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Phytotoxicity of biosolids and screening of selected plant species with potential for mercury phytoextraction

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ABSTRACT

Mercury contaminated stockpiles of biosolids (3.5–8.4 mg kg^{-1} Hg) from Melbourne Water's Western Treatment Plant (MW-WTP) were investigated to evaluate the possibility for their phytoremediation. Nine plant species (*Atriplex codonocarpa*, *Atriplex semibaccata*, *Austrodanthonia caespitosa*, *Brassica juncea*, *Brassica napus*, *Gypsophila paniculata*, *Sorghum bicolor*, *Themeda triandra* and *Trifolium subterraneum*) were screened for phytoextraction potential in Hg-contaminated biosolids from MW-WTP. In addition, the same plant species were germinated and grown in two other substrates (*i.e.* potting mix and potting mix spiked with mercury(II)). Growth measurements and the mercury uptake for all three substrates were compared. Some plant species grown in potting mix spiked with mercury(II) grew more vigorously than in the other two substrates and showed higher levels of sulphur in their tissues. These results suggested that the mercury stress activated defence mechanisms and it was hypothesised that this was the likely reason for the enhanced production of sulphur compounds in the plant species studied which stimulated their growth. Some species did not grow in biosolids because of the combined effect of high mercury toxicity and high salt content. *Atriplex conodocarpa* and *Australodanthonia caespitose* proved to be the most suitable candidates for mercury phytoextraction because of their ability to translocate mercury from roots to the above-ground tissues.

1. Introduction

Biosolids (sewage sludge) are the stabilized organic residues from domestic and industrial wastewater treatment [1]. To avoid environmental and economic costs of disposal and taking up space in landfills, reuse of biosolids has been advocated. Typical biosolids are rich in N, P, macro- and micro-nutrients, and organic matter, thus making them suitable for land applications where it is expected to increase the soil organic content and improve soil stability, porosity and water filtration rates [2]. Biosolids can also be used in power generation, either directly or after conversion to methane [3,4]. The manufacture of bricks and cement allows both safe disposal and reuse of biosolids with high levels of contaminants [5]. Currently, the main use of biosolids is as fertilizers or composts in land applications to improve and maintain soil productivity, stimulate plant growth and establish sustainable vegetation at mine sites [6]. This is not only beneficial to agriculture but also eliminates disposal costs [7]. However, there are environmental and public health concerns related to the biosolids applications. These are mainly related to the presence of pathogenic microorganisms and hazardous compounds [8]. Heavy metals and metalloids are of particular concern as they are frequently present at elevated concentrations in biosolids. Their high toxicity and potential mobility can result in surface and groundwater contamination. In addition, heavy metals do not decompose in the environment as organic contaminants do and they can be translocated into plants and further transferred into animal and human food chains [9,7]. Among the heavy metals frequently present in biosolids, mercury is arguably of the highest environmental and public health concern. This is due to its extremely high toxicity in both organic and inorganic forms and to its ability to bioaccumulate, thus further increasing the risks to exposure even at trace levels [10].

Since mercury is one of the most toxic heavy metals, mitigation of its effects is required. Phytoremediation is a technology which utilises plants to remove or make innocuous pollutants and it is the most innovative and environment-friendly technique [11]. However, mercury has a very limited solubility in soil, low availability for plant uptake and does not have any known biological function [12]. This may explain why Hg-hyperaccumulating plant has not been identified yet. An approach involving the use of thiol containing ligands to induce Hg accumulation in

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Phaseolus vulgaris, *Brassica juncea* and *Vicia villosa* [13,14] has been proposed as a potential strategy for the removal of Hg from contaminated sites and for increasing the translocation of this metal to the above-ground plant tissues. Uptake of Hg has been shown in mosses, lichens, fungi and in wetland, woody and crop plants [15]. Other studies on the phytoremediation of mercury in contaminated soils have been reported using different plant species such as saltbush (*Atriplex canescens*) [16], *Rumex induratus* and *Marrubium vulgare* [17], lupin [11,16], wheat [18], pea [12,19], sorghum [15], vetiver grass [20], an aquatic fern [21], and rice [22]. Few studies however, have been carried out on biosolids phytoremediation. Investigations are therefore needed to understand the uptake, translocation and fate of Hg during biosolids phytoremediation.

Melbourne produces about 925 million litres of sewage per day. About 92% of Melbourne's sewage is treated at two treatment plants: the Western Treatment Plant in Werribee and the Eastern Treatment Plant in Bangholme. The network of main sewers transfers sewage from the retail interface points by gravity and pumping to the two treatment plants. Around 10% of flows are divertible between the two treatment plants and this capability is used to optimise system performance and costs. The remaining sewage is treated by local treatment plants, which are owned and operated by the retail water companies. Melbourne Water Company is owned by the Victorian Government and manages and operates both the Western and Eastern Treatment Plants [23].

Melbourne Water's Western Treatment Plant (MW-WTP) has an important place in the history of Melbourne Water, being the first project of the Melbourne and Metropolitan Board of Works following the creation of the Board in 1891. MW-WTP is now the largest sewage treatment facility in Australia producing 175,000 t $year^{-1}$ of dry biosolids and covering 10,823 ha in the past 100 years, 6950 ha of which are utilised for sewage treatment [23].

The MW-WTP serves about 1.6 million people in the inner northern and western suburbs of Melbourne, north and west of the Yarra River and around Hobsons Bay and treats about 52% of Melbourne's domestic sewage and 70% of Melbourne's industrial waste. Sewage is transported from domestic and industrial sources to Melbourne's treatment plants via pipes and pumping stations. On average, the WTP processes about 500 million litres of sewage (66% of the State's sewage) a day before discharging treated and not recycled effluent into Port Phillip Bay under an EPA (Environmental Protection Authority) Victoria licence agreement [23].

Three natural sewage processes were historically used to produce effluent that consistently met EPA Victoria licence requirements. They were lagoon treatment, land filtration in the summer months (land filtration bays are grazed by cattle and sheep all year round) and grass filtration in the winter months (grass filtration paddocks are grazed in the summer months). Since 2004 these three methods were completely replaced by enhanced lagoon treatment technology to meet EPA Victoria requirements. This follows installation of covers on the initial ponds of these lagoon systems and construction of an effluent reuse delivery scheme. Consequently, raw sewage is no longer applied directly to the land, significantly reducing greenhouse gas emissions, and removing a significant amount of nitrogen in effluent treated at the plant, and therefore decreasing nitrogen flowing into the Bay. Furthermore, the land that is freed up by the cessation of raw sewage treatment is now mainly irrigated with the treated effluent from the lagoons. This effectively reduces discharges of effluent to Port Phillip Bay by reusing the effluent for irrigation purposes [23].

Biosolid stockpiles from the MW-WTP exhibit varying degrees of mercury contamination, with the older sludge generally containing higher levels of Hg. The main sources of mercury in these biosolids derive from the chlor-alkali industry, where Hg is used as the cathode in the electrolysis of salt, and from combustion processes using fossil fuels, mercury thermometers, mercurial disinfectants and dental Hg amalgam fillings.

The present paper reports on a comparative study of the mercury phytoextraction potential of 9 plant species grown in biosolids from MW-WTP. The plant Hg uptake and growth rate in Hg-contaminated biosolids were compared to the results obtained in potting mix with and without Hg spiking.

2. Experimental

2.1. Reagents

Mercury stock solution (1000 mg L^{-1} Hg) was prepared by dissolving $HgCl_2$ (Ajax Finechem, Australia) in 0.1 M HCl (BDH, England) and kept in a sealed container at 4 °C. Sulphur stock solution (1000 mg L^{-1}) was purchased from Choice Analytical Pty Ltd., Australia. Diluted $HgCl_2$ and S solutions were made up daily by appropriate dilutions of the corresponding stock solution. Calibration solutions (5–40 μg L^{-1} Hg) of $HgCl_2$ or (5–100 mg kg^{-1}) of S were prepared daily by diluting the corresponding stock solution and 5 mL of digestion reagent consisting of reverse *aqua regia* and H_2O_2 (Chem-Supply, Australia) in 5:1 ratio [24] to 100 mL using deionized water. The mercury solution used for spiking the potting mix was made by diluting the Hg stock solution to 260 mg L^{-1} Hg in deionized water. All reagents were of analytical reagent grade and were used as received. Deionized water (18 MΩ cm, Millipore, Synergy 185, France) was used for the preparation of all solutions.

Reverse *aqua regia* was prepared by mixing concentrated nitric acid (Merck Pty, Australia) and hydrochloric acid in 3:1 ratio.

The washing solution used to rinse plant material prior to digestion in concentrated nitric acid was prepared by dissolving 2 mL Triton X-100 detergent (Bran + Luebbe, Australia) in 1 L deionized water.

2.2. Biosolids collection and preparation of substrates

The upper layer (<20 cm) of biosolids aged between 2 and 30 years was sampled in 6 different areas of the MW-WTP (A–F) (Fig. 1). Fresh samples from each location were collected randomly, crushed, sieved through a 2 mm mesh sieve, homogenized by a soil mixer, and stored in polypropylene containers at 4 °C prior to measurements of pH, conductivity and mercury concentration.

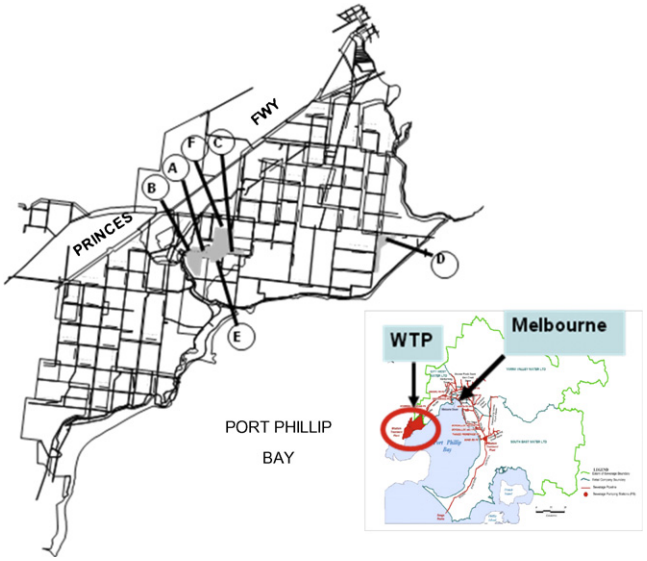

Fig. 1. Sampling points of biosolids stockpiles from MW-WTP (A–F).

Table 1
Hg concentrations (mg kg^{-1}), pH, conductivity and moisture content of biosolids samples from six locations of MW-WTP (A–F).

Sample	Hg concentration ± SD[a] (mg kg^{-1})	pH ± SD[a]	Conductivity ± SD[a] (mS cm^{-1})	Moisture ± SD[a] (%)
A	8.4 ± 1.2	4.6 ± 0.2	0.8 ± 0.3	3.8 ± 0.7
B	8.1 ± 0.3	4.7 ± 0.1	2.4 ± 0.3	3.0 ± 0.3
C	4.4 ± 0.3	5.8 ± 0.2	5.9 ± 0.2	4.1 ± 1.3
D	3.8 ± 0.1	5.3 ± 0.1	2.3 ± 0.3	6.9 ± 1.2
E	3.5 ± 0.2	5.7 ± 0.4	0.7 ± 0.3	10.4 ± 1.2
F	4.5 ± 0.3	5.0 ± 0.2	2.8 ± 0.4	16.4 ± 2.7

[a] Standard deviation.

Mercury-spiked potting mix was prepared by spraying 0.7 kg of dry standard potting mix (Seed raising mix, Debco, Australia) located in a barrel with 50 mL of the $HgCl_2$ spiking solution (260 mg L^{-1} Hg). $HgCl_2$ was chosen as the Hg source because of its high water solubility. The barrel was then closed and tumbled for 5 min to homogenize the Hg-spiked potting mix which was then transferred to 1.3 L plastic pots (140 mm in diameter) for the phytoremediation pilot study. The calculated Hg concentration in the spiked potting mix was 17.3 mg kg^{-1}. Because of its high porosity and hydrophobicity, the biosolids used in the germination test or the phytoremediation pilot study were sprayed with 50 mL soil-wetting agent solution to improve the penetration of water. This solution was prepared by dissolving 15 mL of concentrated soil-wetting agent (Easy Wetta, Brunnings, Australia) in 9 L deionized water. For consistency, all potting mixes studied were also sprayed with the same wetting agent.

2.3. Biosolids characteristics

The Hg concentrations, pH, conductivity and moisture content of biosolids from A–F locations are summarised in Table 1 [25]. The organic matter content of the biosolids samples ranged between 36 and 44% (w/w). These samples also contained Cl^-, SO_4^{2-}, nitrate N, orthophosphate P, K, Ca and Mg in the range 70–1117, 358–7493, 388–3290, 174–288 mg kg^{-1}, and 5.8–15.8, 1.8–3.3, 1.6–3.9 g kg^{-1}, respectively [25]. The concentrations of heavy metals such as Cd, Pb, Cu, Mn, Co, Ni, Fe and Zn were in the following ranges: 14.1–22.4, 311–1398, 596–1025, 44.7–169, 9.67–14.8, 104–158 mg kg^{-1}, and 11.5–21.5, 1.36–1.91 g kg^{-1}, respectively. In addition, a microbial activity test using fluorescein diacetate showed that the concentration of fluorescein released was in the range 14.4–23.3 μg g^{-1}. The particle size distribution of the biosolids is summarised in Table 2. All measurements were conducted in triplicate and the results regarding the concentrations of Hg and other elements have been reported on a dry weight basis.

2.4. Germination test

A germination test was carried out for 13 d in Petri dishes. Fifty seeds of 'Iceberg' lettuce or 'Scarlet Globe' radish were germinated in a Petri dish containing 100 g of potting mix (control) or biosolids from the six different locations (A–F) of MW-WTP studied. This test was carried out in a glasshouse with a climate regime of 24/23 °C and 14/10 h photoperiod. The temperature during the night was 23 °C. Each substrate was tested in triplicate. The seeds were moistened with tap water each day and those that had germinated were counted and removed.

Table 2
Particle size distribution (PSD) of biosolids from MW-WTP.

PSD fraction	Fraction PSD range	Percentage (%) Mud soil
Clay fraction	Particle size ≤ 2 μm	0
Silt fraction	2 μm < particle size ≤ 75 μm	17.5
Sand fraction	75 μm < particle size ≤ 2 mm	32.5
Gravel fraction	2 mm < particle size	50.0

2.5. Plant growth trial

The suitability of the plant species studied for growth in this type of biosolids was determined on the basis of their tolerance to: high salinity, high Hg concentration and a dryland climate with high summer temperatures [11,13,14,22]. On the basis of these criteria the following plant species were selected for further testing: *Atriplex codonocarpa*, *Atriplex semibaccata*, *Austrodanthonia caespitosa*, *B. juncea* (cv. 426308), *Brassica napus* (cv. 'Mounty'), *Gypsophila paniculata*, *Sorghum bicolor*, *Trifolium subterraneum* and *Themeda triandra*. *B. juncea*, *B. napus*, *G. paniculata* and *T. subterraneum* [26] are enriched in S compounds which may be expected to act as Hg-binders [27]. These plants are also high biomass crop species. *A. codonocarpa* and *A. semibaccata* are salt-tolerant species [28], while *Au. caespitosa* and *Th. triandra* are native Australian drought-resistant grasses [29,30].

Twenty-five seeds per species were sown into pots filled with potting mix, Hg-spiked potting mix or biosolids from location A. These biosolids were chosen because they had the highest Hg concentration among the biosolids from the six locations studied and seeds were fully capable of germination as reported in Section 3.2. Seeds of the two *Atriplex* species were removed from their bracteoles and placed under running water overnight to release their salt content to facilitate the germination, and then sown [31,32].

To ensure a similar level of nutrition, the potting mix (with and without Hg spiking) was fertilized with 5 g kg^{-1} of Osmocote Plus (NPK slow-release fertilizer, Scotts Australia Pty Ltd.). The fertilizer contained 7.5% NH_4–N, 8.5% NO_3–N and 3.5% available PO_4. As a result of the application of this fertilizer the potting mix with or without Hg spiking contained 800 mg kg^{-1} N (as NH_4–N and NO_3–N) and 175 mg kg^{-1} P (as PO_4). Plants were watered daily to maintain field capacity. Dishes were placed under each pot in order to collect any leachate which was subsequently reapplied to the corresponding pots. The trial was set up in autumn in a glasshouse on the university campus at an ambient temperature of 24/23 °C day/night and 14 h photoperiod. One week after germination, each pot was thinned to leave six individual plants. Pot positions were randomized on a periodic basis (2 weeks) to equalize light exposure. All plants were harvested after 60 d from germination.

2.6. Chemical analysis

Conductivity and pH of all three substrates were determined after extraction in deionized water in 1:5 biosolids:water mass ratio (w/v) [33] by a combined pH-conductivity meter (SmartChem-Lab, TPS, Australia).

The concentrations of Hg in biosolids and potting mixes (with and without Hg spiking) were determined after digestion of 0.4 g of fresh material (AIM500 digestion block, A.I. Scientific, Australia) in 12 mL reverse *aqua regia*–H_2O_2 (5:1 ratio) [24].

Mercury concentrations were measured in roots, stems and leaves of *B. juncea*, *B. napus*, *S. bicolor* and *T. subterraneum* and

in roots and shoots of *A. codonocarpa*, *A. semibaccata*, *Au. caespitosa*, *G. paniculata* and *Th. triandra*. All samples were acid digested after 2–5 d from harvesting. Concentrated nitric acid (5 mL) was used in the digestion of the corresponding plant material (0.2 g) [16] after rinsing with diluted Triton solution and drying at 60 °C for 48 h. This temperature was chosen to minimize Hg volatilization according to previous studies [14,34]. A preliminary test comparing the results for Hg in fresh and dried (60 °C) plant material confirmed these findings. Blank digests were analyzed for possible mercury contamination during the acid digestion procedure.

Prior to analysis, the substrate samples were filtered (Whatman No. 40 filter paper) and the digests diluted to 200 mL with deionized water. Plant digests were centrifuged at 14,500 rpm for 7 min and evaporated to 0.5 mL on a 60 °C hotplate. The digests were subsequently diluted to 10 mL with deionized water to decrease the acidity prior to the analytical measurement. The Hg concentrations in the digests of the three substrates were measured by atomic fluorescence spectrometry (Millennium Merlin, PSA, England, detection limit 0.6 $\mu g\,L^{-1}$ Hg), while in plant material they were determined by inductively coupled plasma-mass spectrometry, ICP-MS (ULTRAMASS 700, Varian, Australia Pty Ltd., detection limit 0.4 $\mu g\,L^{-1}$ Hg). Sulphur concentrations in plant material were measured in the same digests prepared for Hg determination by inductively coupled plasma optical emission spectrometry, ICP-OES (Varian Vista AX CCD, Australia Pty Ltd.). All measurements were conducted using the calibration curve method and were reported on a dry weight basis (60 °C). The calibration curves used in the quantitative analytical measurements were periodically checked with analyte standards. The reproducibility of the analytical methodologies was characterized by the standard deviation of the measurements.

2.7. Plant growth measurements

Plant growth measurements (biomass, number of leaves and leaf area) were carried out for all plants grown in each substrate. Leaf area was measured using a portable area meter (LI-3000A, LI-COR, Australia). The 10 largest leaves of each species were used in measuring the leaf area. In addition, the total number of leaves of each species in each pot was counted.

Biomass was measured in roots, stems and shoots of *B. juncea*, *B. napus*, *S. bicolor*, and *T. subterraneum* and in roots and shoots of *A. codonocarpa*, *A. semibaccata*, *Au. caespitosa*, *G. paniculata* and *Th. triandra*. All biomass measurements were conducted on plant material dried at 60 °C for 48 h.

2.8. Statistical analysis

The experiment was conducted in a block randomized design to test for the effects for Hg phytoextraction, S concentrations, biomass, leaf area and number of leaves of the plant species studied. Two randomized factors were plant species (*A. codonocarpa*, *A. semibaccata*, *Au. caespitosa*, *B. juncea*, *B. napus*, *G. paniculata*, *S. bicolor*, *T. subterraneum* and *Th. triandra*) and treatments (Hg-free potting mix, Hg-spiked potting mix and biosolids). Results (means ± SD) were presented as the means from three replicates of three independent experiments except for the determinations of the leaf area. All leaf area measurements were conducted considering the 10 largest leaves of each species in three independent experiments. The significance of differences among means was determined by one-way ANOVA. Comparisons among means were performed using Duncan's test ($P \leq 0.05$).

3. Results and discussion

3.1. Mercury analysis, conductivity and pH measurements

The Hg concentrations in biosolids from A to F locations of MW-WTP (Table 1) were indicative of Hg contamination, in particular at sites A ($8.4 \pm 1.2\,mg\,kg^{-1}$) and B ($8.1 \pm 0.3\,mg\,kg^{-1}$) where the Hg concentration was higher than the regulatory safety level for land applications ($5\,mg\,kg^{-1}$, Australian and Victorian State Biosolids Guidelines). Although the Hg concentrations in the remaining four biosolids sites were slightly below this safety level, their re-use is not recommended. The Hg concentration in the potting mix was found to be $0.3 \pm 0.2\,mg\,kg^{-1}$.

The concentration of Hg in spiked potting mix was determined as $17.3 \pm 0.4\,mg\,kg^{-1}$ 2 weeks after spiking. This value agreed well with the theoretically calculated concentration of $17.3\,mg\,kg^{-1}$. All biosolids had high salinity (measured as conductivity, Table 1), in particular biosolids from location C ($5.9 \pm 0.2\,mS\,cm^{-1}$). In contrast, the conductivities of potting mix and Hg-spiked potting mix were 0.12 ± 0.02 and $0.15 \pm 0.01\,mS\,cm^{-1}$, respectively. All substrates were acidic and the pH of both potting mix and Hg-spiked potting mix was found to be 5.3 ± 0.1.

3.2. Germination test

The results of the germination test (Fig. 2) with 'Iceberg' lettuce and 'Scarlet Globe' radish showed that seeds germinated more readily in biosolids substrates from locations A and E. In these two substrates the Hg concentration was 8.4 and $3.5\,mg\,kg^{-1}$, respectively. These were the biosolids with the highest and lowest Hg concentrations. At the same time these were the substrates with the lowest salinity. The pH and P concentration in all substrates had similar values. The N concentration varied with salinity. Poor germination was observed at high salinity and therefore it was concluded that salinity was most likely the main factor affecting the

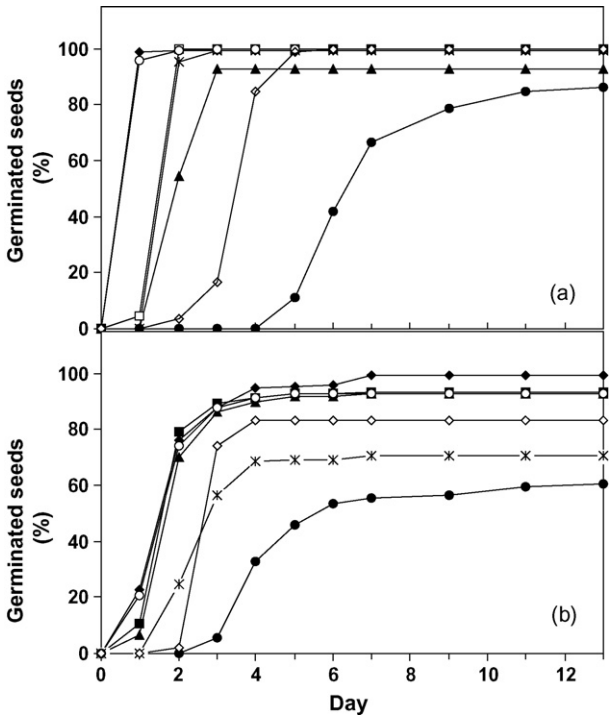

Fig. 2. Germination test results based on the number of germinated seeds of 'Iceberg' lettuce (a) and 'Scarlet Globe' radish (b) in the control (♦) and biosolids (■) A, (▲) B, (○) E, (●) C, (*) D, (◊) F.

germination process. This conclusion was also supported by the fact the slowest and most incomplete seed germination was observed in biosolids from location C which was characterized by the highest salinity.

3.3. Plant growth and mercury uptake

All plants germinated in all three treatment substrates. While all species grew in potting mix and Hg-spiked potting mix, *B. juncea*, *B. napus* and *G. paniculata* could not tolerate the high concentration of salts in the biosolids. After 2 weeks of germination in biosolids both *Brassica* spp. showed symptoms of salt stress, *i.e.* the plants produced fewer leaves which appeared dehydrated. *G. paniculata* plants died within the first 10 d of germination while all *B. juncea* and *B. napus* plants did not survive the first 4 weeks. As the pH, N and P concentrations in biosolids from location A and potting mix with or without Hg spiking had similar values [25] and the Hg concentration in spiked potting mix was statistically significantly higher (twice) than that in biosolids, the likely explanation for the poorer plant survival and growth in biosolids was thought to be the higher salinity of the biosolids. The effect of salinity on plant growth is well known and it is considered a major abiotic stress facing plant agriculture worldwide [35]. High salt stress disrupts homeostasis in water potential and ion distribution. This disruption of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death [35].

As observed by other authors [22], Hg was found to bioaccumulate mainly in root tissues, with very little translocation to the above-ground tissues of all plants tested (Table 3) in all substrates. *A. codonocarpa*, *A. semibaccata* and *Au. caespitosa* grew slowly in biosolids and consequently they did not produce enough root biomass to allow the detection of Hg uptake after acid digestion. Low Hg concentrations were measured in the roots of *T. subterraneum* and *S. bicolor* grown in potting mix but there was no translocation to the stems and leaves. By contrast, *B. juncea*, *B. napus*, *A. semibaccata*, and *Au. caespitosa* had detectable Hg concentrations in leaves for the first two species and in shoots for the other two. It is possible that the two *Brassica* species have taken up Hg from the atmosphere [36]. The same two species also accumulated the highest amount of Hg in their roots when grown in Hg-spiked potting mix but only a small fraction of this amount was translocated to stems and leaves. However, in terms of translocation, *A. codonocarpa* grown in Hg-spiked potting mix showed the highest translocation index (Ti = 19.0%), calculated as a percentage of the ratio of Hg located in the above-ground tissues and the total (roots + shoots) Hg in the plant. The Ti values of *A. semibaccata*, *Au. caespitosa*, *B. juncea*, *B. napus*, *G. paniculata*, *T. subterraneum*, and *Th. triandra* grown in Hg-spiked potting mix were 6.3, 15.9, 0.1, 0.1, 13.5, 2.9 and 4.1%, respectively. Ti could not be determined for *S. bicolor* due to Hg in its stems and leaves being below detection levels. The Ti of plants grown in biosolids could be determined only for *T. subterraneum* (Ti = 8.1%) and *Th. triandra* (Ti = 13.2%) because of the very low Hg concentration in the other species. Further, *A. codonocarpa* and *A. semibaccata* showed the highest Hg concentrations in shoots (Table 3) despite their slow growth in biosolids. Based on the results outlined above it was concluded that *A. codonocarpa* could be a suitable candidate for Hg phytoremediation studies.

The root biomass of all plant species grown in biosolids was significantly smaller compared to plants grown in Hg-spiked potting mix. This explains the lower Hg uptake in plants grown in biosolids in comparison to those grown in Hg-spiked potting mix. However, it should be pointed out that the stress endured by plants in biosolids was also due to the high salinity content and not only to the presence of mercury as in the case of Hg-spiked potting mix where the conductivity was about five times lower than that in biosolids from location A. The high salinity in the biosolids studied reduced the Hg plant uptake. Similar results regarding the effect of salinity in the substrate were observed by other authors [26].

3.4. Plant growth measurements and observations

Because of the complexity of the biosolids matrix, to evaluate only the Hg effect on the species studied, plants grown in Hg-free potting mix were compared with those grown in Hg-spiked potting mix. The only difference between these two substrates was the presence of mercury in the Hg-spiked potting mix. The data for biomass (Fig. 3), leaf area (Fig. 4a) and number of leaves (Fig. 4b) showed that some of the plants studied (*B. juncea*, *B. napus*, *A. codonocarpa*, *A. semibaccata*, *T. subterraneum* and *Th. Triandra*) grew more vigorously in Hg-spiked potting mix than in the original potting mix. This was also shown by the fact that after 60 d from germination, 30% of *B. napus* had flowered in Hg-spiked potting mix in comparison to only 11% of the plants grown in Hg-free potting mix.

Table 3
Hg concentration (mg kg^{-1}) in plant tissues (measurements conducted by ICP-MS).

Species	Hg concentration ± SD[a] (mg kg^{-1})								
	Substrate: potting mix			Substrate: Hg-spiked potting mix			Substrate: biosolids from location A		
	Roots	Stems	Leaves	Roots	Stems	Leaves	Roots	Stems	Leaves
B. juncea	BDL[b]	BDL[b]	$(9.7 \pm 1.4) \times 10^{-3}$	32.3 ± 13.9	$(10.1 \pm 7.3) \times 10^{-3}$	$(25.9 \pm 10.8) \times 10^{-3}$	Not grown		
B. napus	BDL[b]	BDL[b]	$(4.2 \pm 3.0) \times 10^{-3}$	30.2 ± 5.3	$(11.9 \pm 4.2) \times 10^{-3}$	$(26.8 \pm 10.9) \times 10^{-3}$	Not grown		
S. bicolor	$(9.9 \pm 9.1) \times 10^{-3}$	BDL[b]	BDL[b]	1.44 ± 0.26	BDL[b]	BDL[b]	0.12 ± 0.02	BDL[b]	BDL[b]
T. subterraneum	$(14.2 \pm 3.35) \times 10^{-3}$	BDL[b]	BDL[b]	1.69 ± 0.51	$(23.3 \pm 7.3) \times 10^{-3}$	$(27.8 \pm 6.9)10^{-3}$	0.49 ± 0.02	BDL[b]	$(42.6 \pm 36.9) \times 10^{-3}$

Species	Hg concentration ± SD[a] (mg kg^{-1})					
	Substrate: potting mix		Substrate: Hg-spiked potting mix		Substrate: biosolids from location A	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Au. caespitosa	BDL[b]	$(14.2 \pm 3.6)10 \times ^{-3}$	1.01 ± 0.05	0.19 ± 0.07	ND[c]	$(57.3 \pm 25.9) \times 10^{-3}$
A. codonocarpa	BDL[b]	BDL[b]	0.87 ± 0.06	0.20 ± 0.07	ND[c]	$(74.3 \pm 1.1) \times 10^{-3}$
A. semibaccata	BDL[b]	$(9.9 \pm 3.3) \times 10^{-3}$	3.34 ± 0.13	0.22 ± 0.06	ND[c]	$(80.3 \pm 6.6) \times 10^{-3}$
G. paniculata	BDL[b]	BDL[b]	0.45 ± 0.02	$(70.1 \pm 11.9) \times 10^{-3}$	Not grown	
Th. triandra	BDL[b]	BDL[b]	1.61 ± 0.10	$(69.9 \pm 10.3) \times 10^{-3}$	0.16 ± 0.06	$(24.7 \pm 3.5) \times 10^{-3}$

[a] Standard deviation.
[b] BDL: below detection limit.
[c] ND: not detectable because of insufficient material for Hg digestion.

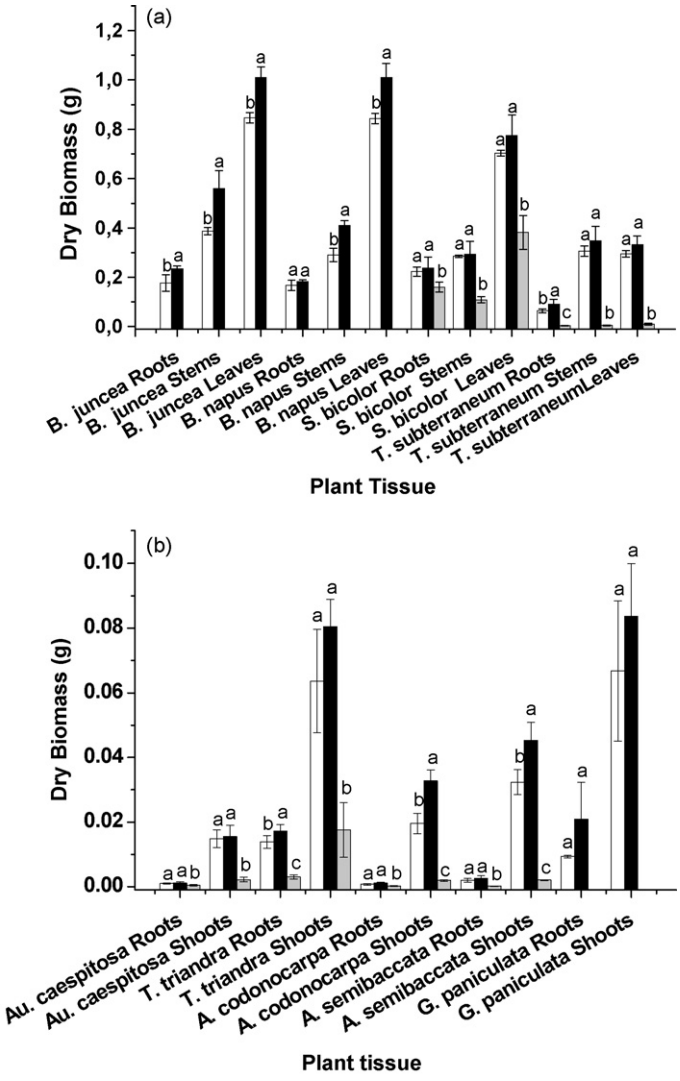

Fig. 3. Dry biomass of (a) *B. juncea*, *B. napus*, *S. bicolor* and *T. subterraneum*; and (b) *Au. caespitosa*, *A. codonocarpa*, *A. semibaccata*, *G. paniculata* and *Th. triandra* grown in potting mix (□), Hg-spiked potting mix (■) and biosolids (▩). Means followed by different letters are significantly different by ANOVA ($P \leq 0.05$).

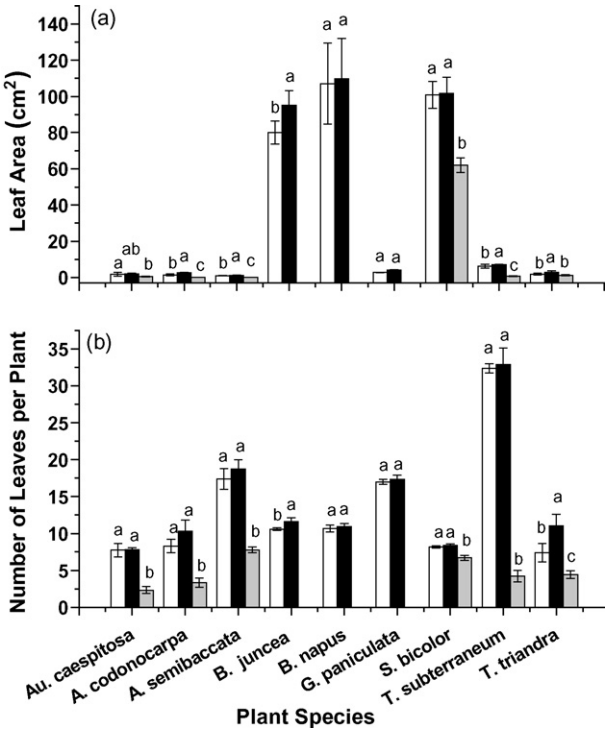

Fig. 4. Leaf area (cm^2) (a) and number of leaves per plant (b) of *Au. caespitosa*, *A. codonocarpa*, *A. semibaccata*, *B. juncea*, *B. napus*, *G. paniculata*, *S. bicolor*, *T. subterraneum* and *Th. triandra* grown in potting mix (□), Hg-spiked potting mix (■) and biosolids (▩). Means followed by different letters are significantly different by ANOVA ($P \leq 0.05$).

In addition, the concentrations of S (Table 4) in the above-ground tissues of *B. juncea*, *B. napus*, *A. codonocarpa* and *A. semibaccata* grown in Hg-spiked potting mix was significantly higher (Table 5) than those grown in Hg-free potting mix. A possible correlation between the biomass increase and the sulphur content was hypothesised in *B. juncea*, *B. napus*, *A. codonocarpa* and *A. semibaccata* grown in Hg-spiked potting mix in comparison with those grown in Hg-free potting mix. Sulphur is located predominantly in the thiol groups of plant proteins (*e.g.*, cysteine and methionine residues) which affect the protein structure, conformation and functionality [37]. Sulphur is also required for the synthesis of other compounds,

Table 4
Sulphur concentration ($g\,kg^{-1}$) in plant tissues (measurements conducted by ICP-OES).

Species	S concentration ± SD[a] ($g\,kg^{-1}$)								
	Substrate: potting mix			Substrate: Hg-spiked potting mix			Substrate: biosolids from location A		
	Roots	Stems	Leaves	Roots	Stems	Leaves	Roots	Stems	Leaves
B. juncea	5.13 ± 0.58	4.98 ± 0.56	8.82 ± 2.28	6.40 ± 0.93	8.40 ± 0.87	16.1 ± 1.3	Not grown		
B. napus	5.27 ± 1.47	4.90 ± 0.84	7.82 ± 0.86	7.09 ± 0.04	6.06 ± 0.89	16.3 ± 2.0	Not grown		
S. bicolor	4.68 ± 0.33	2.80 ± 0.29	2.24 ± 0.43	4.94 ± 0.97	2.91 ± 0.28	2.31 ± 0.35	7.65 ± 0.49	5.58 ± 0.87	4.08 ± 0.06
T. subterraneum	6.91 ± 0.76	2.90 ± 0.26	8.23 ± 2.26	7.11 ± 1.74	4.43 ± 1.67	8.29 ± 1.61	4.34 ± 0.09	8.20 ± 0.54	10.9 ± 0.4

Species	S concentration ± SD[a] ($g\,kg^{-1}$)					
	Substrate: potting mix		Substrate: Hg-spiked potting mix		Substrate: biosolids from location A	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Au. caespitosa	5.73 ± 0.51	6.78 ± 2.15	6.01 ± 0.89	10.2 ± 0.5	ND[b]	14.0 ± 0.8
A. codonocarpa	6.87 ± 0.06	5.20 ± 0.57	6.99 ± 0.49	9.63 ± 1.41	ND[b]	14.3 ± 0.8
A. semibaccata	3.06 ± 0.12	5.09 ± 0.25	3.48 ± 0.09	6.66 ± 0.33	ND[b]	12.0 ± 0.7
G. paniculata	4.20 ± 0.28	6.14 ± 0.63	7.25 ± 0.61	9.33 ± 1.80	Not grown	
Th. triandra	3.76 ± 0.64	2.96 ± 0.09	4.73 ± 0.74	3.23 ± 0.77	6.56 ± 0.67	6.20 ± 0.47

[a] Standard deviation.
[b] ND: not detectable because of insufficient material for digestion.

Table 5
Statistically significant difference in the S concentration of plant tissues corresponding to the three substrates studied.

Species	Roots			Stems			Leaves		
	Potting mix	Hg-spiked potting mix	Biosolids (location A)	Potting mix	Hg-spiked potting mix	Biosolids (location A)	Potting mix	Hg-spiked potting mix	Biosolids (location A)
B. juncea	a	a	Not grown	b	a	Not grown	b	a	Not grown
B. napus	a	a	Not grown	b	a	Not grown	b	a	Not grown
S. bicolor	b	b	a	b	b	a	b	b	a
T. subterraneum	a	a	b	b	b	a	b	b	a

Species	Roots			Shoots		
	Potting mix	Hg-spiked potting mix	Biosolids (location A)	Potting mix	Hg-spiked potting mix	Biosolids (location A)
Au. caespitosa	a	a	ND[a]	c	b	a
A. codonocarpa	a	a	ND[a]	c	b	a
A. semibaccata	b	a	ND[a]	c	b	a
G. paniculata	b	a	Not grown	a	a	Not grown
Th. triandra	b	b	a	b	b	a

[a] ND: not detectable because of insufficient material for digestion.

such as thiols (*e.g.*, glutathione, GSH), sulpholipids and secondary S compounds (*e.g.*, alliins, glucosinolates, phytochelatins) which play an important role in plant metabolism and in protection and adaptation of plants to stress [38]. In response to excess heavy metals such as Hg and salinity, plants activate inducible detoxification mechanisms, *e.g.*, they induce non-protein thiols, such as GSH and phytochelatins [38,39]. Higher GSH concentrations in foliar tissues of plants exposed to environmental stress have been interpreted as an acclimation response, which strengthens the antioxidative defence system [40].

It has been demonstrated that metals can be toxic to plants if their accumulation exceeds the detoxification capacity of the plant tissue [41]. In this study the Hg concentrations in the spiked potting mix did not prove toxic to all species. As the pH values of both potting mix with and without Hg spiking were comparable and the leachate water did not show any detectable Hg, it was supposed that the presence of Hg in the in Hg-spiked potting mix activated in some plants (*e.g.*, *B. juncea*, *B. napus*, *A. codonocarpa* and *A. semibaccata*) resistance mechanisms producing more S to react to the Hg stress. However, because the stress intensity was not too high it was hypothesised that the overall result was a stimulation of plant growth compared to that in potting mix without Hg spiking. In favour of this suggestion is the fact that S, as a component of amino acids, *e.g.*, methionine and cysteine, is a requisite for protein synthesis necessary for plant growth [42]. A strong correlation between S concentration in plant tissue and biomass increase has been found by other authors [43]. Further, it has been shown that biomass increase can be induced by non-toxic concentrations of Hg [44] and other heavy metals, *e.g.*, Pb [45].

However, if the induced metabolic responses are insufficient or the stress intensity is too high, the plant is expected to deteriorate [39]. This effect was observed with plants grown in biosolids. All species underwent a higher intensity stress because of the combined effect of high Hg concentration and high salinity. This explains the slow growth process and the stress symptoms observed. In addition, in *Brassica* species and *G. paniculata*, the resistance mechanisms were not strong enough to allow plant survival. All species tested also showed necrosis of leaf margins which is a symptom of salt-burn.

T. subterraneum nodulated normally when grown in Hg-free potting mix. However, *Rhizobia* in the nodules of legumes are known to be sensitive to metals [20] and consequently *Trifolium* roots grown in Hg-spiked potting mix and biosolids were not effective in N-fixation. *S. bicolor* grown in biosolids soon developed a red pigmentation in the leaf sheaths, which indicated nutritional stress.

4. Conclusions

This study demonstrated the possibility of mercury phytoremediation of the biosolids from MW-WPT which could allow its subsequent re-use for land applications. The phytotoxicity of biosolids was assessed by a germination test involving 'Iceberg' lettuce and 'Scarlet Globe' radish seeds and it showed that here salinity played the most important role for the germination process, delaying or inhibiting it.

Atriplex conodocarpa showed the highest potential for Hg phytoremediation in terms of the highest translocation index and its capability of tolerating the high salt content in the biosolids studied, despite its slow growth. However, *Au. caespitosa* exhibited also a high translocation factor in comparison with the other species studied. It has the advantage of producing a relatively high biomass because it can be cut several times per year thus further increasing the amount of mercury phytoextracted.

In addition, some plant species grown in potting mix spiked with mercury grew more vigorously than in the other two substrates suggesting that the Hg stress activated defence mechanisms and this was a possible explanation for the enhanced production of S compounds in the plant species studied. Further studies on the correlation between S concentration in plant tissue and biomass increase induced by Hg are currently been undertaken in our laboratory.

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