



## Metal-binding particles alleviate lead and zinc toxicity during seed germination of metallophyte grass *Astrebla lappacea*

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### ABSTRACT

Combining metal-binding particles and metal-tolerant plants (metallophytes) offers a promising new approach for rehabilitation of heavy metal contaminated sites. Three types of hydrogel metal-binding polymer particles were synthesized and their effects on metal concentrations tested *in vitro* using metal ion solutions. The most effective of the tested polymers was a micron-sized thiol functional cross-linked acrylamide polymer which reduced the available solution concentrations of Pb<sup>2+</sup> (9.65 mM), Cu<sup>2+</sup> (4 mM) and Zn<sup>2+</sup> (10 mM) by 86.5%, 75.5% and 63.8%, respectively, and was able to store water up to 608% of its dry mass. This polymer was not toxic to seed germination. In deionised water, it enhanced seed germination, and at otherwise phytotoxic Pb<sup>2+</sup> (9.65 mM) and Zn<sup>2+</sup> (10 mM) concentrations, it allowed normal germination and root elongation of the metallophyte grass *Astrebla lappacea*. We conclude that the polymer has the potential to facilitate restoration of heavy metal contaminated lands by reducing the concentration of metal cations in the soil solution and improving germination rates through reduced toxicity and enhanced plant water relations.

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### 1. Introduction

Over the past century, mining, industrial activities, manufacturing and disposal of metals and metal-containing materials have left toxic footprints over large areas of land that now carry elevated levels of heavy metals [1,2]. It is estimated that worldwide metal contamination from mine tailings only, ranges from 10,000 to 600,000 metric tons of metals including cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn) and metalloid arsenic (As) [3]. In Australia decontamination of >110,000 contaminated sites will cost in excess of A\$ 5 billion [4], and fewer than 5% of contaminated sites have been cleaned-up using bioremediation or other methods [4,5]. Metal contaminated sites frequently hinder establishment of vegetation and are therefore prone to erosion and contaminant dispersion into adjacent areas. Many approaches have been used to address metal contamination but current remediation methods are unsustainable or do not adequately address the risks posed by contaminants [6]. Contaminated soils are a risk to ecosystems and human health via exposure through food chain, drinking water and air, and exposure to toxic metals at elevated bioavailable con-

centrations can contribute to cancers and degenerative diseases [7–10].

Establishment of vegetation cover is a key to remediation and to achieve this, metal contaminated sites may require substrate amelioration prior to planting to ensure adequate germination and plant establishment rates [11]. Several substrate ameliorants have been trialled to precipitate or increase metal sorption in soils and therefore decrease the metal concentration in soil solution including lime [12], organic matter [13], vermiculite [14] and zeolite [15]. Hydrogels have been used to ameliorate plant growth in arid conditions [16–20] and saline or metal-contaminated soils [21–23] by providing a temporary source of water during germination and seedling establishment and diluting soil ion concentrations to tolerable levels [24].

Nanotechnology offers a further approach to metal remediation through design and use of micron- or nano-size metal-binding polymer particles with very high specific surface area [25] and high reactivity [26]. Such polymer particles have the capacity to selectively and irreversibly bind metal ions in soil and water [27], thus reducing their availability for plant uptake. Hydrogel polymers with capacity to sequester metal cations have the potential to enhance establishment of vegetation in water-limited and metal contaminated sites by improving water relations, reducing metal availability and/or providing a nutrient reservoir. Acrylamide polymer provides an ideal material for the synthesis of cross-linked

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hydrogel particles for environmental applications because such hydrogels have a high water retaining capacity (>90% of wet mass), good mechanical strength and resistance to microbial attack [28]. Slow degradation (~10% per year) occurs mainly through physical breakdown [28,29]. In addition, acrylamide polymers are considered to not pose an environmental threat [29], although more research is needed to ensure that soil organisms are not negatively affected indirectly or through degradation products. Synthesis of acrylamide-based polymers is relatively simple and cheap, their water holding properties can be easily manipulated through well known synthesis techniques [30,31] and the incorporation of metal binding moieties into hydrogel networks has been previously demonstrated [32–34]. It is these combinations of factors that make them novel and ideal for use in large-scale *in situ* remediation of contaminated soils.

In addition to ameliorating soil conditions, plant species tolerant to metals can assist in establishing vegetation cover and stabilising metal-contaminated lands. Several thousand so-called metallophytes have evolved globally on soils naturally rich in metals [35,36], but Australian legislation prohibits the import and use of non-native plant species for the purpose of remediation due to their potential to invade habitats and decrease regional plant biodiversity [37,38]. Fortunately, Australia has an extremely high diversity of endemic metallophytes [39] which are adapted to the local climate, especially its predominantly arid and semiarid environments, and are tolerant to drought, chloride salinity and low soil fertility [40]. Native metallophyte grasses can achieve rapid ground coverage to stabilise tailings/soils [41–43] while native metallophyte shrubs and trees develop canopies that attenuate erosive forces and establish more extensive and deeper root systems that prevent erosion over the long term [44,45]. However, rehabilitation of phytotoxic soils with metallophytes can be problematic when bioavailable concentrations of contaminants in the surface soils exceed the plants' toxicity thresholds and prevent plant establishment and growth [40,46] and adequate root formation [11]. Inhibited germination and root and shoot elongation are the most common symptoms of metal toxicity [47] and reduced root extension is commonly the first visible symptom [48]. Combining metallophytes and metal-binding particles may offer a powerful new approach for rehabilitation of heavy metal contaminated sites. In this study, we (i) assessed binding capacities of three types of synthesized hydrogel particles for the efficiency to reduce bioavailability of Cu, Zn, As and Pb, (ii) determined water-holding capacities of the particles, and (iii) examined the effect of particle treatment on germination and early root growth of *Astrelba lappacea* (a tussock-forming perennial metallophyte grass native to semi-arid Australia which possesses a robust root system able to stabilise the soil surface) under phytotoxic concentrations of Pb and Zn.

## 2. Materials and methods

### 2.1. Synthesis of cross-linked acrylamide polymers

Three cross-linked acrylamide micron-size polymers of varying functionalities (non-functional X1, xanthate functional X2 and thiol functional X3 particles) were synthesised as described in Scheme 1. Acrylamide (Fluka, 98%) and N,N' methylene bisacrylamide (Fluka, 98%) were used as received. Azo-bis-isobutyronitrile (AIBN, Reidelde-Haen) was recrystallised from methanol before use. Surface functionality and metal-binding moieties of X2 and X3 were introduced by the addition of a reversible addition-fragmentation chain transfer (RAFT) located within the structure. The RAFT mediating species employed for the synthesis of the X2 and X3 particles was the O-ethylxanthyl ethyl benzene (xanthate). Xanthate was synthesised according to procedure described by Bell et al. [27]. This

approach negated the need to synthesise specific metal binding monomers that have been used previously [32–34,49–51]. Xanthate can easily be transformed into thiol groups by facile treatment with hexylamine [27] (Scheme 1C). Thiol binding groups on solid supports are well known for binding heavy metal ions strongly and selectively [52,53], while showing little affinity for lighter metals. The specificity for particularly important toxic heavy metal ions such as Hg<sup>2+</sup>, Pb<sup>2+</sup>, and Cd<sup>2+</sup>, is a consequence of the soft Lewis acid–soft Lewis base interactions [54,55].

#### 2.1.1. Synthesis of micron-size non-functional polymer X1 (Scheme 1A)

A polymerisation stock solution was prepared by dissolving acrylamide monomer (1) (18 g, 0.253 mol), bisacrylamide (2) (1.1 g, 0.0071 mol) and AIBN (0.04 g, 0.00024 mol) into 20 mL dimethyl formamide (DMF). The stock solution was transferred into 20 mL polymerisation vials which were then sealed with rubber septa and the solution degassed via nitrogen sparging for 15 min. Polymerisation was carried out in a 40 °C water bath for a period of 24 h, and then allowed to continue at 60 °C for a further 24 h. The cross-linked polymer was then removed and coarsely ground in a mortar and pestle. Residual monomer and initiator was removed by stirring the ground material in a large excess of DMF for 24 h. The polymer was recovered by filtration, washed with DMF and methanol. The final material was dried at 25 °C under vacuum for 24 h. This purified material was subsequently milled to micron-sized particles using cryogenic crushing at liquid nitrogen temperatures to yield a fine, free-flowing powder.

#### 2.1.2. Synthesis of micron-size xanthate functional polymer X2 (Scheme 1B)

X2 polymer was prepared exactly as described above for X1 (see Section 2.1.1) except that xanthate (3) (0.3 g, 0.00133 mol) was added to the polymerisation stock solution before transfer into polymerisation vials.

#### 2.1.3. Synthesis of micron-size thiol functional polymer X3 (Scheme 1C)

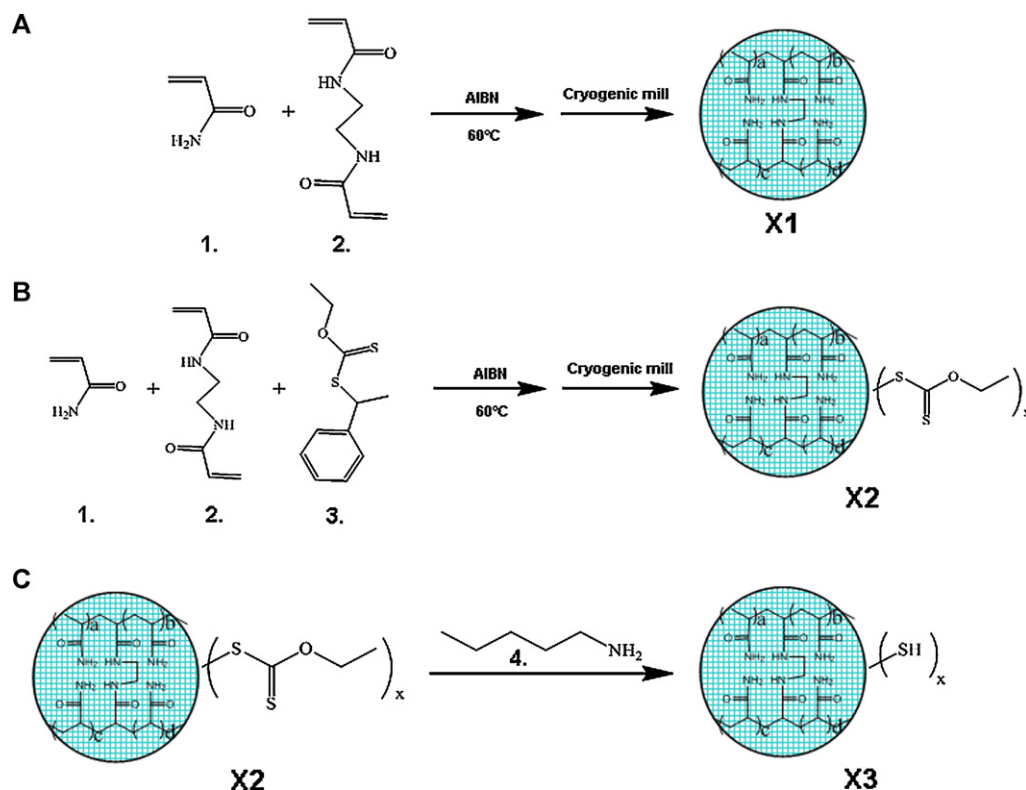
20 g of polymer X2 was stirred in a 5% (v/v) solution of hexylamine (4) in DMF for 72 h to convert the xanthate to thiols through aminolysis. The recovered polymer was then stirred with fresh DMF for 48 h with frequent changes of the DMF supernatant to ensure complete removal of all potentially phytotoxic reagents. The final material was recovered by filtration, washed with methanol and dried for 24 h under vacuum at 25 °C.

### 2.2. Determination of particle size

Dynamic light scattering measurements were performed using a Malvern Zetasizer Nano Series running DTS software and operating a 4 mW He–Ne laser at 633 nm. Analysis was performed at an angle of 90° and a constant temperature of 25 °C. Dilute particle concentrations ensure that multiple scattering and particle–particle interactions can be considered negligible during data analysis. X1, X2 and X3 polymer particles all had dynamic light scattering diameters ranging from 10 to 500 μm.

### 2.3. Determination of metal binding capacities of particles

Mixing experiments were conducted to test the efficiencies of X1, X2 and X3 particles in sequestering As, Cu, Pb and Zn from a single solution of each. The initial metal concentrations were selected from previous experiments (data not shown) to represent toxicity thresholds for the grass *A. lappacea* and included: As, 0.667 mM; Cu, 4 mM; Pb, 9.65 mM and Zn, 10 mM. As (Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O, 98%) was purchased from Sigma–Aldrich (Germany), Cu (CuCl<sub>2</sub>·2H<sub>2</sub>O,



**Scheme 1.** Synthetic procedure for the preparation of (A) non-functional X1, (B) xanthate functional X2 and (C) thiol functional X3 particles. (1) Acrylamide monomer, (2) bisacrylamide, (3) xanthate, (4) hexylamine. AIBN (free radical initiator azo-bis-isobutyronitrile).

98%), Zn ( $\text{ZnCl}_2$ , 97%) from Chem-Supply Pty. Ltd. (Australia) and Pb ( $\text{PbCl}_2$ , pure) from Lomb Scientific (Australia). Metal ion solutions were freshly prepared in sterilised deionised water (SDIW). All glassware was acid washed before use in a dilute solution of nitric acid and rinsed thoroughly in SDIW. The weight of particles required for the mixing experiments was calculated by using a 1:2 molar ratio of functionality (RAFT group, i.e., xanthate) to metal for Cu, Zn and Pb, and 1:2 and 10.2:1 for As. Metal solution (10 mL) with or without particles was added into a 20 mL glass vial and gently shaken over night (12 h). The experiment was replicated three times. Solutions were then transferred to 15 mL falcon tubes and centrifuged (3000 rpm for 30 min at 22 °C). The supernatant fraction, which contained the free metal ions (the particle pellet fraction contained the bound metals), was carefully pipetted into 10 mL ICP-OES (inductively coupled plasma optical emission spectroscopy) sample tubes. To digest the sample, 25  $\mu\text{L}$  of nitric acid (70%) was added per 1 mL of supernatant with stirring. The samples were then stored at 4 °C until analysis of total soluble metal concentrations via ICP-OES against standards. The  $r^2$  for all calibration curves was 0.9999. Particle metal binding efficiencies were calculated as follows:

$$\% \text{Metal adsorption} = \frac{[\text{Metal concentration in solution without particles}] - [\text{Metal concentration in solution with particles}]}{[\text{Metal concentration in solution without particles}]} \times 100 \quad (1)$$

#### 2.4. Determination of deionised water holding capacities of particles

Known amounts of X1, X2 and X3 polymers were placed into tared glass vials and hydrated with excess deionised water for 12 h. Three replicates of each polymer were weighed before and after hydration (each polymer type was blotted onto filter paper to remove surplus water before re-weighing). Hydrated polymers were allowed to air-dry for 48 h, then dried to constant weight under vacuum at 25 °C for 24 h, and re-weighed to obtain the

dry weight of the particles. The deionised water holding capacity (DWHC) was calculated as the difference between the hydrated ( $W_h$ ) and dry ( $W_d$ ) polymer weights, expressed as a percentage of polymer dry weight as follows:

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

#### 2.5. Effect of polymer treatment on germination percentages, mean germination times and root tolerance indices of *A. lappacea* seeds

Seeds of the metallophyte grass *A. lappacea* were obtained from AustraHort (Australia). Seeds had been hand collected from Northern Western Plains (Australia), and stored at 21 °C for more than 12 months for post-harvest ripening and to break dormancy. The germination of *A. lappacea* seeds in the presence or absence of micron-size thiol functional X3 polymer was assessed at metal concentrations known to be phytotoxic to this species (Rossato, unpublished data) including  $\text{Pb}^{2+}$  (9.65 mM and 4.825 mM in SDIW) and  $\text{Zn}^{2+}$  (10 mM in SDIW).

Seeds were surface-sterilised in 20% sodium hypochlorite for 10 min, rinsed three times in SDIW for 1 min, and placed in disposable plastic Petri dishes (9 cm) containing two autoclaved (15 min, 121 °C) 84 mm filter paper discs (Advantec). Ten mL of metal solution was added to each treatment Petri dish and SDIW was used for the control treatment. For particle treatments, X3 particles were added to the top filter paper prior to adding the metal solution or SDIW, and the amount of X3 particles applied was calculated by using a 1:2 molar ratio of xanthate to metal. The particles were suspended in 50 mL deionised water and evenly distributed onto

**Table 1**

Percentage reduction (–) or increase (+) in solution metal concentrations by particles is given as the mean  $\pm$  standard error (SE) for supernatants ( $n=3$ ); nt stands for “not tested”.

Molar ratio RAFT to metal	Particle type	Change in metal solution concentration (%)			
		As <sup>2-</sup> (0.667 mM)	Cu <sup>2+</sup> (4 mM)	Pb <sup>2+</sup> (9.65 mM)	Zn <sup>2+</sup> (10 mM)
–	X1	+3.2 $\pm$ 1.9	–25.1 $\pm$ 2.2	–49.0 $\pm$ 1.0	–23.7 $\pm$ 1.7
1:2	X2	+1.5 $\pm$ 2.4	–27.1 $\pm$ 1.1	–43.9 $\pm$ 2.4	–29.2 $\pm$ 3.0
1:2	X3	+13.0 $\pm$ 0.7	–75.5 $\pm$ 0.7	–86.4 $\pm$ 0.5	–63.8 $\pm$ 0.7
10.2:1	X2	+66.4 $\pm$ 1.2	Nt	Nt	Nt

the filter paper through filtration using a Buchner funnel. The filter paper manipulated with the X3 polymer was placed on top to ensure contact with the seeds. Twenty-five seeds were placed in each Petri dish and the experiment was replicated five times. The Petri dishes were closed, sealed with Parafilm and placed in a transparent plastic zip resealable bag to reduce water loss. They were then incubated in a controlled temperature cabinet at day and night temperatures of 30 °C and 25 °C, respectively, with 12:12 h white light and dark conditions, and with moisture non-limiting. Germinated seeds were counted and removed daily, within a laminar flow cabinet to minimise fungal and bacterial infection, until the maximum germination percentage was reached (13 days in our experiment). Seeds were considered germinated when 2 mm of radicle had emerged.

Mean germination times (MGT) were calculated using the formula of Ellis and Roberts [56].

Radicle lengths were measured from the root–shoot junction to the tip of the longest root on day 2 and expressed as root tolerance index (RTI) using the following formula:

$$RTI = \frac{\text{Length of the longest radicle in treatment}}{\text{Length of the longest radicle in control}} \times 100 \quad (3)$$

Data were tested for significant differences between treatments using one-way analysis of variance (ANOVA,  $p < 0.05$ ) and mean separation performed using the Tukey's multiple comparison test and 95% confidence interval ( $p < 0.05$ ) (Matlab<sup>®</sup> Statistics Toolbox, version 2007b, The Mathworks, USA).

### 3. Results

#### 3.1. Metal-binding capacities of the particles

The micron-size thiol functional cross-linked polymer (X3) was the most efficient of the three tested polymers at adsorbing metals and reduced the initial solution concentrations of Pb<sup>2+</sup> (9.65 mM), Cu<sup>2+</sup> (4 mM) and Zn<sup>2+</sup> (10 mM) by 86.4%, 75.5% and 63.8%, respectively (Table 1). Arsenic (As<sup>2-</sup>) was not adsorbed by any of the particle types tested, and its concentration in the supernatant solution increased by 66.4% using X2 with a molar ratio of xanthate to As of 10.2:1 (Table 1). This confirms that the particles tested here cannot bind to As anions. On the contrary, we speculate that the

**Table 2**

Deionised water holding capacity of particle types as a percentage of dry weight. Data are presented as the mean  $\pm$  standard error (SE) ( $n=3$ ).

Particle type	Water holding capacity (%)
X1	470.5 $\pm$ 38.9
X2	764.1 $\pm$ 16.8
X3	607.8 $\pm$ 30.1

As<sup>2-</sup> was excluded from the polymer network but water was still taken up and effectively “removed” from the solution, reducing the volume of the supernatant but not altering the number of moles of As, thereby increasing the As concentration in the supernatant.

#### 3.2. Deionised water holding capacities of the particles

The capacity to hold deionised water of particles X1, X2 and X3 was tested to investigate the potential of the polymers to provide a water source for seed imbibition and germination. The tested particles exhibited a high water holding capacity ranging from 470% to 608% of dry mass (Table 2).

#### 3.3. Effects of X3 particles on metal bioavailability and seed germination

The potential for the X3 particles (the most efficient particles at adsorbing metals, see Section 3.1) to bind heavy metals and to reduce bioavailable metal concentrations was tested in a germination experiment using *A. lappacea*. Maximum germination percentage (MaxGerm) after 13 days was reduced from 78.4% in the deionised water control to 8.8% in the presence of Pb 4.825 mM, and it was totally inhibited (0%) with Pb 9.65 mM (Table 3). When X3 particles were added, germination of seeds in the presence of Pb increased greatly and MaxGerm recorded were two-thirds to three-quarters of those in the X3 + deionised water control (94.4%) for Pb 4.825 mM (72.0%) and Pb 9.65 mM (65.3%), and not significantly different ( $p > 0.05$ ) from the deionised water control (78.4%, Table 3).

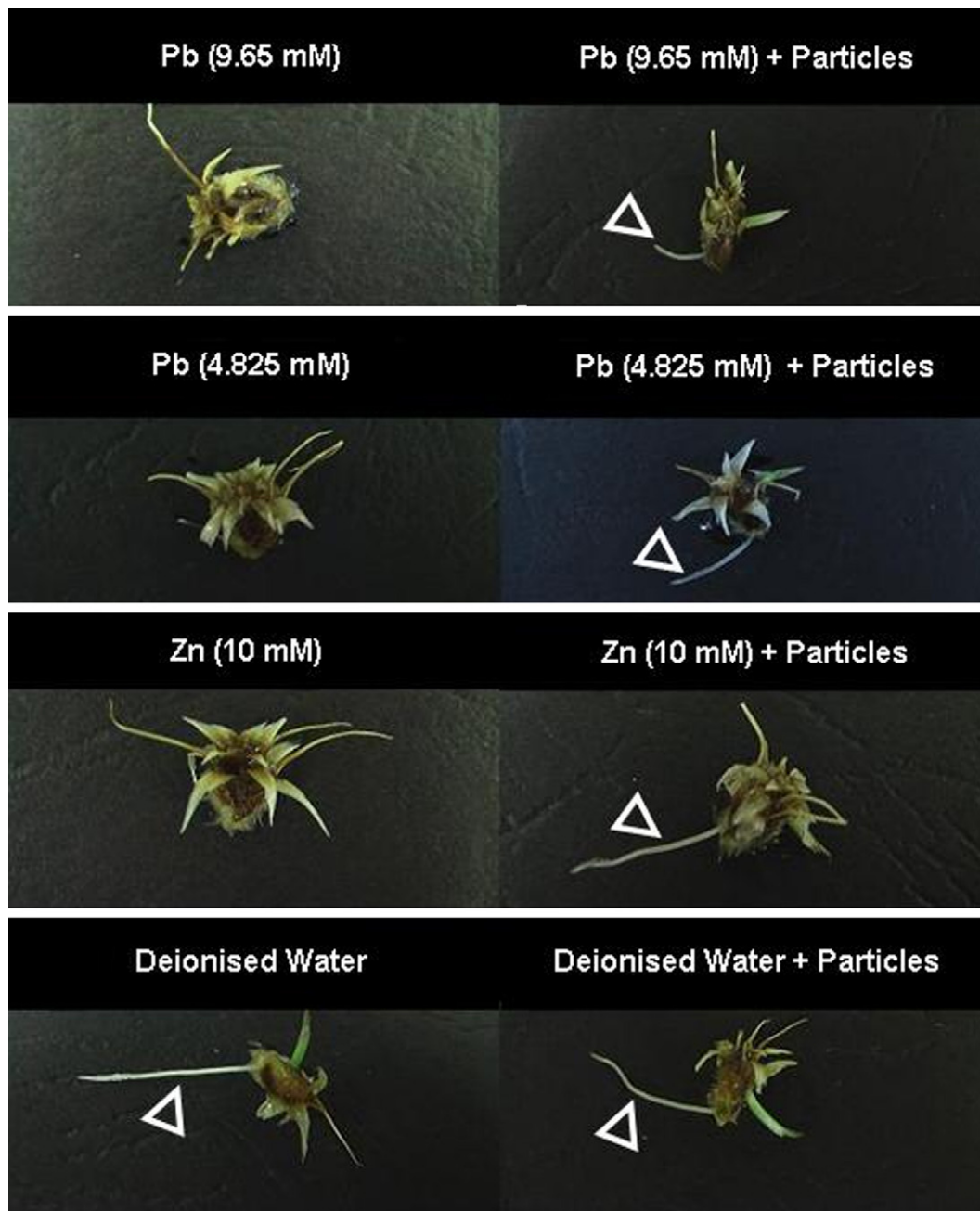
The addition of particles in the absence of metals increased the MaxGerm to 94.4% (Table 3) and also accelerated germination (mean germination time decreased from 2.9 to 2.1 days, Table 3).

**Table 3**

Maximum germination percentage (MaxGerm), mean germination time (MGT) and root tolerance index (RTI) of *Astrebla lappacea* in the presence of lead or zinc with or without addition of micron-size thiol functional X3 particles. Results are given as the mean  $\pm$  standard error (SE) for  $n=5$ . For each attribute, values followed by the same letter were not significantly different ( $p > 0.05$ ).

Treatment	MaxGerm (%)	MGT (days)	RTI (%)	
			Control = deionised water	Control = deionised water + X3
Deionised water	78.4 $\pm$ 2.0 <sup>a</sup>	2.9 $\pm$ 0.2 <sup>a</sup>	100 $\pm$ 0.0 <sup>a</sup>	–
Deionised water + X3	94.4 $\pm$ 3.9 <sup>c</sup>	2.1 $\pm$ 0.1 <sup>b</sup>	96.5 $\pm$ 2.7 <sup>a</sup>	100 $\pm$ 0.0 <sup>a</sup>
Pb (9.65 mM)	0.0 $\pm$ 0.0 <sup>b</sup>	–	0.0 $\pm$ 0.0 <sup>b</sup>	–
Pb (9.65 mM) + X3	65.3 $\pm$ 1.3 <sup>a</sup>	2.2 $\pm$ 0.0 <sup>a</sup>	–	29.3 $\pm$ 3.3 <sup>b</sup>
Pb (4.825 mM)	8.8 $\pm$ 3.8 <sup>b</sup>	2.7 $\pm$ 0.1 <sup>a</sup>	15.5 $\pm$ 9.8 <sup>b</sup>	–
Pb (4.825 mM) + X3	72.0 $\pm$ 4.3 <sup>a</sup>	2.2 $\pm$ 0.0 <sup>a</sup>	–	54.5 $\pm$ 5.6 <sup>b</sup>
Zn (10 mM)	4.8 $\pm$ 1.9 <sup>b</sup>	3.0 $\pm$ 0.0 <sup>a</sup>	0.0 $\pm$ 0.0 <sup>b</sup>	–
Zn (10 mM) + X3	76.0 $\pm$ 5.2 <sup>a</sup>	2.3 $\pm$ 0.0 <sup>a</sup>	–	71.5 $\pm$ 7.6 <sup>b</sup>





**Fig. 1.** Effect of Pb and Zn on the root elongation capacity of *Astrebla lapacea* seeds germinated in the presence or absence of micron-size thiol functional X3 particles. Radicle elongation was totally inhibited in seeds treated with heavy metals only. White arrows indicate emergent radicles in all other treatments.

These germination responses show that addition of particles did not cause toxicity to imbibing or germinating seeds and reduced soluble (i.e., available) concentrations of Pb, which in turn allowed the seeds to germinate in the presence of Pb concentrations that are normally toxic to this grass species. Similar results were found with zinc (Table 3). In the presence of 10 mM Zn and X3 particles, the MaxGerm (76%) was three-quarters that of the X3 + deionised water control (94.4%), and similar ( $p > 0.05$ ) to that of the deionised water control (78.4%). In the absence of X3 particles, this concentration of Zn dramatically decreased the MaxGerm from 78.4% in the deionised water control to 4.8% (Table 3).

Root tolerance index (RTI) was used here as a sensitive indicator of the alleviation of heavy metal toxicity by particle addition. Radicle elongation was totally inhibited in seeds treated with heavy metals in the absence of X3 particles (RTI=0%, Table 3 and Fig. 1)

with the exception of Pb 4.825 mM (RTI =  $15.5 \pm 9.8$ ). The large standard error in this treatment was caused by the variable radicle extension, with three of the five replicates not producing a radicle. Addition of particles did not affect radicle elongation as RTI of seeds germinated with deionised water in the presence of particles (96.5%) was statistically similar ( $p > 0.05$ ) to the deionised water control (100%). When particles were added to the metal solution, RTI increased to 29.3%, 54.5% and 71.5% for Pb 9.65 mM, Pb 4.825 mM and Zn 10 mM, respectively (Table 3).

#### 4. Discussion

The three X polymers studied were effective in adsorbing zinc, copper and lead cations that commonly occur in phytotoxic concentrations in contaminated sites. However, our study demonstrated

that the thiol functional X3 hydrogel particles were the most effective (Table 1). X3 significantly reduced initial soluble concentrations of  $Zn^{2+}$ ,  $Cu^{2+}$  and  $Pb^{2+}$  through strong and selective interactions between the thiol binding groups and the heavy metals. Both the non-functional (X1) and xanthate functional (X2) cross-linked polyacrylamide particles tested had some tendency to remove metal ions from aqueous solutions through both specific and non-specific pathways. It has been reported that the carbonyl group has a tendency to attract the electron cloud of the N atom in the amide groups [57,58]. Therefore, the electron cloud density of the N atom can be reduced. This makes it difficult for the N atom in the amide to participate in chelating bonds with some heavy metals. However, due to the special structure of mercury and the out layer electron cloud arrangement, mercury has extraordinary affinity for amide groups and is able to form strong covalent bonds with them. Therefore, these polyacrylamide gels have found specific application for adsorbing mercury over other heavy metals [57,58]. The residual absorption of other metal ions in these studies, such as  $Cd^{2+}$ ,  $Pb^{2+}$ ,  $Zn^{2+}$  and  $Fe^{3+}$  has been attributed to the precipitation of the metal hydroxides, due to the weak basicity of the amide group, rather than through specific interactions with the metal ions [57]. Non-functional cross-linked polyacrylamide particles also bind with  $Cu^{2+}$  through weak interactions with the amide-N. The ability of the amide nitrogen atom to participate in these binding events has been found to be heavily influenced by the network structure of the gel, i.e., porosity controlled through cross-linking [59].

None of the polymers synthesized here could bind to As anions as the  $As^{2-}$  was found to be physically excluded from the polymer network. Cross-linked polyacrylamide gels like the ones investigated herein are used in a variety of applications. Of particular interest to observed exclusion of arsenic from the gel network, is the use of such gels as a diffusion conduit in metal speciation techniques such as diffusion gradient in thin films (DGT) method [60]. The assumption is always that the gels are uncharged and inert with respect to the ions of interest. However, gels of this type possess inherent structural charge [61–63] and it is this network charge that has the capacity to influence the concentration of all ionic species within the cross-linked gel network. The cause for our observation that arsenic is excluded from the gel and caused a concentration increase in the supernatant is evident in the results presented in Table 1. In the presence of a typically negative structural charge within the gel, the concentration of positive ions will be enhanced in the gel relative to their respective populations in the bulk solution in equilibrium with the gel, resulting in a pre-concentration within the gel phase. Similarly, the concentration of negative ions (such as arsenic in this case) in the gel will be diminished. This structural charge has previously been attributed to presence of charged initiator moieties concomitant in the gel structure. The initiator used in our study does not possess inherent negative charge, AIBN (neutral) c.f. the APS (negative) water soluble initiator which has been used widely. We speculate that structural charge can also either manifest via acrylic acid monomer present either by contamination of acrylamide monomer or through hydrolysis of a small number of acrylamide moieties in the synthesis process [64]. The magnitude of this effect depends on the excluded volume of the gel, water content and therefore network structure. The precise origin of this structural charge is currently under further investigation. However, this effect may also have implications for the designed partitioning of other anions, such as sulfate and fluoride contaminants which frequently occur in post-mining soils at phytotoxic concentrations. This effect may also explain the reduction in supernatant concentrations of cationic metal species in the X1 and X2 particles. In this case, the cationic nature of the gel network would effectively increase the concentration of these positively charged species within the negatively charged gel network. This

notion is supported by results found by Bicak and Sherrington [57] who showed that linear acrylamide, with no network structure, did not to specifically bind to  $Cu^{2+}$ ,  $Cd^{2+}$  or  $Zn^{2+}$ .

The introduction of the xanthate group to the gel network did not significantly alter the partitioning behaviour of the metals studied. We propose that non-specific pathways dominate the partitioning of these metals either through the precipitation of the metals or via structural charge. Specific metal binding events in these gels are related to water content and porosity which is defined by the amount of cross-linker and the hydrophilic nature of the gel network. It is not surprising then that the metal binding results found for these cross-linked, porous, water swollen particles (X2) differ from those reported by Bell et al. [27] who examined the metal binding of nanoparticles which have the same xanthate functionality used in X2 particles. The particles used by Bell et al. [27] consisted of xanthate groups distributed through a highly hydrophobic surface layer having ester groups. In our study the xanthates are distributed through water swollen porous network of amide groups, speculated to have some negative structural charge. However, once the xanthate has been converted to the thiol functionality a significant increase in the partitioning of  $Cu^{2+}$ ,  $Zn^{2+}$  and  $Pb^{2+}$  in the particles is evident (Table 1) reflecting the enhanced interaction of the metal ions with the thiol groups.

Since the early 1990s, water soluble (i.e., not cross-linked) polyacrylamide polymers (PAM) have been applied as soil conditioners in agriculture as an effective strategy for minimising water run-off, stabilization of soil structure by preventing clay dispersion [29,65], water quality protection [66] and reduction of crusting and erosion [67]. The cross-linked acrylamide polymers used in our study are not water soluble but are capable of absorbing large quantities of water (470–608% of dry mass) to form a gel, suggesting that they could increase the water holding capacity of the soil and enhance the water supply to plants. However, the water holding capacities of the particles were determined in deionised water and the presence of impurities in water, and associated increasing osmotic potentials, would be expected to decrease water-holding capacity but this remains to be assessed. Abd El-Rehim et al. [68] reported that acrylamide based hydrogels improved the water holding capacity of sandy soils for cultivation purposes, due to their ability to absorb and retain water up to 1000-times their weight. The authors found that the addition of acrylamide based polymers allowed reduction of the watering frequency of the plants, enhanced water retention of the soil matrix and increased plant growth and performance. Furthermore, the addition of acrylamide polymers to the soil matrix was shown to increase [69] and prolong [70] water availability for plant use when irrigation ceased. Other beneficial effects of adding acrylamide based hydrogels to soils include reduction of erosion, water run-off and compaction tendency, and increase of the soil aeration, microbial activities and seedling survival [24,68,71].

In the last decade, use of synthetic cross-linked polyacrylate polymers has been proposed as a new technique for *in situ* remediation of metal contaminated soils [24,72]. As a soil amendment, addition of synthetic cross-linked polyacrylate hydrogels increases soil water holding capacity, reduces metal availability, protects plant roots from metal toxicity and prevents the entry of toxic metals into roots [73]. However, in Pb-contaminated mine soil (0.87 mg/kg extractable Pb), the water holding capacity of the polymer decreased progressively as the polymer sorbed Pb [24]. Soil urease activity was impaired by polymer application whereas other soil enzyme activities including dehydrogenase, phosphatase,  $\beta$ -glucosidase, protease and cellulase increased suggesting that polymers altered soil microbial activity [24]. In our study, addition of thiol functional cross-linked acrylamide polymer (X3) was not toxic to imbibing or germinating seeds. In deionised water, it accelerated and enhanced the completeness of seed germination, and at otherwise highly phytotoxic metal concentrations, it

alleviated metal toxicity and allowed normal germination rates and increased radicle elongation rates of *A. lappacea*. The experimental Pb concentrations at which the polymer was effective exceeded concentrations of contaminated soils [74]. The polymer may have potential for use in the restoration of contaminated land by reducing soil solution concentrations of metal cations, and improving plant germination and early establishment through reduced metal toxicity and increased soil water availability.

## 5. Conclusions

Our study shows that the three X polymers tested were effective in reducing metal cation concentration in solution but the micron-size thiol functional cross-linked acrylamide polymer (X3) was the most effective. X3 improved germination of *A. lappacea* in the presence of Pb and Zn, and increased the RTI indirectly through reducing metal ion concentrations in solution. If the X3 particles can maintain metal concentrations in the soil solution below the toxicity thresholds for plants, vegetation could be established on contaminated sites. In addition, the water-holding properties of X3 polymers may contribute to vegetation performance by increasing water supply to plants. Future research relates to whether the X3 particles are effective in binding to heavy metals in contaminated soils or tailings, with a particular focus on stability and durability of the binding. The question of potential toxicity of the particles to soil microbes and fauna will also be addressed.

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