.7[abd]	13.5 ± 1.7[cd]	2.5 ± 0.2[a]
Tailings + water limited	0.9 ± 0.9[c]	4*	6.5 ± 1.3[e]	4.4 ± 0.4[b]
Tailings + field capacity	12.9 ± 2.4[de]	11.5 ± 1[c]	8.0 ± 1.5[e]	7.5 ± 1.8[b]
Tailings + X3 + water limited	10.4 ± 3.6[e]	3.4 ± 0.9[ab]	21.5 ± 4.9[ad]	5.0 ± 0.3[b]
Tailings + X3 + field capacity	23 ± 2.4[a]	6.7 ± 0.3[d]	13.5 ± 1.5[cd]	5.0 ± 0.3[b]

4.4.3 *Effect of X3 particles on radicle elongation of* A. lappacea *and* A. scabra *in mine substrates*

X3 significantly enhanced radicle elongation of both species in contaminated waste rock and tailings (Figure 2 and Table 6). In general, radicle elongation of *A. lappacea* was greater under water limited conditions but the opposite was true for tailings. In *A. scabra*, the longest radicles were observed in amended waste rock at field capacity and in amended tailings under water limited conditions. Under the restricted water regime, radicle length of *A. lappacea* was up to 19 and 13.5 mm in amended waste rock and tailings respectively as compared with 2 and 3 mm in unamended treatments. In *A. scabra,* radicle length was up to 5.5 and 4.5 mm in amended waste rock and tailings respectively versus 0 and 1 mm in unamended treatments. At field capacity, radicle length of *A. lappacea* reached 5 and 17.5 mm in amended waste rock and tailings respectively compared to 0.5 and 0.0 mm in unamended treatments. Radicle length of *A. scabra* reached 7 and 3.5 mm in waste rock and tailings respectively as compared to 0.0 and 1.0 mm in unamended treatments.

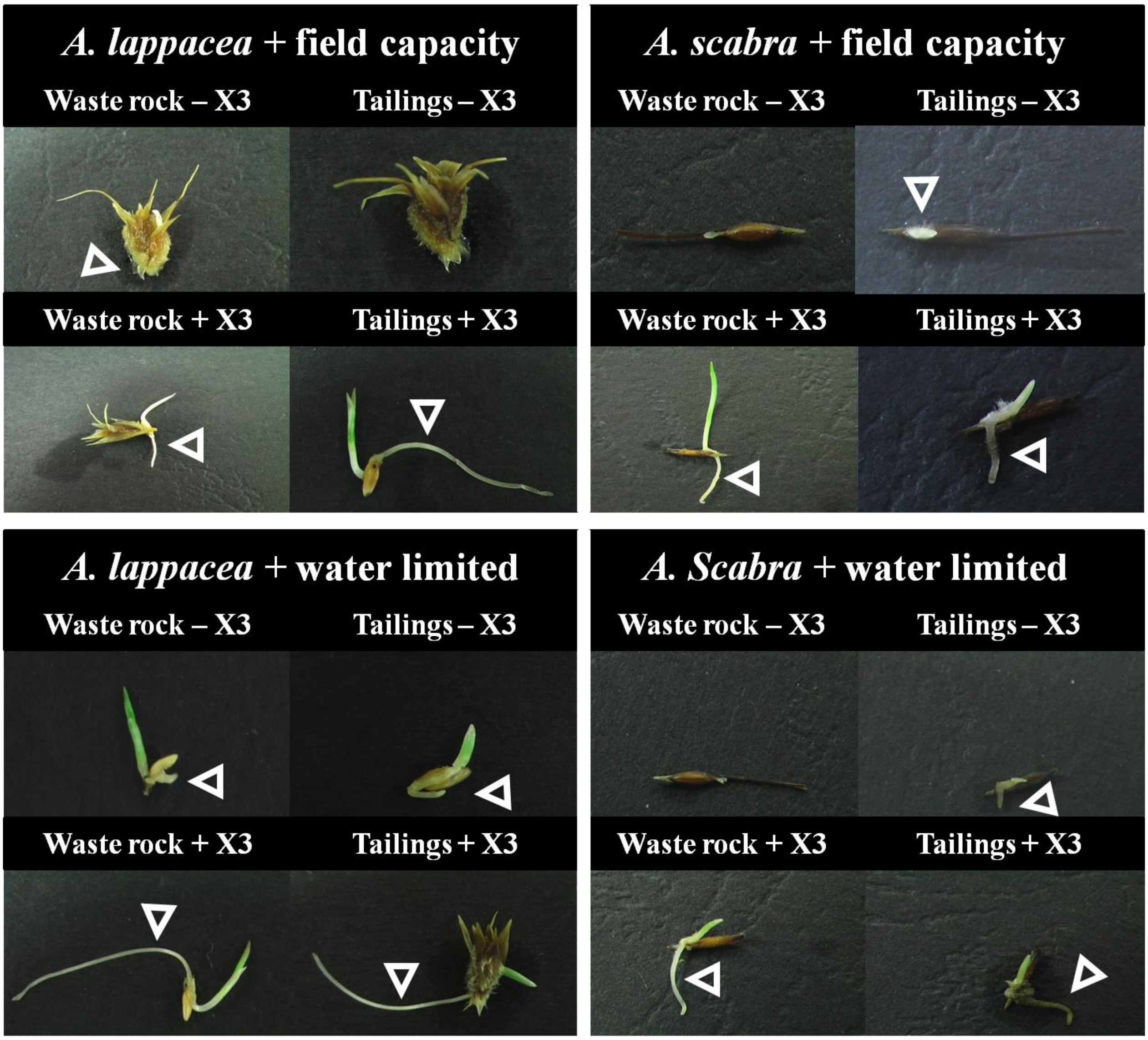

Figure 2 Radicle elongation of *A. lappacea* after four days and *A. scabra* after three days at field capacity and in water limited regime on unamended and X3 amended waste rock (18.4% DW) and tailings (3.2% DW). Arrows indicate emergent radicle

Radicle tolerance index (RTI) of *A. lappacea* and *A. scabra* was also greatly increased by the addition of X3 in both waste rock and tailings (Table 6). In the presence of X3, the highest RTI of *A. lappacea* was recorded in tailings at field capacity (138%) which was significantly (P<0.05) higher than the sand control (100%), followed by waste rock under water limited regime (108%) which was similar (P>0.05) to the sand control (100%). Although the RTIs of the species in X3 amended waste rock at field capacity (almost 53%) and tailings under water limited condition (46%) were lower than in control sand (100%), they were significantly (P<0.05) higher than in unamended waste rock (1.1%) and tailings (13.6%) under the same water regime. Regardless of water supply treatment, the RTI of *A. scabra* in waste rock and tailings in the presence of X3 was not significantly different (P>0.05) from the sand control. Large standard errors in some of the treatments were due to variable radicle extension.

Table 6 RTI of *A. lappacea* and *A. scabra* in unamended and X3 amended waste rock (18.4% DW) and tailings (3.2% DW of X3). Results are given as the mean ± SE (n = 5). Treatments followed by the same letter do not differ significantly (P>0.05); asterisks indicate values based on one replicate only

	RTI (%)			
	A. Lappacea		*A. Scabra*	
Treatment	**Control = Sand**	**Control = Sand + X3**	**Control = Sand**	**Control = Sand + X3**
a) Waste rock, water limited				
Sand	100 ± 0.0[a]	–	100 ± 0.0[a]	–
Sand + X3	222 ± 80.3[a]	100 ± 0.0[a]	67.7 ± 27.3[a]	100 ± 0.0[a]
Waste rock	32.3 ± 22.9[b]	–	0 ± 0[b]	–
Waste rock + X3	–	108 ± 29.5[a]	–	65.5 ± 40.6[a]
b) Waste rock, field capacity				
Sand	100 ± 0.0[a]	–	100 ± 0.0[a]	–
Sand + X3	33 ± 21[a]	100 ± 0.0[a]	34.8 ± 22.3[b]	100 ± 0.0[a]
Waste rock	1.1 ± 1.1[b]*	–	0 ± 0[b]	–
Waste rock + X3	–	52.8 ± 2.8[b]	–	85 ± 50.6[a]
c) Tailings, water limited				
Sand	100 ± 0.0[a]	–	100 ± 0.0[a]	–
Sand + X3	324 ± 124.7[a]	100 ± 0.0[a]	150 ± 38[a]	100 ± 0.0[a]
Tailings	13.6 ± 13.6[b]*	–	24.7 ± 10.4[b]	–
Tailings + X3	–	46.1 ± 18.7[b]	–	74.5 ± 24.8[a]
d) Tailings, field capacity				
Sand	100 ± 0.0[a]		100 ± 0.0[a]	
Sand + X3	34.2 ± 19.7[b]	100 ± 0.0[a]	129 ± 29.3[a]	100 ± 0.0[a]
Tailings	0 ± 0[c]		6.7 ± 6.7[b]	
Tailings + X3	–	138 ± 73.2[b]	–	103 ± 22.5[a]

5 Discussion

This study was the first to assess the capacity of micron-sized thiol functional cross-linked acrylamide polymer hydrogel particles (X3) to bind metals in contaminated waste rock and tailings, and to increase water holding capacities of the substrates. It demonstrated that addition of novel X3 particles led to improved seed germination in both phytotoxic mine substrates.

In this study, the plant available concentrations of Al and Cu in mine waste rock were significantly reduced by the addition of X3 to below the phytotoxicity thresholds for both native metallophyte grasses *A. lappacea* and *A. scabra*, thereby allowing the seeds to germinate. This has previously been shown to be due to the irreversible adsorption of these metals by the thiol functional group of X3 (Rossato et al., 2011). The increase of pH in the waste rock amended with X3 might also have contributed to the reduction of metal availability to seeds as Al and Cu are more available in low pH (Adriano, 2001; Kikui et al., 2005; Meda and Furlani, 2005). Falatah et al. (1996) also reported increased pH of soil with the addition of 0.2 to 0.6% (w/w) of polymers (Broadleaf P4, Agrihobe, Aquasorb and hydrogel). Other studies have investigated the use of micron-size polymers for mine soil remediation. For example, Qu and de Varennes (2010) reported a considerable decrease of water-extractable Pb, Cu and Zn in mine soils following application of cross-linked polyacrylate hydrogel polymer. However, in Pb contaminated soil, the metal-binding capacity of the polymer was found to compete with its water holding capacity which decreased progressively as the polymer sorbed Pb (Guiwei et al., 2008). Activities of soil enzymes such as urease were also impaired by the addition of the polymer, which suggested alteration of soil microbial functionality (Guiwei et al., 2008). Addition of polymer also promoted the formation of microsites rich in water and nutrient, which were favourable to roots and microorganisms (Qu and de Varennes, 2010).

X3 remarkably increased water holding capacities of both waste rock and tailings. This increase is crucial as it could provide water to plants in drought conditions by slowly releasing water to the roots (Rossato et al., 2009) and enhancing plant establishment particularly in metal contaminated sites (Mendez and Maier, 2008). Previous studies demonstrated that the water absorbing capacity and the degree of swelling in hydrogels decreased with increasing salinity (Hussien et al., 2012; Qu and de Varennes, 2010). Our study shows that the effectiveness of X3 particles in increasing WHCs of the extremely saline waste rock and tailings was not affected by the presence of salt. Abd El-Rahim et al. (2006) studied the absorbency of superabsorbent hydrogel (polyacrylamide/potassium polyacrylate) in different salt solutions and showed that the absorbent capacity of the hydrogel decreased rapidly at lower pH (4–2). In the current study, pH did not have any effect on the capacity of X3 to increase the water holding capacities of waste rock and tailings at or above pH 3.2.

Our study shows that with the addition of X3 particles, seeds of *A. lappacea* and *A. scabra* were able to germinate on extremely saline and metal contaminated waste rock and tailings which were previously phytotoxic. Seed germination and root elongation have been used in the past to evaluate toxicity of nanoparticles on higher plants (Ma et al., 2011) as this is the first physiological stage of plant growth affected by metals (Shanker et al., 2005). In contrast with other nanoparticles which had toxic effects on seed germination, such as zero-valent iron and nanosilvers on shoot growth of ryegrass, barley and flax (El-Temsah and Joner, 2012), nano-scale Fe_3O_4, TiO_2 and carbon particles on root growth of cucumber (Mushtaq, 2011) and nanomaterial quantum dots on germination of rice seeds (Nair et al., 2011), our study confirms that the X3 particles are not toxic to seed germination and supports the previous findings by Rossato et al. (2011). Addition of X3 to waste rock and tailings significantly increased germination percentages, root length and RTI of both grass species which suggests alleviation of toxicity in amended substrates (Torres and de Varennes, 1998). Enhanced germination of the seeds and radicle elongation in the presence of X3 hydrogel polymer particles under water restricted conditions confirm the ability of X3 to store and progressively release water to plant roots and to improve plant water relations under drought conditions. This is consistent with other studies on hydrogels which have been reported to stimulate root proliferation and aggregation, leading to increased contact with moisture and improved water use efficiency (Agaba et al., 2011) which in turn increased shoot and root biomass (Davies and Castro-Jimenez, 1989; Dorraji et al., 2010). Addition of acrylamide based hydrogels to sandy soils for cultivation purposes was also found to improve water retention of the soil matrix, reduce plant watering frequency and increase plant growth and performance (Abd El-Rehim et al., 2004). Additional benefits of amending soils with acrylamide based polymers include increased (Blodgett et al., 1993) and prolonged (Huttermann et al., 1999) plant water availability when irrigation ceased, reduction of soil erosion, water runoff and compaction, and increased aeration, microbial activity and plant survival (Abd El-Rehim et al., 2004; Guiwei et al., 2008).

6 Conclusion

Our study shows that X3 micron-size particles significantly ameliorated the quality of mine waste substrates in such a way as to alleviate metal toxicity to radicles, and provide moisture in water restricted conditions. This led to improved imbibition and the subsequent germination and radicle elongation of *A. lappacea* and *A. scabra* seeds in otherwise phytotoxic mine waste rock and tailings. Further research will investigate the interactions between the X3 polymer, water and ions in a long-term glasshouse experiment on contaminated mine wastes and their effects on plant establishment in phytotoxic conditions. Sustainability of the X3 particles in mine spoils will also be tested.

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