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Growth of selected plant species in biosolids-amended mine tailings

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ABSTRACT

Biosolids stockpiles from sewage treatment plants are a valuable source of organic matter which could be utilized to improve the nutritional status and physical properties of Au mine tailings and support the growth of vegetation planted in the tailings. However, biosolids often contain elevated concentrations of heavy metals including Hg while Au mine tailings would usually contain residual Au. Therefore, it would be beneficial to select plants capable of both tolerating and phytoextracting Hg and/or Au.

This paper reports on a glasshouse-based screening study which examined the growth of plant species known for their ability to phytoextract Hg and/or Au which can grow on substrates consisting of biosolids, Au mine tailings, or different combinations of both. The germination and establishment of plants over 8-12 weeks were monitored for *Brassica juncea* (Indian mustard), *Daucus carota* (carrot), *Lupinus albus* (white lupin), *Beta vulgaris* (sugar beet), *Solanum tuberosum* (potato), and *Manihot esculenta* (cassava).

Each plant species exhibited differential responses in terms of germination, seedling quality, leaf area, specific leaf area, root and shoot biomass, and percentage dry matter partitioning to the roots. Both the Indian mustard and carrot grew successfully in the biosolids-mine tailings substrate combinations while white lupin, sugar beet, cassava, and potato failed to grow in most of the substrate combinations. The most suitable biosolids-mine tailings combination was determined to be 75% biosolids – 25% mine tailings, wherein most of the abovementioned growth parameters did not differ significantly from those of the plants grown in the control potting mix.

Keywords: Phytoremediation, Mercury, Gold, Biosolids, Gold mine tailings

1. Introduction

The use of plants to remove, detoxify, and contain heavy metals is called phytoremediation (Chaney et al., 1997). Phytoextraction is a type of phytoremediation wherein metals in the growth substrate are effectively extracted in large amounts into the aboveground harvestable parts. The most desirable characteristics for a plant used for metal phytoextraction, is that it should be able to take up and sequester the pollutant in harvestable aboveground tissues and must also be able to tolerate the presence of the pollutant while maintaining normal growth and development. In some phytoextraction efforts a hyperaccumulating plant species is utilized. A metal hyperaccumulator plant is typically defined as a plant species that accumulates a metal to a concentration in the aboveground tissues 100-fold higher than what is normally observed for plants. The accepted criteria for heavy metal hyperaccumulating plants are at least 100 mg/kg (0.01% dry wt.) for Cd or As; 1000 mg/kg (0.1 dry wt.) for Co, Cu, Cr, or Pb; and 10,000 mg/kg (1 % dry wt.) for Zn, Mn, or Ni (Reeves and Baker, 2000). Some hyperaccumulating plants are often small plants that can be challenging to use in larger scale field phytoremediation efforts. High biomass plants capable of moderate accumulation of metals in their aboveground tissues can also be used for phytoextraction (Salt et al 1995, Ebbs and Kochian, 1998; Clemente et al., 2005; Ghosh and Singh, 2005). An advantage of using these high biomass, moderately accumulating plants is that they are often agronomic plants with readily available sources of seed that are easier to propagate.

Mine tailings are generally the fine-grained solid material remaining after the metals and minerals have been extracted from mined ore and the process water. In the case of gold ore, the tailings can contain residual gold which can be extracted using a variation of phytoextraction called phytomining (Anderson et al., 1999, Piccinin et al., 2007). However the success of phytoextraction or phytomining depends first and foremost on whether that substrate can support plant growth. Mine tailings often have physicochemical characteristics that are not conducive to plant growth. Trying to grow plants in mine tailings is difficult because of the lack of oxygen supply to the roots, the poor physicochemical characteristics of the tailings, and the lack of key plant nutrients. There has been considerable work showing that tailings can be improved if blended with other substrates (Madejón et al., 2010). The use of soil amendments like composts, sewage sludge, manure and plant cover is the basis of cost-effective and environmentally sustainable methods to manage landscapes in mined areas (Tordoff et al., 2000; Wong, 2003). In a study by Moreno et al. (2004), pumice was added to improve drainage of the fine-textured mine tailings. The use of fungal endophytes and biosolids enhanced the growth of native grasses on sulphidic arsenical mine tailings (Madejón et al., 2010).

Industrialized gold mining in the Stawell gold fields involved extraction and crushing of sulphidic quartz veined gold ore followed by extraction using carbon in the pulp cyanidation process. This recovery method dates back to the 1880's as a replacement for mercury amalgamation of gold (Lougheed, 1987). Characteristic of the Goldfields region of Victoria, the tailings contain significant quantities of arsenic, present in solid solution in pyrite and arsenopyrite form (Oldmeadow, 2009). This is in contrast to the soils near the mine which show greater concentrations of As, Cr and Pb than those near a regionally determined background. This is attributed to the combination of a natural geochemical halo around mineralization and anthropogenic dispersion due to mining and urbanization. Total As concentrations were between 16 and 946 mg kg^{-1} near the mine in a regional background of 1–16 mg kg^{-1} (Noble et al., 2010). Previous geochemical testing of mine rocks near the historic mine working from where the tailings were obtained showed high sulphur content (> 5% S) and a low but significant acid neutralizing capacity (Miller, 1994). The net acid generation (NAG) tests confirmed that the sulphides were reactive with a final NAG pH of 2.6 indicating a high risk of acid generation in these materials.

Historical gold mining activities especially in the state of Victoria have also left a legacy of Hg contamination of soils, surface and sediments with concentrations up to 130 mg kg^{-1} in soils of residential areas of mining towns (Bycrof et al., 1982). Normal, non-polluted soils usually contain 20-150 μg Hg kg^{-1} (WHO, 1976). In a later report, the concentration of Hg in natural soils, which comprises 93% of all land surfaces, was reported to be approximately 0.05 to 0.08 mg kg^{-1} (World Bank Group, 1998). Because of the elevated levels of Hg in gold mine tailings in the goldfields, exposure to this heavy metal has been considered a potential health risk. Mercury is also very phytotoxic (Boney, 1971; De et al., 1985; Godbold and Huttermann, 1986; Suszcynsky and Shann, 1995; Israr et al., 2006; Zhou et al., 2007; Shiyab et al., 2008; Shiyab et al., 2009). The physiological processes of plants exposed to Hg-contaminated soil, water, or air are generally negatively affected. Toxic metal ions act mainly by blocking functional groups and/or displacing other vital metal ions in plant biomolecules. The biochemical phytotoxicity of Hg is based on its high affinity to sulfhydryl (-SH) groups and disulfide bonds (-S-S-) (Kabata-Pendias, 2011). In a study of exposed two-day-old sporelings of *Plumaria elegans* (red algae) to $HgCl_2$, 50% growth inhibition occurred after 6, 12 and 24 h at concentrations of 1.0, 0.5, and 0.25 mg Hg L^{-1}, respectively (Boney, 1971). Mercury has also been shown to reduce dry weight, decrease chlorophyll, protein and RNA contents, as well as decrease catalase and protease activity, all of which accelerate leaf senescence. This has been observed in *Pistia stratiotes* (water cabbage) when exposed to $HgCl_2$ at concentrations between 0.05 and 20.0 mg Hg L^{-1} for 2 days (De et al., 1985). The pattern of Hg phytotoxicity is virtually the same in terrestrial plants where changes in root tip cell membrane integrity have been observed in *Picea abies* (spruce seedlings) treated with 100 nM $HgCl_2$ and 1.0 nM CH_3HgCl (Godbold and Huttermann, 1986). Inhibition of root and shoot growth occurred in *Nicotiana miersii* exposed to 1.0 mg $HgCl_2$ L^{-1} for 10 days (Suszcynsky and Shann, 1995). Hence, if Hg is also present in tailings targeted for Au phytomining, then Hg phytotoxicity could limit plant growth.

Biosolids are stabilised organic solids, resulting from the treatment of domestic and industrial wastewater (Bright and Healey, 2003). The reuse of biosolids is being promoted due to the excessive space taken up by these materials in treatment plants and landfills, not to mention the large cost of disposal. Typical biosolids are rich in organic matter as well as macro- and micronutrients essential for plant growth and development. Currently, the main use of biosolids is as fertilizers or composts in land applications to improve and maintain soil productivity and stimulate plant growth. Biosolids can also be used to re-establish and sustain vegetation at mine sites (Tian et al., 2006). Reutilization of biosolids also reduces or eliminates issues associated with their disposal (Fresquez et al., 1990). However, there are environmental and public health concerns related to biosolids applications. These are mainly related to the presence of pathogenic microorganisms and hazardous compounds (Oliver et al., 2005). Heavy metals and metalloids are of particular concern as they are frequently present at elevated concentrations in biosolids. The toxicity and potential mobility of these metals can result in surface and groundwater contamination. Heavy metals can also be translocated into plants and further transferred into animal and human food chains (Lavado et al., 2005; Oliver et al., 2005). Among the heavy metals frequently present in biosolids, Hg is arguably of the highest environmental and public health concern. This is due to the extreme toxicity of both the organic and inorganic Hg species and their potential for bioaccumulation (Sloan et al., 2001). Gold can also be present in biosolids as a result of the discharge of waste materials from manufacturing processes (Reeves et al, 1999).

Mining activities produce large quantities of waste materials and tailings that frequently contain toxic concentrations of heavy metals and metalloids. Currently, many strategies and health and safety policies are used to minimize the production, emission and dispersion of pollutants from mine sites. Biosolids stockpiles from sewage treatment plants are a valuable source of organic matter which could be utilized to improve the nutritional status and physical properties of sulphidic tailings.

Most plants are sensitive to heavy metals, especially Hg. However, certain plant species can grow on contaminated habitats because they have developed a range of avoidance and/or tolerance mechanisms by which the excess of heavy metals can be rendered harmless. Mercury compounds have very limited solubility in soil leading to low Hg availability for plant uptake. In addition, Hg does not have any known biological function (Beauford et al., 1977). This may explain why any Hg-hyperaccumulating plant has yet to be identified. Limited uptake of Hg has been shown in mosses, lichens, fungi and in wetland, woody and crop plants (Patra and Sharma, 2000). Other studies on the phytoremediation of mercury in contaminated soils have been reported using different plant species such as *Atriplex canescens* (saltbush) (Patra and Sharma, 2000), *Rumex induratus* and *Marrubium vulgare* (common horehound) (Moreno-Jiménez et al., 2006), white lupin (Ximenez-Embun et al., 2001), *Tritcum aestivum* (wheat) (Cavallini et al., 1999), *Pisum sativum* (pea) (Beauford et al., 1977; Godbold and Huttermann, 1986), *Sorghum bicolor* (sorghum) (Patra and Sharma, 2000), *Chrysopogon zizanioides* (vetiver grass) (Wong, 2003), *Azolla caroliniana* (an aquatic fern) (Bennicelli et al., 2004), and *Oryza sativa* (rice) (Du et al., 2005). Few studies however, have been carried out on biosolids phytoremediation and Hg was found mainly in the roots of the nine plant species tested with very low translocation to the shoot (Lomonte et al., 2010a). In order to find the best plant species for future phytoextraction or phytomining studies, several candidate plant species known for Hg and/or Au uptake have been identified and they include Indian mustard, white lupin, sugar beet, carrot, cassava, and potato.

Indian mustard has been one of the most-studied plant species for metal phytoextraction. This species has high biomass and exhibits rapid growth, both of which are desirable for an effective phytoremediation approach. The tap roots of Indian mustard are strong and easy to harvest (Rakow, 2004), making it an ideal plant for root phytoextraction. Hg uptake/phytoextraction or Au uptake/phytoextraction have already been studied using this plant (Anderson et al., 2005; Shiyab et al., 2008; Lomonte et al., 2010a), but not both elements together. White lupin is also known to take up Hg (Esteban et al., 2008). While not a Hg hyperaccumulator, this species nonetheless shows potential as the roots of this plant do not show any effects of Hg toxicity upon exposure to as high as 10 μmol Hg L^{-1} in solution. Lupin is a legume, fixing atmospheric N_2 in its root nodules, which makes this species suitable for revegetation of mine tailings and for improving soil quality for future reclamation. Sugar beet is another plant with very large storage tap roots. It has also known Hg uptake capabilities, with the Hg ions mainly concentrated in the storage root (Greger et al., 2005). Carrot is a root crop with high biomass yields. It has been shown to have Au uptake capabilities, accumulating as much as 48.3 mg Au kg^{-1} in its tap root (Msuya et al., 2000). Cassava and potato are other crops which have been selected for propagation based on their underground storage organs, ease of planting and cultivation, and their high biomass. Both of these crops have no known Hg or Au uptake capabilities. Cassava was also selected because it is naturally cyanogenic, i.e. it can synthesize and release cyanogenic glycosides (White et al., 1998). Hydrolysis of cyanogenic glycosides produces hydrocyanic acid (HCN), which can potentially mobilise Hg and Au in the substrate and induce metal uptake into the plant, thereby making cassava a potential candidate for future phytoextraction studies.

The use of vegetation cover on unstable degraded land, such as mine-spoils and tailings sites, has been determined to provide an *in situ* cost-effective and environmentally sustainable method of stabilizing and reclaiming waste lands (Tordoff et al., 2000). This use of vegetation to reduce erosion and provide structural support is known as phytostabilization (Singh et al., 2006). Revegetation of mine sites is a well documented tool for long-term surface stabilization as tailings wastes are usually almost completely devoid of vegetation, which increases the likelihood of serious pollution resulting from wind and water erosion of the bare tailings surface (Tordoff et al., 2000). Approaches to revegetation may be categorized as ameliorative or adaptive (Tordoff et al., 2000). Both aim to sustain vegetation, though differ in their methodology in improving the growth substrate (Johnson et al., 1994). The ameliorative approach to revegetation attempts to achieve optimum conditions for plant growth by improving the physical and chemical nature of derelict land. These conditions can be attained using amendments. Numerous amendments have been used to immobilize trace elements in contaminated soils, including lime, zeolites, apatite, Fe and Mn oxides, alkaline composted biosolids, clay minerals, and industrial by-products such as beringite (Tordoff et al., 2000).

This research aimed at investigating the impact of biosolids and mine tailings mixtures on the growth and establishment of Indian mustard, white lupin, sugar beet, carrot, cassava, and potato in biosolids, tailings, or mixtures of these substrates. Both of the substrates used in this study contained elevated concentrations of Hg while mine tailings also contained elevated concentrations of Au. The goal was to identify from the plants mentioned above those that establish successfully when propagated in these substrates. Any potential phytoremediation effort with these species would depend on adequate biomass production, ideally with significant production of the most easily harvestable tissue. Altered patterns of growth and development that either reduce that harvestable biomass or total biomass, would limit the value of that plant species for the remediation of Hg and/or Au in the biosolids and tailings. Characterizing the germination and early growth of these plant species then becomes the essential first step in the development of a potential phytoremediation strategy.

2. Materials and Methods

2.1. Substrate collection, preparation, and characterisation

Historic and current mine tailings materials and oxidic ore were collected at the Stawell Gold Mine (Victoria, Australia). Historic mine tailings, or tailings that were the result of close to 150 years of mining operations, recorded to have an elevated Hg content, were collected from three abandoned sites (i.e., Newington, Magdala, and Big Hill) while current or recent tailings from an operational gold mine were collected from the Davis stockpile. Biosolids samples (i.e., moisture content > 60-70%) were collected from three sites at the Melbourne Water-Western Treatment Plant (MW-WTP) (Victoria, Australia). All substrates were dried to constant mass, crushed, and sieved through a 5 mm-mesh sieve prior to Hg and Au analysis.

Two wet digestion methods were performed and compared to determine the digestion procedure appropriate for both mine tailings and biosolids. Mine tailings and biosolids samples were digested in *aqua regia* (3:1 conc. HCl: conc. HNO_3) and *reverse aqua regia* (1:3 conc. HCl: conc. HNO_3) as described by Lomonte et al. (Lomonte et al., 2008). Acid digests were analysed for Hg by atomic fluorescence spectrometry (PS Analytical Millenium) and for Au by graphite furnace atomic absorption spectrometry (Hitachi Z-2000 Atomic Absorption Spectrometer). All measurements were done in triplicate and the Hg and Au concentrations are expressed on a dry mass basis. Elemental composition (5 replicates) was determined by inductively coupled optical emission spectrometry (ICP-OES) (Optima 4400, Perkin Elmer).

The biosolids and mine tailings samples determined to have the highest Hg and Au concentration were used for plant growth studies. The salinity of the substrates had to be decreased prior to planting as high salinity was expected to affect adversely the plants. A leaching experiment was done in order to find out how much water was needed to decrease the salinity of each of the substrates. This was done by stepwise addition of water to each of the potted substrates, followed by collection of the leachate and measurement of Total Dissolved Salts (TDS) by Multiline P4 universal meter. The TDS of the leachate was measured after each leaching increment until satisfactory soil salinity was achieved (i.e., 0-200 $\mu S\ cm^{-1}$). The mine tailings required 1.1 L of water to achieve a TDS of 172 $\mu S\ cm^{-1}$, while biosolids alone and the mine tailings-biosolids mixtures described below required 5 L water for their salinity to decrease to 147 and 177 $\mu S\ cm^{-1}$, respectively. After leaching, the substrates were air-dried and prepared for the growth experiments described below.

The following physicochemical characteristics were measured for each substrate combination: soil texture, soil stability, pH (Multiline P4 universal meter), and organic matter content. The soil texture was determined as follows. The substrate material was finely pulverised and put into a glass jar until it was 1/4 filled, to which water was added until it was 3/4 filled. One teaspoon of powdered, non-foaming detergent (Spraynex, Environex International) was added to the jar, which was then covered tightly and shaken vigorously to allow the soil aggregates to break apart. The jars were left undisturbed for 3 days to allow the different particles to settle, after which the thickness of each layer was measured (i.e., sand, silt, clay, and total deposit) and the percentage of clay, silt, and sand calculated (Whiting et al., 2003). Soil texture was then determined using the soil texture triangle (McDonald, 1984). Soil stability was determined based on the Emerson Dispersion Test where the breakdown of aggregates in water assigns the substrate material into different classes of stability: Class 1 – very unstable, Class 2 – partly stable, Class 3 – stable, and Class 4 – very stable. The organic matter content was determined by dry-ashing the substrate material in a muffle furnace at 440°C and recording the following masses: dry soil, ashed soil, organic matter (dry soil – ashed soil). The percentage organic matter was calculated as: OM = (organic matter/dry soil) * 100.

The growth substrates consisted of a mixture of biosolids and mine tailings (v/v %): 0% biosolids-100% mine tailings (0% BS); 10% biosolids-90% mine tailings (10% BS); 25% biosolids-75% mine tailings (25% BS); 50% biosolids-50% mine tailings (50% BS); 75% biosolids-25% mine tailings (75% BS); and 100% biosolids-0% mine tailings (100% BS). A seed raising mix and garden potting mix served as the control growth substrates.

2.2. Plant growth study

This study investigated the growth and establishment of the six plant species on the substrate mixtures described above in order to select best plant species and substrate combination for future phytoextraction studies. Two of the candidate plant species (i.e., cassava and potato) were initially cultivated and propagated using potting mix containing a mixture of peat moss, compost, coarse, and fine sand (2-1-1-1 ratio) at the University of Melbourne Glasshouse Complex at an average 24/20ºC day/night temperatures, 14 h photoperiod and daily watering.

The first part of the study assessed the germination of the species that could be grown from seeds. 100 seeds of Indian mustard, carrot, and sugar beet, and 150 seeds of white lupin were sown into shallow 30 x 30 cm planters containing the various biosolids and mine tailings mixtures mentioned above, with 3 replicates per substrate combination. For this experiment, two substrates were used as a control. One was a potting mix containing a mixture of peat moss, compost, coarse, and fine sand (2-1-1-1 ratio, prepared at the University of Melbourne Glasshouse Complex). The second was a Debco Seed Raising & Superior Germinating Mix (light loosely textured mix). This additional substrate is used specifically for seed raising (SRB) and contains a balanced mixture of ingredients to give the ideal combination of moisture retention, soil aeration and drainage. The pots were carefully watered daily to 100% field capacity in the morning. After 5-7 days, the number of emerged seedlings was counted and expressed as percent germination. A qualitative assessment of lupin seedlings was done using the following criteria: 1 – most healthy seedling, with 2 cotyledons and full y-expanded leaves, primary and secondary roots present, 2 – chlorotic leaves and cotyledons, primary and secondary roots present, 3 – late emerging plants 4 – necrotic leaves and roots, and 5 – dead. In parallel to this seed germination experiment, two other species, i.e., potato and cassava, were propagated vegetatively, planted as small seed potatoes or as stem cuttings, respectively. Potato and sugar beet showed 97-100% mortality for all of the tailings-biosolids mixtures so these species were not included in any subsequent plant growth experiments. The cassava stem cuttings did not root at all, suggesting that a prior rooting treatment was required before planting in the substrates.

Two week-old healthy and vigorous seedlings of Indian mustard, lupin and carrot from the germination experiment described above were then transplanted into the surface of 1-L PVC pots filled with the different growth substrates, with 5 replicates per substrate type for each species and one plant per pot. Plants were allowed to grow for 8-12 weeks under the same glasshouse conditions.

At harvest each plant species was separated into roots, stems and leaves. The leaf area for each plant was measured using a leaf area meter (LI-3000C portable area meter, LiCor, USA) before oven-drying. The plant materials were placed in storage bags and dried in an oven for 2 weeks at 60°C. After drying, each sample was weighed to determine total dry biomass. The percentage dry matter partitioning to roots was calculated as (root biomass/total plant biomass) x 100.

2.3. Data analysis

The data were analysed using MATLAB® R2013a and Microsoft Office Excel 2010. The mean, standard deviation and coefficient of variance were calculated using descriptive statistics. The Kruskal-Wallis H test or one-way analysis of variance was used to determine significant differences among the different substrate combinations. Differences at p-value < 0.05 were considered significant. Fisher's LSD test was performed to assess significance between groups.

3. Results and Discussion

Pure mine tailings were dark reddish brown to dark brown in colour and was equivalent in texture to a fine to heavy silty loam. The tailings had pH of 2.8 and 0.67 wt% organic matter (Table 1), and contained 11.67 mg Hg kg^{-1} and 3.37 mg Au kg^{-1} (Table 2). Pure biosolids were pale yellowish to brown sandy particles with pH 6.6 and 64.7 wt% organic matter (Table 1). Table 1 also provides information about the texture, pH and organic matter concentration of the different mine tailings with biosolids amendments used in this study. The concentrations of Hg and Au were found to be 5.74 mg kg^{-1} and 2.39 mg kg^{-1}, respectively (Table 2).

Table 1. Physicochemical characteristics of the mine tailings with biosolids amendments (Substrate composition in v/v %; **PM** - potting mix; **BS** - biosolids; **MT** - mine tailings; s.e. – standard error (N=3)).

Substrate	Texture	Stability	pH	s.e.	Organic matter (wt %)	s.e.
PM	sandy clay debris	Unknown – no soil aggregates	6.8	0.09	86.3	0.15
0% BS+100% MT	silty loam	Class 1 – unstable	2.8	0.03	0.67	0.00
25% BS+75% MT	silty loam	Class 2 – partly stable	3.2	0.11	4.2	0.09
50% BS+50% MT	loamy sand	Class 3 – stable	4.7	0.09	28.1	0.09
75% BS+25% MT	loamy sand	Class 3 – stable	5.5	0.03	55.3	0.20
100% BS+0% MT	sandy debris	Class 1 – unstable	6.6	0.06	64.7	0.15

Table 2. Hg and Au concentration in biosolids and mine tailings (s.e. – standard error (N=3)).

Substrate	Hg (mg kg^{-1})	s.e.	Au (mg kg^{-1})	s.e.
Pure mine tailings	11.67	0.36	3.37	0.27
Pure biosolids	5.74	0.33	2.39	0.47

The chemical composition of the biosolids, determined earlier by us (Lomonte et al., 2010b), is shown in Tables 3 while Table 4 presents the elemental analysis of the tailings material conducted as part of the present study. The pH and electrical conductivity of the tailings were determined as 5.10 ± 0.25 and 2.42 ± 0.12 mS cm^{-1}, respectively. Both pure substrates are highly unstable and would likely be problematic substrates for plants (Emerson, 1991).

Table 3. Concentrations ± standard deviations (SD) of heavy metals, phosphorus, sulphur, soluble anions and cations in biosolids from MW-WTP (Lomonte et al., 2010b) (LoD – Limit of detection).

Conc. ± SD [mg kg^{-1}]

As	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**
19.5±1.1	21.8 ±1.8	9.7±1.3	951±31	868±15	44.7±2.0	115±7	1121± 84
F^-	**Cl^-**	**NO_2^-**	**Br^-**	**NO_3^-**	**PO_4^{3-}**	**SO_4^{2-}**	
1.4± 0.4	111±2	1.9±0.3	< LoD	456±5	181±3	1119±21	

Conc. ± SD [mg kg^{-1}]

Al	**Fe**	**Zn**	**Ca^{2+}**	**K^+**	**Na^+**	**Mg^{2+}**	**P**	**S**
18.7±0.1	11.5±0.9	1.5±0.3	5.8±1.2	1.8±0.3	0.6± 0.0	1.6±0.3	9.1±0.6	7.2±0.9

Table 4. Concentrations ± standard deviations (n=5) [mg kg^{-1}] of metals, phosphorus and sulphur in mine tailings (LoD – Limit of detection).

As	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
561± 23	< LoD	1.2±0.2	4.2±0.2	102±20	391±20	9.30±1.02	16.2±0.8	123±9
Al	**Fe**	**P**	**S**	**Ca^{2+}**	**K^+**	**Na^+**	**Mg^{2+}**	
3,548±142	14,793±470	109±32	406±29	5,142±233	786±34	982±218	930±52	

The primary purpose of this study was to investigate the establishment and early growth of the candidate plant species from 0 to 8-12 weeks. Plant establishment in this context involves not only whether the plant survives or not but whether it demonstrates an initial pattern of growth that provides an indication that the plant is able to tolerate the conditions in the amended biosolids. If plant survival and establishment do not occur, then there is little hope for successful phytoextraction.

There were no significant differences across the different substrate types for the germination percentage of Indian mustard, except for the ones grown in the 25% biosolids – 75% mine tailings substrate and 50% biosolids – 50% mine tailings (Table 5). Carrot germinated well in the 100% biosolids substrate, similar to the seeds sown in both the seed-raising mix and potting mix, followed by the ones grown in the 75% biosolids – 25% mine tailings substrate and declining as the amount of mine tailings material was increased (Table 5). Despite good germination rates, white lupin seedlings exhibited early onset of senescence symptoms, hence the quality of seedling growth was closely monitored and recorded. Seedling quality of lupins was strongly affected by the poor quality of the substrate materials (Figure 1), which in turn affected the subsequent growth parameters that were measured. Nevertheless, seedlings grown in the 75% biosolids – 25% mine tailings substrate were initially healthier and most similar to the control compared to all the other combinations (Figure 1).

Table 5. Germination percentage of Indian mustard, carrot, and white lupin seeds grown in the biosolids-amended mine tailings. Data were evaluated using a one-way ANOVA with post-hoc analysis. Within a plant species, treatments with the same letter are not statistically different from each other. (**PM** = potting mix, **BS** = biosolids; **MT** - mine tailings; s.e. – standard error).

Substrate	**Germination percentage (%)**					
	Indian mustard	s.e.	Carrot	s.e.	White lupin	s.e.
Seed-raising mix	91.3^{a}	1.76	72.3^{a}	1.66	98.7^{a}	0.20
Potting mix	93.0^{a}	1.00	73.3^{a}	0.91	$91.3^{a,b}$	0.61
0% BS+100% MT	92.3^{a}	0.67	25.3^{b}	0.86	74.0^{c}	1.27
25% BS+75% MT	81.7^{b}	1.33	30.3^{c}	0.81	$80.0^{b,c}$	1.09
50% BS+50% MT	82.3^{b}	0.33	36.3^{d}	0.15	68.7^{c}	1.32
75% BS+25% MT	87.0^{c}	2.08	57.3^{b}	1.07	$90.0^{a,b}$	0.38
100% BS+0% MT	93.3^{a}	1.20	71.0^{a}	0.93	72.7^{c}	1.26
N	3		3		15	
p-value	< 0.05		< 0.05		< 0.05	
Fisher's LSD	4.0		12.0		1.2	

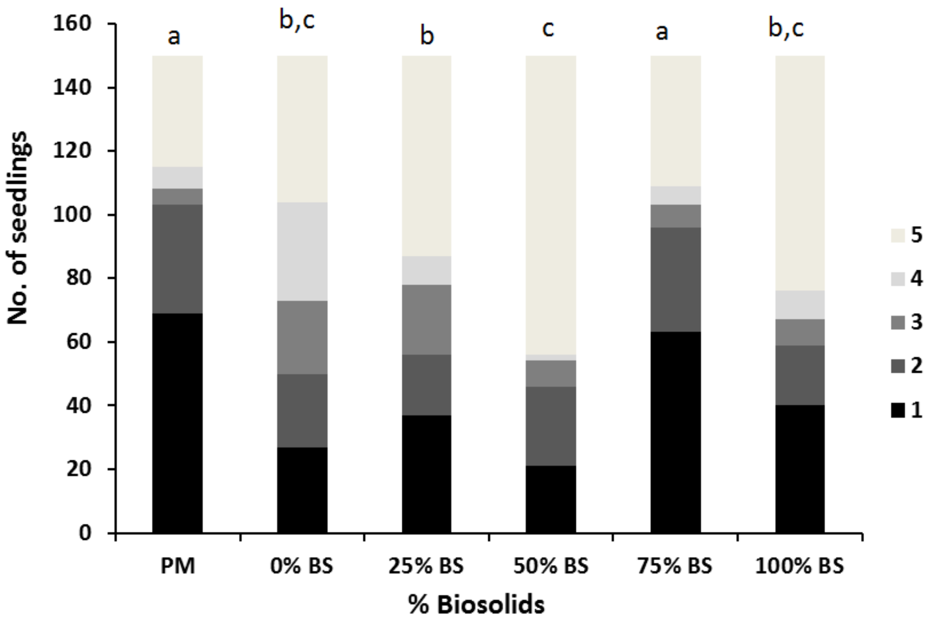

Fig. 1. Seedling quality of white lupins grown in the biosolids-mine tailings substrate combinations, ranked from 1-5 according to the following qualitative criteria: **1** – most healthy seedling, with 2 cotyledons and fully-expanded leaves, primary and secondary roots present, **2** – chlorotic leaves and cotyledons, primary and secondary roots present, **3** – late emerging plants, **4** – necrotic leaves and roots, **5** – dead. Data were evaluated using the Kruskal-Wallis H test with post-hoc analysis. Treatments with the same letter are not statistically different from each other. (**PM** = potting mix, **BS** = biosolids).

The total leaf area of Indian mustard plants increased with increasing the amount of biosolids material but there was no significant difference between the leaf area of the plants grown in the 50% and 75% biosolids substrate (Table 6). The same was observed for the carrot plants, which peaked at 75% biosolids – 25% mine tailings substrate but drastically declined when 100% pure biosolids were used (Table 6). Leaf area was also significantly larger in the substrate-grown carrot plants than in the potting mix-grown plants. White lupin plants exhibited a reduced leaf area when grown in all of the substrates as compared with the control (Table 6). Overall, the presence of biosolids-amended mine tailings enhanced plant leaf area of Indian mustard and carrot but the use of pure biosolids was not beneficial.

Both Indian mustard and carrot showed increased shoot and root biomass as the amount of biosolids was increased from 0 to 75%, and declined sharply when grown in 100% biosolids (Table 6). Plants grown in the substrates generally had higher biomass than those grown in the control potting mix, indicating possible tolerance or coping mechanisms or some growth promoting effect of the biosolids-mine tailings mixtures. White lupin plants on the other hand had significantly lower shoot and root biomass when grown in the different substrates as compared to those grown in the potting mix (Table 6). No trend was observed between the different substrate combinations.

The percentage dry matter partitioning to roots (Fig. 2) is of interest as heavy metals mainly accumulate in roots or underground storage organs (Msuya et al., 2000; Anderson et al., 2005; Esteban et al., 2008; Shiyab et al., 2008; Lomonte et al., 2010a). In terms of biomass allocation to roots, there was no significant difference among the Indian mustard plants grown in the different substrates, ranging from 6-13% only (Fig. 2a). Dry matter partitioning to the roots of carrot plants was statistically significantly higher (95% confidence level) in the biosolids-amended mine tailings than in pure biosolids or mine tailings. For the plants grown in the 75% biosolids- 25% mine tailings combination (Fig. 2b) the partitioning was 63% which did not differ statistically (95% confidence level) from the partitioning in plants grown in the control potting mix. Lastly, white lupin plants grown in the substrate combinations allocated significantly more biomass to the roots compared to those grown in potting mix, with the exception of the plants grown in pure biosolids (Fig. 2c). In the plants grown in the 25% biosolids- 75% mine tailings combination the highest percentage of dry matter partitioned to roots was 24% only. This gives an indication of the possible efficiency of the roots of carrot plants for metals accumulation.

Table 6. Leaf area, shoot biomass, and root biomass of Indian mustard, carrot, and white lupin plants grown in the biosolids-amended mine tailings. Data were evaluated using a one-way ANOVA with post-hoc analysis. Within a plant species and for each measurement, treatments with the same letter are not statistically different from each other. (**PM** = potting mix, **BS** = biosolids; **MT** - mine tailings; s.e. – standard error).

Plant species	**Substrate**	**Leaf area (cm^2)**	**s.e.**	**Shoot biomass (g)**	**s.e.**	**Root biomass (g)**	**s.e.**
Indian mustard	PM	270.71^{b}	21.87	1.30^{d}	0.75	0.20^{b}	0.11
	0% BS+100% MT	267.69^{b}	27.05	0.90^{e}	0.52	0.06^{c}	0.04
	25% BS+75% MT	449.61^{b}	44.18	2.26^{c}	1.30	0.21^{b}	0.12
	50% BS+50% MT	1180.39^{a}	17.61	2.83^{b}	1.64	0.23^{b}	0.13
	75% BS+25% MT	1165.68^{a}	17.17	3.99^{a}	2.30	0.52^{a}	0.30
	100% BS+0% MT	121.03^{c}	21.87	0.38^{e}	0.22	0.05^{c}	0.03
N		3		5		5	
p-value		< 0.05		< 0.05		< 0.05	
Fisher's LSD		81.8		0.35		0.16	
Carrot	PM	307.78^{d}	20.11	1.51^{d}	0.87	3.45^{d}	1.99
	0% BS+100% MT	287.62^{d}	1.31	3.17^{c}	1.83	2.06^{e}	1.19
	25% BS+75% MT	659.69^{c}	29.34	4.52^{b}	2.61	6.63^{c}	3.83
	50% BS+50% MT	792.31^{b}	8.37	6.24^{a}	3.60	9.85^{b}	5.69
	75% BS+25% MT	1030.25^{a}	11.90	6.58^{a}	3.80	11.58^{a}	6.69
	100% BS+0% MT	328.07^{d}	16.15	1.81^{d}	1.04	2.21^{e}	1.28
N		3		5		5	
p-value		< 0.05		0		0	
Fisher's LSD		52.5		0.47		0.72	
White lupin	**PM**	958.38^{a}	48.80	3.40^{a}	1.52	0.22^{b}	0.10
	0% BS+100% MT	253.25^{e}	233.54	1.31^{c}	0.59	0.26^{b}	0.12
	25% BS+75% MT	371.75^{c,d}	27.31	1.59^{b,c}	0.71	0.48^{a}	0.22
	50% BS+50% MT	303.04^{d,e}	75.51	2.03^{b}	0.91	0.43^{a}	0.19
	75% BS+25% MT	527.38^{b}	17.20	2.22^{b}	0.99	0.22^{b}	0.10
	100% BS+0% MT	439.75^{b,c}	42.38	2.99^{a}	1.34	0.10^{c}	0.05
N		3		5		5	
p-value		< 0.05		< 0.05		< 0.05	
Fisher's LSD		93.5		0.43		0.09	

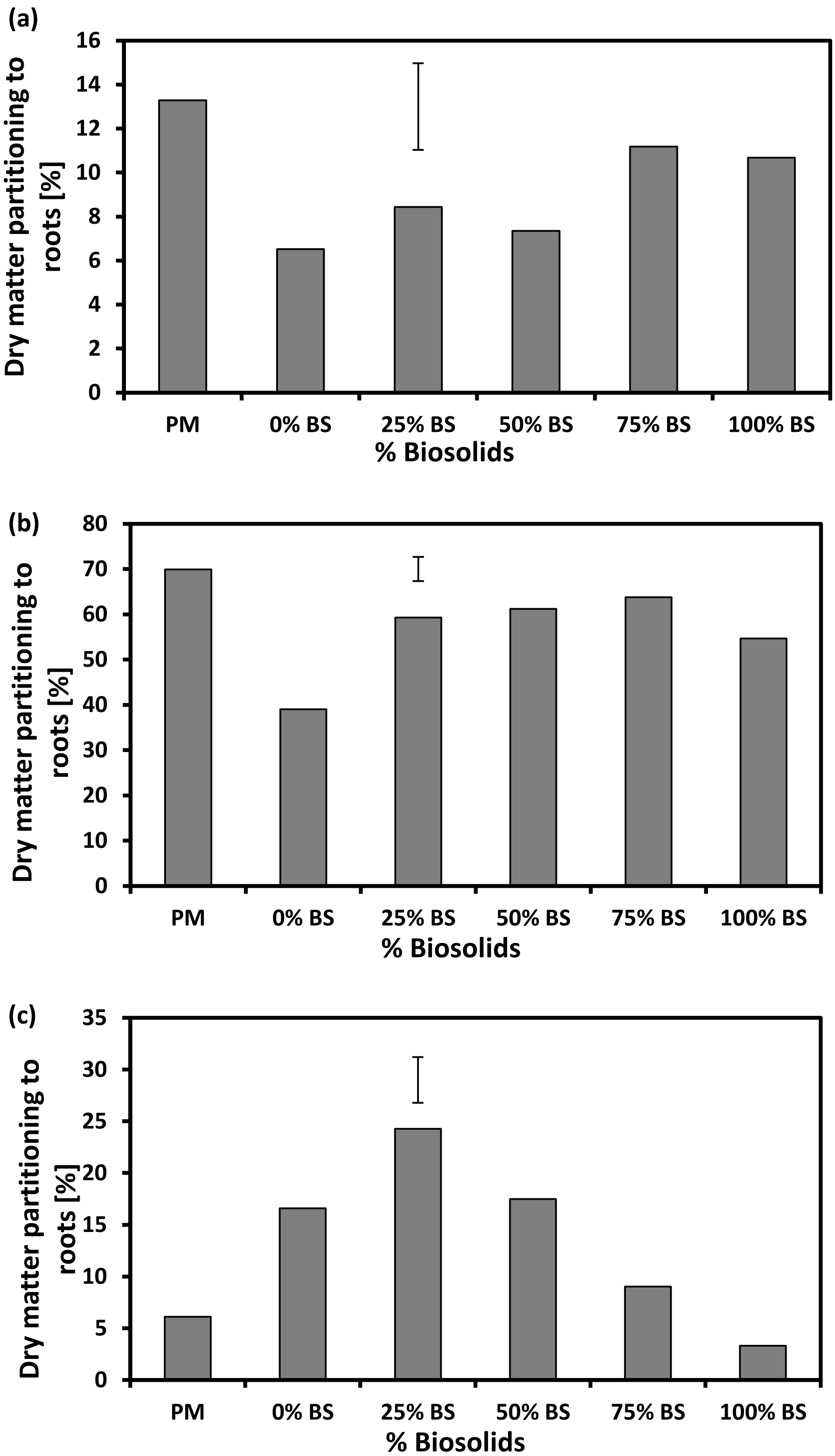

Fig. 2. Percentage dry matter partitioning to roots in **(a)** Indian mustard, **(b)** carrot, and **(c)** white lupin plants grown in the biosolids-amended mine tailings. One-way ANOVA was used to determine the p-value where $p < 0.05$ was considered significant. The Fisher LSD bar is shown for multiple comparisons (**PM** = potting mix, **BS** = biosolids).

4. Conclusions

The results of this study demonstrated that Indian mustard and carrot could be successfully established in amended with biosolids mine tailings. Overall, the most suitable biosolids-mine tailings combination was determined to be 75% biosolids - 25% mine tailings, wherein most of the growth parameters did not differ significantly from those of the plants grown in the control potting mix. The experimental results demonstrated that the addition of biosolids to mine tailings improved the physicochemical properties of the substrate in terms of it better supporting plant growth and development. It is likely that the addition of the biosolids provided a number of benefits including, but not limited to, an increase in organic matter content, improved water infiltration rate and increased concentration of essential macro- and micronutrients. These results indicate that future phytoextraction studies with the selected plant species Indian mustard and carrot should use the 75% biosolids - 25% mine tailings combination to maximize plant growth, and potentially also maximize Hg and Au phytoextraction.

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