The potential of four legume trees for mercury phytoremediation and the role of arbuscular mycorrhizal fungi

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Abstract

Artisanal and small-scale gold mining (ASGM) in low- and middle-income countries often lack adequate safety measures, leading to significant health risks and environmental mercury pollution. Phytoremediation, a plant-based method that utilizes plants to accumulate soil-borne contaminants such as heavy metals, has been verified to restore land for ecosystem services or even future farming. Therefore, this study evaluates the potential of four legume species typically found in Ghana, the world's second largest gold exporter - *Acacia mangium*, *Gliricidia sepium*, *Leucaena leucocephala* and *Senna siamea* - for the removal of mercury from contaminated soils, as well as potential trade-offs related to eco-physiological processes. It was further investigated whether arbuscular mycorrhizal fungi (AMF) inoculation could enhance mercury removal capacity. Predominantly, *A. mangium* consistently exhibited the highest mercury uptake and did not show signs of mercury toxicity. *G. sepium* showed moderate mercury uptake but suffered considerable physiological damage. *L. leucocephala* was resistant to mercury but accumulated only small amounts. *S. siamea* exhibited moderate mercury accumulation without physiological impairment. AMF inoculation did not significantly increase mercury uptake but appeared to mitigate physiological stress under mercury exposure. These results indicate that reforestation of abandoned gold mines with *A. mangium* may be a suitable starting point for phytoremediation of mercury and inoculation with AMF can provide additional protection against mercury toxicity.

Introduction

Artisanal and small-scale gold mining (ASGM) is the largest anthropogenic source of mercury pollution, which is caused by the amalgamation process used in gold extraction (Donkor et al. 2009; Boateng 2014). It was estimated that these mining activities emitted 838 tons of mercury to air in 2015, accounting for 38% of global anthropogenic mercury emissions (UNEP 2019). Mercury amalgamation is the preferred method of almost all ASGM activities because it is simple and inexpensive (Donkor et al. 2009, Boateng et al. 2014). In addition to mercury contamination, gold mining drastically degrades soil health by removing the topsoil, which is rich in nutrients and organic matter (Aryee et al. 2003). Furthermore, the removal of vegetation has the effect of disrupting the flow of ecosystem services and matter fluxes, as well as increasing the risk of soil erosion (Schueler et al. 2011). What remains are “lunar” landscapes with rubbish dumps, abandoned excavations and vast, barren stretches of land (Aryee et al. 2003).

In Ghana, where gold mining is a predominant economic activity, the repercussions are particularly pronounced. In 2022, gold accounted for 48% of total exports in Ghana (OEC 2024). But mining, with its environmental impact, directly competes with agriculture, which was the largest employment sector for the Ghanaian people at 35.3% in 2022 (FAO 2024). The loss of farmland due to mining-related changes often forces farmers to move to new locations. As a result, farmers clear forests to create new farmland, indicating spillover effects of mining on neighboring areas (Schueler et al. 2011).

Contaminated soils can be remediated using chemical, physical or biological techniques. Chemical and physical treatments may irreversibly alter soil properties, destroy biodiversity, and are costly (Padmavathiamma and Li 2007). Since contaminated areas are extensive and funding is limited, a cost-effective biological soil remediation technique is needed to remove mercury without compromising soil fertility (Mertens et al. 2004). Phytoremediation, a process that uses plants to remove, transfer, stabilize or degrade

contaminants in soil, is environmentally friendly and cost-effective (Hughes et al. 1997; Padmavathiamma and Li 2007). Such phytoremediation techniques include phytoextraction, phytostabilization, phytovolatilization, phytofiltration, and phytodegradation (Alkorta et al. 2004). Phytoextraction (also known as phytoaccumulation) is the uptake of contaminants from soil by plant roots and their translocation and accumulation in the aboveground biomass (Yoon et al. 2006). Translocation of metal ions into shoots is a crucial biochemical process and desirable for effective phytoextraction, as harvesting root biomass is difficult. Phytovolatilization describes a process whereby heavy metals are absorbed and transpired by plants (Yoon et al. 2006). Suitable plants for the phytoextraction of mercury should be tolerant to high mercury concentrations and should accumulate high concentrations of mercury in their harvestable above-ground tissues. Moreover, they should have a rapid growth rate with particularly high production of above-ground biomass, develop a well-branched root system, be adapted to the prevailing environmental and climatic conditions and should be easy to cultivate and harvest (Garbisu and Alkorta 2001; Ali et al. 2013).

Plants adapted to thrive in heavy metal-rich soils, known as ´metallophytes´, are categorized into metal excluders, metal indicators, and metal accumulators (Ali et al. 2013; Baker 1981). Metal excluders store heavy metals in their roots but restrict their transport to aerial parts, maintaining low metal concentrations in their shoots (Baker 1981). Metal indicators accumulate heavy metals in their aerial parts and generally reflect heavy metal concentrations in the substrate (Sheoran et al. 2011). Metal accumulators are preferred for phytoremediation since they gather high concentrations of metals in their above-ground biomass. This leads to efficient removal of contaminants from the environment (Baker 1981). The efficacy of metal accumulator plants can be assessed by the bioaccumulation factor (BAF) calculated as the heavy metal concentrations in plant shoots divided by the heavy metal concentrations in corresponding soils (Liu et al. 2020). Further, a high translocation factor (TF) calculated as heavy metal concentrations in plant shoots divided by the heavy metal concentrations in plant roots, is another key parameter (Xun et al. 2017). Ideal metal accumulators do not show high reduction in plant biomass when plants are grown in soils contaminated with heavy metals. This can be evaluated by the tolerance index (TI) calculated as the biomass of treated plants divided by the biomass of control plants (Diwan et al. 2010). Although over the past 20 years many scientists from different countries have investigated more than 200 plant species for their ability to accumulate and translocate mercury, no accumulator has been identified yet (Liu et al. 2020).

Generally, mercury is toxic for plants, it inhibits photosynthesis, nutrient uptake, nutrient transportaffecting plant growth and biomass production (Lenti et al. 2002; Mei et al. 2021). To counteract those negative effects, plants may be inoculated with plant strengthening agents such as arbuscular mycorrhizal fungi AMF (Ferrol et al. 2016; Pirsarandib et al. 2022). Several studies have reported that certain AMF species, like *Glomus mosseae*, enhance the transport of heavy metals into the aboveground plant biomass (Weissenhorn et al. 1995; Singh et al. 2019). However, other studies have shown that some AMF species, like *Funneliformis mosseae*, inhibit the translocation of heavy metals to the aboveground biomass (Shabani et al. 2016; Chamba et al. 2017; Salazar et al. 2018). This was explained by the observation that some heavy metals are bound to fungal hyphae, arbuscules, vesicles or vacuoles, which inhibits the transport of heavy metals to the plant tissue (Garg and Singh 2018; Motaharpoor et al. 2019).

The use of nitrogen-fixing legumes in the remediation of mercury-contaminated soils has the added benefit of increasing the biomass of subsequent plant species and thus the biomass production of the whole plant

community (Frérot et al. 2006). The four legume tree species, *Acacia mangium* Willd, *Gliricidia sepium* Jacq, *Senna siamea* Lam (synonym *Cassia siamea*) and *Leucaena leucocephala* Lam, were selected for their rapid growth and ability to establish and survive on degraded land in Ghana (Tetteh et al. 2015; Festin et al. 2019; Kusumaningtyas et al. 2021). In addition, *A. mangium*, *G. sepium*, and *L. leucocephala* are nitrogen-fixing tree species, making them ideal for reforestation of degraded sites (Macedo et al. 2008; Tetteh et al. 2015).

While the majority of studies focus on the phytoextraction of heavy metals by grasses and herbs, this work is dedicated to tropical trees, which could potentially produce a higher biomass and consequently remove a larger amount of mercury (Liu et al. 2020). This study aims to investigate the phytoremediation potential of four tree species from Ghana in mercury-contaminated soils within gold mining areas. The optimal candidate should accumulate a high concentration of mercury in the above-ground biomass while remaining unharmed (Alkorta et al. 2004). Specifically, the objectives are: (1) to assess the capacity of these species to accumulate mercury in their biomass, particularly in above-ground tissues, under varying levels of soil contamination; (2) to evaluate the physiological responses of these species to mercury exposure, including growth rates and photosynthesis; and (3) to explore the influence of AMF on the effectiveness of mercury uptake and resistance to mercury toxicity. Through this research, we identify and characterize species optimal for reforestation and ecological restoration of areas degraded by mercury pollution.

Materials and Methods

- Plant cultivation

To prepare the seedlings, the dormancy of the seeds of *A. mangium* (Am), *L. leucocephala* (Ll) and *S. siamea* (Ss) was broken by scarification of the seed coat, acid, or hot water treatment. *G. sepium* (Gs) did not require a pretreatment to break seed dormancy. After pretreatment, seeds were soaked in water for 24 h. Seeds were placed in germination trays and exposed to a 12/12 h light/dark cycle at temperatures of 25/20°C in a climate chamber for 11 days. Subsequently, seedlings were transplanted into planting trays and placed in a greenhouse. A mixture of sand and vermiculite (5:3 by volume) was used as substrate. Prior to use, sand and vermiculite were autoclaved at 120°C for 15 minutes and then completely dried at 80°C. Greenhouse conditions were maintained at 26°C with an average relative humidity of 56%. To provide artificial illumination, high-pressure sodium lamps were utilized (SONT Agro 400W; Philips, Amsterdam, Netherlands) with a photoperiod of 12 hours (PAR, 210–270 µmol m^{-2} s^{-1}).

- Arbuscular mycorrhizal fungi (AMF)

The AMF strain *Rhizophagus irregularis* Błaszk., Wubet, Renker & Buscot, 2009 QS81 was supplied by INOQ GmbH (Schnega, Germany). The inoculum of *R. irregularis* was prepared from arbuscular mycorrhizal root fragments of *Trifolium pratense* grown in sand: vermiculite (35:65 by volume). The inoculum contained 100 million propagules per kg of powder (as vesicles and spores according to INOQ GmbH).

- Experimental design

Two consecutive greenhouse experiments were carried out. The greenhouse conditions were as described above. In the first experiment, the effect of a lower (12.5 mg kg^{-1}) and a higher (25.0 mg kg^{-1}) mercury

concentration in the substrate on the tree species was investigated. This experiment is referred to as the ‘mercury concentration experiment’. Based on the results of the ‘mercury concentration experiment’ experiment, the higher mercury concentration was chosen for the second experiment, in which AMF was grown with only one mercury concentration. This experiment is referred to as the "mercury-AMF experiment".

Mercury-concentration experiment: Seedlings were transplanted into their final pots 11 weeks after germination pretreatment. To prevent mercury leaching from drainage water, 1 L plastic buckets without holes in the bottom were used. The substrate was a mixture of sand and vermiculite (19:1 by weight) with the addition of 1 g kg^{-1} controlled-release fertilizer (Osmocote® Exact Mini 3-4M, NPK 15:3.9:9.1 + 1.2 Mg + trace elements, ICL, Netherlands). Prior to use, sand and vermiculite were autoclaved at 120°C for 15 minutes and then completely dried at 80°C. Of the species *A. mangium*, *G. sepium*, and *S. siamea*, 15 plants were included in the experiment. Due to poor germination rate 9 plants of the species *L. leucocephala* were included. Arrangement of pots was a randomized block design with five blocks. Each block had three plants of each species; *L. leucocephala* had only three blocks. Of these three plants, one served as a control, one was treated with 12.5 mg Hg kg^{-1} (Hg 12.5) and one with 25.0 mg Hg kg^{-1} (Hg 25.0). Mercury was applied in the form of a mercury chloride ($HgCl_2$) solution, with the solution volume adjusted to achieve a final mercury concentration in the substrate of either 12.5 mg kg^{-1} or 25 mg kg^{-1}14 weeks after germination. Eight weeks after mercury treatment, plants were harvested and separated into roots, stems, and leaves. Roots were shortly washed three times in deionized water. Plant samples were air dried at room temperature for dry weight determination.

Mercury-AMF experiment: Six weeks after germination, plants were divided into control (x) and AMF-inoculated (AMF) groups and transplanted into 1.0 L pots with 750 g of substrate. The substrate was a mixture of sand and vermiculite (19:1 by weight) with the addition of 0.5 g kg^{-1} controlled-release fertilizer (Osmocote® Exact Mini 3-4M, NPK 15:3.9:9.1 + 1.2 Mg + trace elements, ICL, Netherlands). For the AMF-treated plants, the substrate was mixed with 0.075 g AMF inoculum (a ratio of 0.1 g kg^{-1}). A total of 64 plants were arranged in a complete randomized block design with four blocks. A three factorial design was used, with the factors of mercury concentrations (0 and 25 mg Hg kg^{-1}) and AMF (presence or absence) studied across the four tree species. Twelve weeks after germination 5 mg Hg kg^{-1} (3.75 mg pot^{-1}) was applied as a mercuric chloride ($HgCl_2$) solution every week for a period of 5 weeks to reach a final concentration of 25 mg Hg kg^{-1}. Six weeks after the first mercury application, the plants were harvested as mentioned in the “mercury-concentration experiment” above. A subsample of the roots from the AMF-treated plants was stored at -80°C to determine AMF root colonization. For this, roots were cut into 1 cm lengths and cleared with 10% NaOH in a 70°C water bath for 45 min and then soaked in 1% HCl for 1 min at room temperature. Roots were stained with 2% Parker Quink blue ink (Yon et al. 2015) in a 70°C water bath for 30 min. The roots were rinsed with tap water and then stored in a lactoglycerol solution (lactic acid, glycerol, H_2O in a ratio of 1:1:1). Thirty fragments from each plant were randomly selected, placed on a microscope slide, and examined under a light microscope for mycorrhizal colonization (Trouvelot et al. 1986).

- Mercury concentration

Plant samples were ground and homogenized using a mill with zirconium oxide grinding balls (MM40, Retsch GmbH, Haan, Germany). Subsequently, 0.2 g of the homogenized material were moistened with 1 mL of deionized H_2O and digested in 2.5 mL of 69% HNO_3 in a microwave-heated UltraCLAVE III digestion unit (MLS-

MWS GmbH, Leutkirch, Germany). One mL of the microwave pressure digestion solution was added to 1 mL of dilution solution (2% cysteine, 7% isopropanol made up to 1000 mL with 1% HNO_3) and 0.1 mL of 50 ppb rhodium standard solution and then made up to 10 mL with ddH_2O. The mercury concentration was analyzed using a NexION 300 × inductively coupled plasma mass spectrometer (PerkinElmer LAS GmbH, Rodgau, Germany).

Gas exchange and biomass determination

All plants were analyzed weekly for their stem height using a scale and defined as the height from the substrate surface to the top of the main shoot.

The gas exchange (CO_2 and H_2O) of the youngest fully developed leaf was determined weekly (GFS-3000, Heinz Walz GmbH; 3010-S standard measuring head with 3040-L LED light source, head area of 2.5 cm^2). The area of leaves that did not fill the measuring head was determined using ImageJ.

The Tolerance Index (TI) was calculated in this work using the following equations (Diwan, Ahmad, and Iqbal 2010). TI values above 1 indicate a net increase in biomass and imply that the plants have developed heavy metal tolerance, while TI values below 1 indicate a net decrease in biomass and a stressed state of the plants.

$$TI = \frac{Biomass\ of\ treated\ plants\ \left(g\ plant^{-1}\right)}{Biomass\ of\ control\ plants\ \left(g\ plant^{-1}\right)}$$

The Bioaccumulation Factor (BAF) was calculated using the following equation (Liu et al., 2020). The initial Hg concentration in the substrate (12.5 or 25.0 mg Hg kg^{-1}) was used to calculate the BAF.

$$BAF = \frac{Hg\ concentration\ in\ plant\ shoot}{Hg\ concentration\ in\ substrate}$$

The Translocation Factor (TF), *i.e*. the ability of a plant to translocate metal ions from the roots to the shoots, was calculated as follows:

$$TF = \frac{Hg\ concentration\ in\ plant\ shoot}{Hg\ concentration\ in\ plant\ root}$$

Statistical analysis

Mercury concentration, assimilation rate, stem height, biomass, TI, BAF, TF, and AMF were analyzed using ANOVA with SAS software 9.4 (SAS Institute, Cary NC, USA) with proc mixed. Least squares means were calculated for comparison between control and treated plants. The significance level was set at $p \leq 0.05$. Model assumptions such as normality, homoscedasticity, and independence were verified, when necessary log-transformation was applied, and *post hoc* comparisons were conducted using the least significant difference (LSD) test to ensure accurate interpretation of the differences.

Results

- Mercury accumulation

Our analysis of mercury accumulation across different plant components (roots, stems and leaves), shows that the highest concentrations consistently occurred in the root system, irrespective of the species studied (Fig. 1). Mercury accumulation in the trees followed the treatment with lowest concentrations at the 12.5 Hg treatment and highest at the 25 Hg treatment (Fig. 1b). The 12.5 Hg treatment did not lead to high Hg accumulation in the leaves, stems, or roots, except for the stems of *A. mangium*, which showed high Hg accumulation (Fig. 1b). The 25 Hg treatment showed accumulation in all tree species. In the leaves, highest Hg concentration was detected in *G. sepium* with 95.8 mg kg^{-1} and in *A. mangium* with 77.0 mg Hg kg^{-1}. Stems of *A. mangium* (445 mg Hg kg^{-1}) and *G. sepium* (360.3 mg Hg kg^{-1}) accumulated highest mercury concentrations. *L. leucocephala* accumulated the lowest mercury concentration in both leaves and stem. Mercury accumulation in the above-ground biomass has a clear pattern: Am > Gs > Ss > Ll (Fig. 1b). Consideration of the increase factor of mercury accumulation shows that a doubling of mercury treatment leads to a multiple increase in mercury accumulation (Table 1). This phenomenon is particularly evident in the stems, with *A. mangium* exhibiting a 49-fold higher Hg accumulation in Hg 25.0 than in Hg 12.5.

Table 1
Increase factor of mercury accumulation when comparing Hg 25.0 and Hg 12.5 in leaves, stems, and roots of *A. mangium*, *G. sepium*, *L. leucocephala*, and *S. siamea*.

	A. mangium	***G. sepium***	***L. leucocephala***	***S. siamea***
Leaves	4	7	2	3
Stem	49	33	5	20
Root	2	2	2	1

- Plant physiological responses to mercury accumulation

Table 2 displays the total dry weight, mean assimilation rate, and stem growth for each tree species. *G. sepium* showed significant and particularly pronounced reduction in assimilation rate, stem height, and total dry weight with increasing mercury treatment. *A. mangium* showed a significant decrease in assimilation rate and stem height, but total dry weight remained unchanged. *L. leucocephala* and *S. siamea* appeared resistant to mercury effects on assimilation, stem height, and dry weight, with stable assimilation rates higher mercury concentrations.

Table 2
Total dry weight, mean assimilation rate per plant species and stem growth over the mercury treatment period (8 w). Significant differences of treatment effects indicated by different letters (LSD, $p \leq 0.05$). Statistical analysis was performed individually for each tree species.

Species	Mercury-Treatment (mg Hg kg^{-1})	Mean Assimilation ($\mu mol\ m^2\ s^1$)		Stem Growth (cm)		Total Dry Weight (g per plant)	
	0.0	11.1 (± 0.7)	a	11.9 (± 1.7)	a	3.31 (± 0.5)	a
A. mangium	12.5	8.9 (± 0.6)	ab	11.6 (± 1.8)	a	3.91 (± 0.3)	a
	25.0	7.9 (± 1.9)	b	6.6 (± 2.1)	b	3.19 (± 0.9)	a
	0.0	5.1 (± 0.1)	a	9.3 (± 0.5)	a	7.88 (± 0.2)	a
G. sepium	12.5	3.6 (± 0.8)	ab	5.4 (± 1.4)	ab	5.62 (± 0.9)	b
	25.0	1.5 (± 0.8)	b	2.4 (± 1.7)	b	4.54 (± 0.9)	b
	0.0	5.5 (± 0.5)	a	26.4 (± 2.5)	a	11.35 (± 0.2)	a
L. leucocephala	12.5	5.5 (± 1.0)	a	24.4 (± 3.3)	a	10.01 (± 0.6)	a
	25.0	6.4 (± 0.3)	a	21.7 (± 3.4)	a	9.46 (± 1.2)	a
	0.0	6.8 (± 1.0)	a	6.6 (± 0.6)	a	6.23 (± 0.6)	a
S. siamea	12.5	7.0 (± 0.6)	a	5.9 (± 0.6)	a	6.80 (± 0.2)	a
	25.0	7.6 (± 1.0)	a	4.9 (± 0.4)	a	6.77 (± 1.5)	a

- Phytoremediation assessment indicators

The tolerance index (TI), calculated based on dry weight, was determined for each plant species across different treatments (Table 3). *A. mangium* exhibited a trend in dry weight accumulation at Hg 12.5 and a slight decline at Hg 25 but without statistical significance. The resulting tolerance index exceeding 1 indicated resilience to the medium mercury treatment, while a value slightly below 1 suggested a stress response at high mercury treatment. Furthermore, the 'TI mean' for *A. mangium* was 1.07 and with this the highest value among the four tree species indicating highest mercury tolerance. The dry mass of *S. siamea* was relatively unaffected at Hg 25 or increased at Hg 12.5 compared to control (Table 2). Therefore, the resulting TI values were in the range of 1 for both mercury treatments suggesting no harming effect of mercury on *S. siamea*. *G. sepium* exhibited the most adverse reaction to the presence of mercury, as indicated by reduced total dry weight (Table 2) and high TF of 35.4 (Table 3). The 'TI mean' of *L. leucocephala* was 0.86, which indicated a medium resistance range compared to the other plant species (Table 3).

None of the tree species had a mercury translocation factor (TF) greater than 1.0 (Table 3). For *A. mangium* and *S. siamea*, the differences in TF between treatments were highly significant ($p < 0.001$, data not shown) with highest TF values compared to the other tree species.

The bioaccumulation factor (BAF) is a metric used to calculate the accumulation of mercury in above-ground biomass (shoot) relative to the concentration of mercury in the substrate (Table 3). Except for L. leucocephala, the BAF increased with increasing mercury treatment (Table 3). Notably, the highest values were observed for *A. mangium* and *G. sepium* (Table 3).

Table 3
Translocation factor (TF), bioaccumulation factor (BAF) and tolerance index (TI) per plant species and mercury treatment. BAF mean and TI mean represent the mean value per species averaged over the levels of the mercury treatment. Significant differences of mercury treatment effects are indicated by different letters (LSD, $p \leq 0.05$).

Species	Mercury-treatment ($mg\ kg^{-1}$)	Translocation factor (TF)		Bioaccumulation factor (BAF)		BAF mean		Tolerance index (TI)		TI mean	
	0.0	-						-			
A. mangium	12.5	0.014	a	1.32	b	3.45	a	1.18	a	1.07	a
	25.0	0.106	b	5.57	a			0.96	ac		
	0.0	-						-			
G. sepium	12.5	0.043	a	0.97	b	4.12	a	0.71	bc	0.65	b
	25.0	0.354	b	7.27	a			0.58	c		
	0.0	-						-			
L. leucocephala	12.5	0.024	a	0.47	a	0.54	b	0.88	ac	0.86	ab
	25.0	0.028	a	0.61	a			0.83	ac		
	0.0	-						-			
S. siamea	12.5	0.027	a	0.97	a	3.39	a	1.09	ab	1.04	a
	25.0	0.087	b	2.24	a			1.09	ab		

- Mycorrhizal colonization

A. mangium and *G. sepium* had a successful inoculation with a high frequency of mycorrhizae in the root system without significant differences between control and mercury treatment (Fig. 2). However, *L. leucocephala* showed a very low mycorrhizal colonization rate in the control while the mercury treated plants showed high inoculation capacity. The roots of *S. siamea* were too thick and heavily pigmented to be analyzed for mycorrhizal colonization under the light microscope according to the method of Trouvelot et al. (1986).

- Mercury accumulation depending on arbuscular mycorrhizal fungi

The highest mercury concentrations in aboveground biomass were found in leaves with 202 mg kg^{-1} and in stem with 203 mg kg^{-1} of AMF-treated *A. mangium* (Fig. 2). However, inoculation with AMF showed no significant effect on mercury accumulation in any of the tree species. Mercury accumulation in *G. sepium* and *L. leucocephala* was low in all plant tissues and seemed to be further reduced by inoculation with AMF, although without significant differences. The trends of mercury accumulation in the 'mercury-concentration experiment' were confirmed in the 'mercury-AMF experiment'. The highest mercury concentration was always detected in *A. mangium*, the lowest in *L. leucocephala*.

- Physiological responses to mercury and arbuscular mycorrhizal fungi

The Mercury-AMF experiment aimed to assess different plant physiological parameters such as photosynthesis as indicated by the assimilation rate at week 6 (Table 4). The assimilation of *A. mangium* was significantly reduced by mercury and was increased by inoculation with AMF. The assimilation of *G. sepium* was also significantly reduced by mercury. However, *G. sepium* plants inoculated with arbuscular mycorrhizal fungi (AMF) exhibited a reduction in assimilation due to mercury treatment, though this decrease was not statistically significant. (Table 4). *L. leucocephala* had no significant change in assimilation by either mercury or AMF. In contrast, *S. siamea* had a significant reduction in assimilation in response to mercury, this was mitigated by AMF. However, neither mercury nor AMF showed a significant effect on the total dry weight of *A. mangium* (Table 4).

The mercury treatment showed a significant negative effect on the total dry weight (DW) of *G. sepium* (Table 4), while the inoculation with AMF did not significantly change this reduction of dry weight. The total DW of *L. leucocephala* and *S. siamea* was not significantly affected by either mercury or AMF (Table 4). Regarding the tolerance towards mercury overall, there are no significant differences in TI-values when comparing all treatments and species (Table 4). The TI-values for *A. mangium* were below 1, irrespective of the AMF-inoculation. Species and treatments with TI above 1 were *G. sepium* inoculated with AMF, and *S. siamea* without AMF. The mercury accumulated calculated as BAF showed significant differences between the tree species (Table 4), but the AMF-inoculation did not show significant effects. The highest BAF was found in *A. mangium*, the lowest in *L. leucocephala*.

Table 4

Effect of arbuscular mycorrhizal fungi inoculation (AMF = AMF-inoculated; x = control) on mercury tolerance. Assimilation and total plant dry weight was evaluated 6 w after mercury application; bioaccumulation factor (BAF) and tolerance index (TI). Differences of significance are denoted by varying letters (LSD, $p \leq 0.05$). Statistical analysis was performed separately for each plant species for assimilation and total dry weight. TI and BAF compares all treatments with each other.

Species	Microorganism	Mercury-Treatment (mg Hg kg^{-1})	Assimilation ($\mu mol\ m^2\ s^1$)		Total dry weight (g)		Tolerance Index (TI)		Bioaccumulation factor (BAF)	
A. mangium	x	0.0	15.1 (± 2.8)	a	1.7	a	0.85	a		
	x	25.0	8.4 (± 2.5)	b	1.4	a			6.0 (± 1.0)	a
	AMF	0.0	18.5 (± 2.8)	a	1.4	a	0.68	a		
	AMF	25.0	11.6 (± 3.1)	ab	1.0	a			8.4 (± 1.7)	a
G. sepium	x	0.0	4.7 (± 0.7)	a	7.7	a	0.72	a		
	x	25.0	1.4 (± 0.7)	b	5.6	b			0.8 (± 0.1)	c
	AMF	0.0	4.1 (± 0.8)	ab	6.1	ab	1.15	a		
	AMF	25.0	2.8 (± 0.7)	ab	7.0	ab			0.6 (± 0.1)	cd
L. leucocephala	x	0.0	8.2 (± 1.2)	a	6.0	a	0.84	a		
	x	25.0	9.5 (± 1.3)	a	5.0	a			0.6 (± 0.2)	cd
	AMF	0.0	9.5 (± 1.6)	a	5.2	a	1.00	a		
	AMF	25.0	7.3 (± 1.3)	a	5.2	a			0.5 (± 0.1)	d
S. siamea	x	0.0	6.4 (± 0.6)	a	2.3	a	1.02	a		
	x	25.0	3.0 (± 0.5)	b	2.3	a			2.7 (± 0.8)	b
	AMF	0.0	8.7 (± 1.7)	a	2.8	a	0.91	a		
	AMF	25.0	5.4 (± 1.3)	ab	2.6	a			2.5 (± 1.2)	bc

Discussion

Given the universality of the amalgamation process, similar levels of pollution, as reported by Tomiyasu et al. (2023) may be anticipated in abandoned mining sites in Ghana and other areas exposed to gold mining including amalgamation with mercury. Accordingly, our study investigated the potential of four local leguminous tree species to remediate mercury-contaminated soils. Generally, mercury accumulation in plant tissues has a complex pattern, with roots consistently showing the highest concentrations (Fig. 1), as shown in other studies (Liu et al. 2017; Moreno et al. 2005). However, the accumulation of mercury in aboveground biomass is particularly important for phytoremediation (Ali et al. 2013). Our data revealed that specifically under high mercury treatment, *A. mangium* accumulated highest concentrations in above-ground biomass, closely followed by *G. sepium* (Fig. 1). Therefore, these two species are considered as mercury accumulators. *L. leucocephala* shows comparatively low values in the aboveground biomass (Fig. 1) and may be classified as an excluder (Baker 1981).

The increase in mercury accumulation in the aboveground biomass, especially at the high mercury concentration in soil is of particular interest. Although the mercury concentration in the substrate increased 2-fold (from Hg 12.5 to Hg 25), the concentration in the biomass of all four tree species increased up to 49-fold in stems of *A. mangium* and 7-fold in the leaves of *G. sepium* (Table 1). In the roots, however, 2-fold soil mercury increase led to a 2-fold increase in mercury accumulation - irrespective of the tree species (Table 1). This suggests a threshold above which mercury accumulation becomes more effective but also more harmful for above-ground plant tissue. According to Baker (1981) *A. mangium* and *G. sepium* showed the characteristics of metal accumulators.

- Physiological responses and suitability for phytoremediation

The ideal candidate suitable for phytoextraction should not only accumulate high concentrations of mercury in the aboveground biomass, but also remain unharmed (Alkorta et al. 2004). The high mercury-accumulating species *A. mangium* showed a reduction in assimilation and stem height but no reduction in total dry weight (Table 1). It could be suggested that mercury did not harm the plant but led to a more stunted habitus. On the contrary, *G. sepium* was severely affected; not only did assimilation and stem height decrease with increasing mercury treatment (Table 1), moreover, four out of 10 mercury-treated plants did not survive during the experiment (data not shown). Therefore, *G. sepium* is regarded as a susceptible tree species that is unsuitable for the phytoremediation of mercury-contaminated soils. The physiology of *L. leucocephala* was not affected by mercury; however, it did not absorb a significant amount of mercury (Table 1, Fig. 1). Thus, even though the plant can likely tolerate mercury contaminated soil, it would not qualify as an effective accumulator.

Metal remediation plant indicators have been developed to assist the comparability of different plant species for their phytoremediation potential. It is the main ambition to locate so-called hyperaccumulators, plants that accumulate heavy metals in their high yielding above-ground tissues to levels far above those found in the soil (Memon and Schröder, 2009). Characteristics of hyperaccumulators are high concentration thresholds for heavy metals in plant shoots (*e.g.* 10,000 mg kg^{-1} for zinc (Zn), 1000 mg kg^{-1} for nickel (Ni), and 100 mg kg^{-1} for cadmium (Cd)) (Baker 1981; Baker and Brooks 1989). No threshold levels have yet been set for mercury. Further, the bioaccumulation calculated as bioaccumulation factor (BAF) as well as the translocation factor (TF) should be greater than 1 (Liu et al. 2020; Xun et al. 2017). Finally, the plants should be extremely tolerant

to heavy metals, *i.e*. they should not show significant reduction in biomass as determined by the tolerance with a TI-value of > 1 (Diwan et al. 2010). Despite numerous studies conducted from various countries on over 200 plant species in the last two decades in search of a hyperaccumulator of mercury, none has been successfully identified so far (Liu et al. 2020). Only *Erato polymnioides* and a few other species were labeled as potential mercury hyperaccumulators (Chamba et al. 2017). However, these species are native to the rainforests of Bolivia, Colombia, Costa Rica, Ecuador, Panamá, and Peru—not to West Africa. Introducing non-native plant species can destabilize local ecosystems, as these species often lack natural predators or competitors, allowing them to spread rapidly and become invasive (Zizka et al. 2015). According to the definition, none of the four plant species meets all criteria. *G. sepium* accumulated high levels of mercury, resulting in comparatively high TF values, but suffered considerable damage and plant death due to its sensitivity to mercury, as evident from its low TI values and the reduction in assimilation (Table 2, 3). *L. leucocephala* had the lowest mercury accumulation in the aboveground biomass (Fig. 1) and showed the lowest mercury translocation rates of all species (Table 3). However, *L. leucocephala* did not respond to the mercury treatment in any way. Neither the stem growth, dry weight, nor the assimilation was affected (Table 2, 3, 4), suggesting that it can be classified as an excluder plant. *S. siamea* fulfilled the majority of the set requirements and demonstrated a TI above 1 and a BAF above 2 in both experiments. However, in comparison to *A. mangium*, *S. siamea* exhibited a reduced accumulation of mercury in both experiments, as well as a lower BAF (Table 3, 4, Fig. 1, 3). With regard to the accumulation of mercury, *A. mangium* is the species with the greatest potential for mercury phytoremediation, as it exhibits the highest concentration of mercury in plant tissues and the highest BAF in both experiments (Table 3, 4, Fig. 1. 3). It proved to be resistant to mercury exposure, with a TI greater than 1 in the mercury-concentration-Experiment (Diwan, Ahmad, and Iqbal 2010). Further, its BAF in the 25 Hg treatment was higher than in the 12.5 Hg treatment, corresponding to its increased mercury accumulation (Fig. 1, Table 3). Most importantly, *A. mangium* accumulated by far the highest concentration of mercury in above-ground tissue, even though not being a hyperaccumulator.

A. mangium could therefore be a suitable candidate for the phytoremediation of mercury-contaminated soils in Ghana. The findings of our study align with those of previous research, which have shown that *A. mangium* has the capacity to remediate soils contaminated with heavy metals (Sampanpanish 2018; Rosli et al. 2021; Couic et al. 2021). The concept of diverse forests for phytoremediation is well acknowledged and a fast-growing topic in land restoration research, as reviewed by Gómez et al. (2019). The proposal here is not to exploit the forests directly for short-term gains, but to carefully manage and monitor contamination levels over several decades so that the acacia forest and other organisms can establish a stable ecosystem that facilitates the natural decontamination of anthropogenic pollution. Therefore, *A. mangium* could be accompanied by *S. siamea*, which has also achieved promising results, and *L. leucocephala*, which is not a mercury accumulator but is a robust and fast-growing mercury excluder developing well in mercury-contaminated soils. *L. leucocephala* is a commonly utilized tree in the maintenance of agroforestry systems (Shelton 1998). As such, *L. leucocephala* could potentially contribute to the biodiversity of a remediating forest. Furthermore, previous studies have shown that *S. siamea*, in combination with other plants, provides significant benefits for soil remediation by accumulating heavy metals, improving soil structure and promoting fertility, thus supporting sustainable plant growth in heavy metal-contaminated soils (Vanlauwe et al. 2005; Kusumaningtyas et al. 2021).

- Physiological responses to mercury and arbuscular mycorrhizal fungi

Research indicated that heavy metals typically decrease the mycorrhizal colonization; however, in our case (Fig. 2) the opposite effect was observed, a finding in line with earlier work (Schneider et al. 2017; Garcia et al. 2020). In our study, it is not possible to predict a general trend as to whether mercury accumulation is generally increased or decreased by inoculation with AMF, as no significant difference was found (Fig. 3). Previous findings indicate that inoculation with AMF can lead to both a decrease or an increase in the uptake of mercury by plants (Debeljak et al. 2018; Li et al. 2023). Although inoculation with AMF was successful, it did not result in a significant difference in assimilation or total dry weight as in other studies (Frosi et al. 2016; Estrada-Luna and Davies 2003). Nevertheless, it seems that AMF exerts a 'buffer effect' on the sensitivity of plants to mercury, as the reduction in assimilation in less pronounced in the mercury accumulating species *A. mangium*, *G. sepium* and *S. siamea* (Table 4). Comparing the two experiments, *A. mangium* was able to achieve an even higher mercury accumulation in the mercury-AMF experiment than in the mercury-concentration experiment (Table 3, 4). Thus, the inoculation with AMF seemed to increase the BAF of *A. mangium*, but the TI of *A. mangium* in the mercury-AMF experiment was below 1 both with and without AMF (Table 4). In contrast, the BAF of *G. sepium* in the mercury-AMF experiment was much lower than in the mercury-concentration experiment (Table 2, 4). This was probably due to the reduced mercury accumulation, whereby the TI of *G. sepium* in mercury-AMF was higher than 1. In *A. mangium* and *G. sepium*, the age of the plant and the duration of mercury exposure appeared to influence the process of mercury accumulation. The influence of the duration of mercury exposure on accumulation has already been observed in other research projects (Xun et al. 2017; Millhollen et al. 2006). The mechanisms underlying the ageing effect of plants on mercury accumulation is a subject for future research.

Conclusion

Although it cannot be classified as a hyperaccumulator this study identified *A. mangium* as the most promising species for phytoremediation of mercury-contaminated soils in former gold mining areas. It accumulates the highest concentrations of mercury and shows only minor signs of mercury toxicity making it well suitable for planting on areas with former gold mining. Compared to *A. mangium S. siamea* exhibited lower (moderate) mercury accumulation without physiological impairment. *L. leucocephala* did not accumulate mercury, but also demonstrated no adverse physiological effects and could play an important role as a mercury excluder, thereby promoting biodiversity and ecosystem stability. *G. sepium* showed moderate mercury uptake but suffered considerable physiological damage. Although inoculation with AMF did not significantly increase mercury uptake, it appeared to mitigate physiological stress, supporting plant health rather than directly enhancing mercury removal. These findings support the strategic use of diverse plant species in ecological restoration to optimize mercury removal and promote a stable ecosystem. Further research is needed to identify other suitable plant species that meet all the criteria for mercury hyperaccumulation and can be effectively used in phytoremediation strategies.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, experiments and data collection were performed by Nadine Sommer and Yaqin Guo. Analysis was performed by Nadine Sommer. The first draft of the manuscript was written by Nadine Sommer, Michael Helmut Hagemann and Christian Zörb. Frank Rasche and Yaqin Guo reviewed and edited the manuscript. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

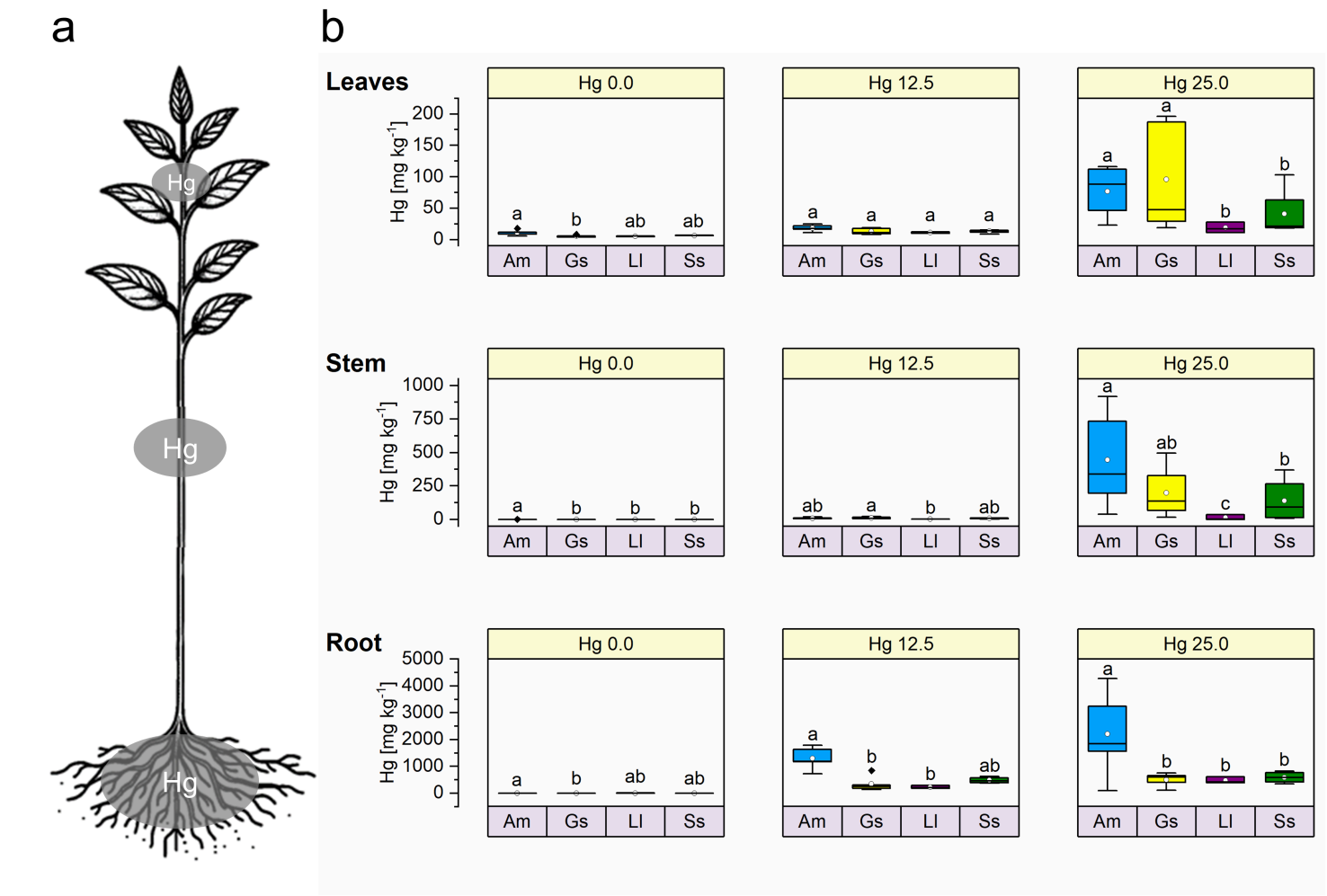

Figure 1

(a) Scheme of total mercury accumulation in leaves, stem, and root. (b) Data on the mercury accumulation per tissue for *A. mangium*(Am, blue), *G. sepium* (Gs, yellow), *L. leucocephala* (Ll, purple) and *S. siamea* (Ss, green). Significant differences in the effects of mercury treatment are indicated by different letters (LSD, $p \leq 0.05$). Statistical analysis was performed individually for each box plot diagram. The box plots represent untransformed data, while the statistical analyses were performed on log-transformed data.

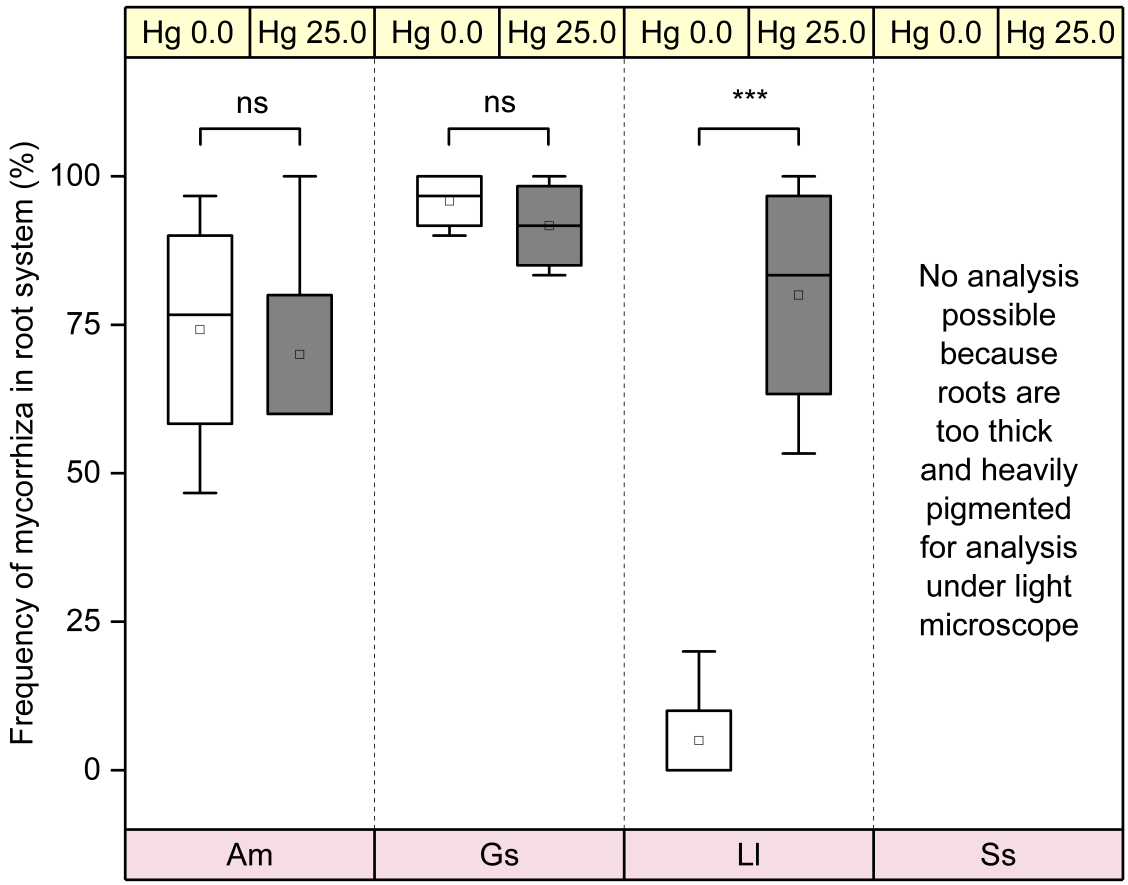

Figure 2

Percentage of mycorrhizal colonization in the root system of plants treated with arbuscular mycorrhizal fungi (Am = *Acacia mangium*, Gs = *Gliricidia sepium*, Ll = *Leucaena leucocephala*, Ss = *Senna siamea*) based on Trouvelot's (1986) method in relation to mercury exposure (Hg 0, control, Hg 25, 25 mg Hg kg^{-1}). The roots of *S. siamea* could not be analyzed because they were too thick and heavily pigmented for analysis under the light microscope. Asterisks indicate a significant difference between Hg 0 and Hg 25 treatments (LSD,*** $p \leq 0.001$).

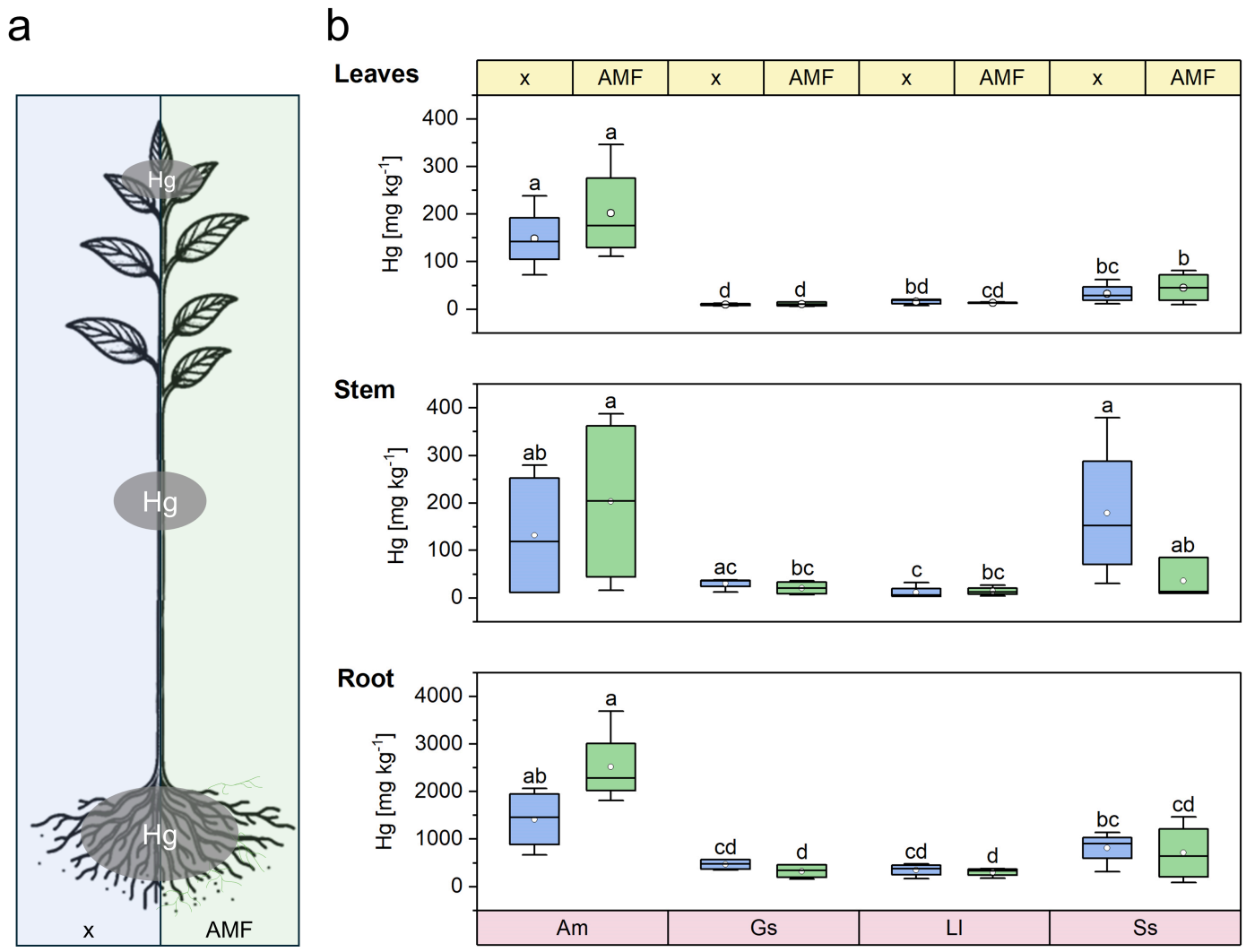

Figure 3

(a) Scheme of mercury accumulation in leaves, stem, and root depending on the presence or absence of arbuscular mycorrhizal fungi (AMF). (b) Mercury accumulation concentrations in the leaves, stems, and roots of plants (Am, *Acacia mangium*; Gs, *Gliricidia sepium*; Ll, *Leucaena leucocephala*; Ss, *Senna siamea*) after inoculation with arbuscular mycorrhizal fungi (AMF, AMF-inoculated; x, control). The data presented pertain solely to plants subjected to mercury treatment (25 mg Hg kg^{-1}). Variations of significance are denoted by distinct letters (LSD, $p \leq 0.05$). Box plots, non-transformed values; statistical assessments with log-transformation.