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Physiological Responses and Tolerance of Three Acacia Species to Cadmium Stress during Germination and Early Seedling Growth

(Tindak Balas Fisiologi dan Toleransi Tiga Spesies Akasia terhadap Tekanan Kadmium semasa Percambahan dan Pertumbuhan Anak Benih Awal)

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ABSTRACT

Soil contamination with cadmium (Cd) is a serious threat to ecosystems. Phytoremediation is a cost-effective green technology that can be used to clean up Cd pollution, and Acacia species are potential phytoremediation agents due to their fast growth, significant biomass, and tolerance to environmental stresses. Most studies of Cd stress on Acacia focused on later vegetative stages and the tolerances during germination and early seedling are elusive. This study aimed to assess the tolerance of *Acacia mangium*, *Acacia crassicarpa*, and *Acacia auriculiformis* to Cd stress during germination and early seedling growth. In response to 250 µM Cd treatment, *A. auriculiformis* germination was slightly increased compared to the control. Leaf organ formation remained largely unaffected by Cd stress, except for the true leaf number in *A. mangium*, which was significantly higher in response to 125 µM Cd than those to 250 µM Cd treatment and control at 8 and 10 weeks after treatment. The onset of phyllode development transition from true leaf was significantly delayed in *A. mangium* compared with the other two Acacia, irrespective of the presence of Cd. There were no significant differences in leaf gas exchange parameters (photosynthesis, stomatal conductance, and transpiration), leaf-level water use efficiency, or leaf chlorophyll content between Cd-treated and control plants, suggesting that the three Acacia species are tolerant to Cd at both germination and early seedling phases. Cd accumulation during the experiment was very low in *A. mangium* seedlings (2.8 mg kg^{-1} upon 250 µM Cd) and insignificant in *A. crassicarpa* and *A. auriculiformis*, indicating non-hyperaccumulation or Cd exclusion at the seedling stage.

Keywords: *Acacia auriculiformis*; *Acacia crassicarpa; Acacia mangium*; cadmium; phytoremediation

ABSTRAK

Pencemaran tanah oleh Cadmium (Cd) merupakan ancaman yang serius bagi ekosistem. Fitoremediasi adalah teknologi hijau yang menjimatkan kos yang dapat digunakan untuk membersihkan pencermaran Cd dan spesies Akasia merupakan agen fitoremediasi yang berpotensi kerana pertumbuhan yang pantas, biojisim yang ketara dan toleransinya terhadap alam sekitar. Kebanyakan kajian tekanan Cd pada Akasia tertumpu pada peringkat vegetatif lanjut sedangkan toleransi semasa percambahan dan fasa anak benih masih belum difahami. Kajian ini bertujuan untuk menilai toleransi *Acacia mangium*, *Acacia crassicarpa* dan *Acacia auriculiformis* terhadap tekanan Cd semasa percambahan dan pertumbuhan awal anak benih. Sebagai gerak balas terhadap rawatan 250 µM Cd, percambahan *A. auriculiformis* meningkat sedikit apabila dibandingkan dengan kawalan. Pembentukan organ daun sebahagian besarnya tidak dipengaruhi oleh tekanan Cd, kecuali bilangan daun sebenar pada *A. mangium*, yang lebih tinggi pada rawatan 125 µM Cd berbanding rawatan 250 µM Cd dan kawalan, pada 8 dan 10 minggu selepas rawatan. Permulaan peralihan perkembangan filod daripada daun sebenar telah terlewat dengan ketara pada *A. mangium* berbanding dua spesies Akasia yang lain, tanpa mengira kehadiran Cd. Tiada perbezaan yang ketara dalam parameter pertukaran gas daun (fotosintesis, kekonduksian stomata, dan transpirasi), kecekapan penggunaan air daun atau kandungan klorofil daun antara tumbuhan yang dirawat dan kawalan, menunjukkan bahawa ketiga-tiga spesies Akasia tersebut adalah toleran terhadap Cd pada kedua-dua fasa percambahan dan awal anak benih. Pengumpulan Cd semasa uji kaji adalah sangat rendah dalam anak benih *A. mangium* (2.8 mg kg^{-1} pada 250 µM Cd) dan tidak ketara dalam *A. crassicarpa* dan *A. auriculiformis*, menunjukkan bukan hiper-pengumpulan atau pengecualian Cd pada peringkat anak benih.

Kata kunci: *Acacia auriculiformis*; *Acacia crassicarpa*; *Acacia mangium*; fitoremediasi; kadmium

INTRODUCTION

Soil contamination by heavy metals poses a significant threat to the ecosystem, endangering plant, animal, and human health through chronic bioaccumulation in the food chain (Jaishankar et al. 2014; Khalid et al. 2017). Heavy metal toxicity triggers the overaccumulation of reactive oxygen species, which can be damaging to cell biomolecules such as membrane lipids, enzymes, and DNA (Ray, Huang & Tsuji 2012). Cadmium (Cd) is a heavy metal on the list of top 10 priority hazardous substances due to its frequency of occurrence, toxicity, and potential for human exposure (ATSDR 2022). Anthropogenic activities, including mining, fuel combustion emissions, and the use of Cd-containing fertilizers in agriculture, primarily contribute to soil contamination with Cd (Kubier, Wilkin & Pichler 2019)

Phytoremediation utilizing various plant species to remove heavy metal contamination from the soil is an eco-friendly and cost-effective approach for large-scale application (Khalid et al. 2017; Pilon-Smits 2005; Suman et al. 2018). Cd and other heavy metal pollutants mainly exist as elemental ions in soil, rendering them non-biodegradable and necessitating stabilization or removal (Suman et al. 2018). For a plant to be a suitable phytoremediation agent, it must possess three essential properties: (1) tolerance to heavy metals, (2) ability to accumulate or stabilize metals, and (3) significant biomass production (Krämer 2010; McGrath & Zhao 2003; Suman et al. 2018)

Acacia species show promise for phytoremediation due to their rapid growth, substantial biomass, and extensive rooting. Additionally, these trees form symbiotic nodules with nitrogen-fixing bacteria, enhancing soil fertility (Galiana et al. 1990). Acacia species, such as *Acacia mangium*, *A. crassicarpa*, and *A. auriculiformis*, which are native to Indonesia, have gained popularity for their high biomass production and other commercial values (Griffin et al. 2011). Acacias are used in agriculture and forestry to improve soil nitrogen, sequester carbon, and restore nutrient cycling in degraded lands and forests (Koutika & Richardson 2019). More importantly, multiple studies have reported Acacia species tolerance to heavy metals, as well as their growth responses and Cd accumulation capacity in toxic Cd-containing environments (Ang et al. 2010; Hossain, Huda & Hossain 2009; Majid, Islam & Mathew 2012; Mok et al. 2012; Ng et al. 2018; Nirola et al. 2015; Sari, Giyanto & Sudadi 2016; Shabir et al. 2018; Zhang et al. 2022).

When selecting plant species for phytoremediation of heavy metal-contaminated soil, it is important to consider different growth and developmental stages, as they may respond differently to toxic metal stress. Generally, Cd negatively affects seed germination and the newly emerged seedlings growth is more vulnerable compared to later stages (Bae, Benoit & Watson 2016; Huybrechts et al. 2019). Interestingly, certain metal-tolerant plants such as *Dorycnium pentaphyllum*, *Prosopis laevigata*, and *Silene sendtneri* were able to maintain their germination in response to Cd and even showed enhanced germination under low Cd concentrations (Buendía-González et al. 2010; Karalija et al. 2021; Lefevre et al. 2009).

Previous studies on Acacia species have primarily focused on their responses to Cd during later vegetative stages (Hossain, Huda & Hossain 2009; Majid, Islam & Mathew 2012; Mok et al. 2012; Ng et al. 2018; Shabir et al. 2018; Zhang et al. 2022). However, their tolerance at germination and early vegetative growth is elusive. Therefore, this study aimed to examine the physiological responses and tolerance of *A. mangium, A. crassicarpa*, and *A. auriculiformis* to Cd stress during germination and early seedling growth. The results of this study will provide evidence for the practical feasibility of direct Acacia seed introduction for successful phytoremediation revegetation in targeted Cd-contaminated sites.

MATERIALS AND METHODS

PLANT MATERIALS

Seeds of *A. mangium*, *A. crassicarpa*, and *A. auriculiformis* were obtained from a certified local producer in Bogor, West Java, Indonesia. The experiments were conducted in the greenhouse facility of the National Research and Innovation Agency of Indonesia (BRIN) in Cibinong, West Java (6°29'39.9156'' S 106°51›2.8836››E).

ACACIA SEEDS GERMINATION ON Cd-CONTAMINATED MEDIA

The effect of Cd on seed germination was tested in two settings: petri dishes and soil. For the petri dish experiment, 50 seeds of each Acacia species were sown on tissue paper moistened with 0 (control), 125, or 250 µM cadmium chloride ($CdCl_2$) solution in a ø 90 mm petri dish. The dishes were incubated at 26 °C under natural light conditions (12 h light/12 h dark) for 30 days (4 weeks). For the soil experiment, 50 seeds of each Acacia species were sown and germinated in a plastic container (30×45×15 cm) without perforation filled with a media mixture of topsoil and manure (1:1). Cd was added one time by saturating the 5 kg media in each container with 10 L of 125 µM or 250 µM $CdCl_2$ solutions for 2 weeks in the greenhouse before seed planting. The seeds were grown in the container in a greenhouse with a daily air temperature of 30 °C and a relative humidity of 85% for 30 days, with daily watering to maintain soil moisture at field capacity. All experiments were conducted in triplicate, and the germination percentage was determined by calculating the number of germinated seedlings divided by the total number of seeds sown in each treatment condition.

GROWTH AND PHYSIOLOGICAL RESPONSES OF ACACIA SEEDLINGS ON Cd-CONTAMINATED SOIL

Four-week-old seedlings with uniform size and vigor from the previous soil germination experiment were selected and further grown in the same containers with respective Cd treatment conditions for an additional 6 weeks. The experiment involved two factors: Acacia species and Cd treatments. Each group had four or more individuals as biological replicates. At the end of the extended experimental period (week 10), various parameters were analyzed, including seedling height, leaf number (true leaf and phyllode), seedling fresh biomass, relative chlorophyll content (measured using an SPAD 502 Chlorophyll meter, Konica Minolta, Japan), and leaf gas exchange parameters such as photosynthesis rate, transpiration rate, and stomatal conductance (measured using a Li-6400 portable photosynthesis system, Li-Cor, USA).

Cd CONTENT IN ACACIA SEEDLINGS GROWN ON Cd-CONTAMINATED SOIL

Cd quantification was done in bulk by merging individual plant or soil samples from each treatment group into a composite sample without replication. Soil or plant (root and shoot) samples were dried in an oven at 70 °C for 3 days and then ground into fine powder. Cd content in the soil and plant biomass were determined by Atomic Absorption Spectrometry (AAS) using GBC 932AA (GBC Scientific, Victoria, Australia) following the previously described protocol (Ulusu, Öztürk & Elmastaş 2017). The whole plant bioconcentration factor (BCF) for Cd was determined as the ratio of Cd concentration in plants to Cd concentration in soil media.

RESULTS

ACACIA SEED GERMINATION UNDER Cd STRESS

A. mangium, A. auriculiformis, and *A. crassicarpa* seeds were germinated under 125 µM and 250 µM $CdCl_2$ to assess their response to Cd. Germination experiments were conducted in petri dishes and soil. In the soil experiment, *A. mangium* (42.33–52%) showed higher germination than *A. crassicarpa* (15.33–25%) but was similar to *A. auriculiformis* (36.5–52.5%) (Figure 1(A)). *A. auriculiformis* had higher germination than *A. crassicarpa* overall (Figure 1(A)). However, there were no differences in germination between Cd-treated and control groups in petri dish experiments for each Acacia species (*A. mangium* 20.67-26.67%, *A. crassicarpa* 17.33-24%, *A. auriculiformis* 14.67-32%) (Figure 1(B)). These results indicate that high Cd soil concentrations did not significantly reduce seed germination in *A. mangium, A. crassicarpa,* and *A. auriculiformis*. Interestingly, *A. auriculiformis* seed germination percentage in soil was slightly increased in response to 250 µM Cd treatment (52.5%) compared to control (36.5%).

Cd EFFECTS ON THE GROWTH OF ACACIA SEEDLINGS

The growth of Acacia seedlings from the germination experiment was analyzed to examine their early vegetative response to Cd toxicity. In total, seedlings were grown in Cd-contaminated soil for 10 weeks. The seedlings primary growth under Cd treatments followed a linear trend similar to the control group (Figure 2). Acacia species with the tallest seedlings was *A. mangium* (30-34 cm), followed by *A. auriculiformis* (21.57-26.92 cm) and *A. crassicarpa* (10-13.63 cm) (Figure 2). However, compared to control, there were no significant differences in the seedling height of each Acacia species under 125 µM and 250 µM Cd treatments (Figure 2).

Seedling fresh weight recorded 10 weeks after planting showed no significant difference between Cd-treated and control groups (Figure 3). This is true for shoot, root, and total biomass (Figure 3(A), 3(B) and 3(C)). The root-to-shoot biomass ratio remained consistent regardless of Cd presence (Figure 3(D)). Overall, *A. mangium* (9.19-11.14 g) showed the largest fresh biomass among the three Acacia, while *A. crassicarpa* (3.37-5.31 g) and *A.*

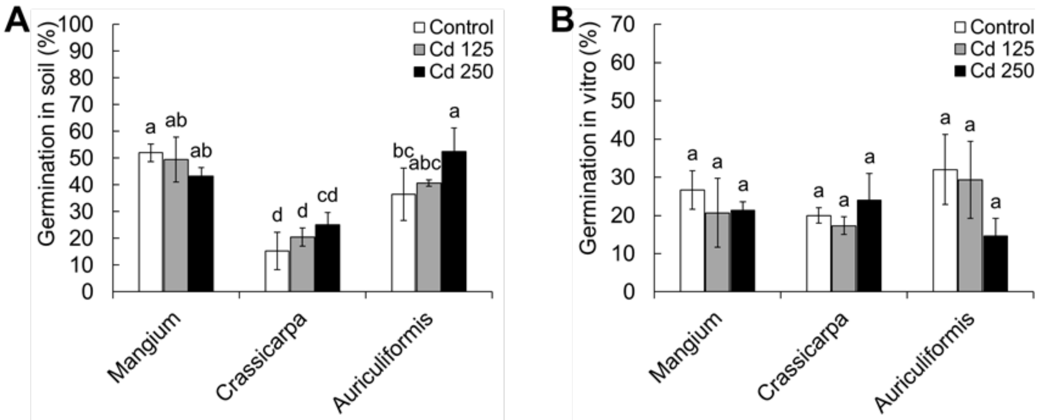

FIGURE 1. Germination percentage of *A. mangium, A. crassicarpa* and *A. auriculiformis* seeds in response to the application of 0 (control), 125 and 250 µM CdCl2 grown for 4 weeks in (A) petri dish and (B) in soil (mean ± SD). Different letters indicate significant differences as determined by ANOVA and Tukey test at a significance level of 5%

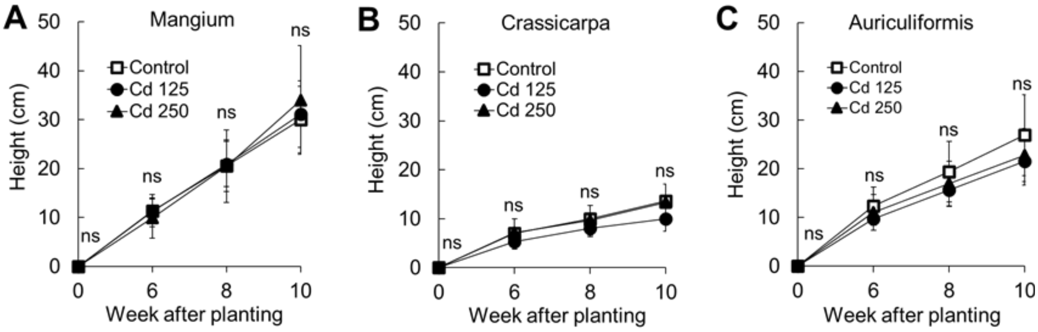

FIGURE 2. Height of (A) *A. mangium*, (B) *A. crassicarpa* and (C) A. auriculiformis seedlings grown in soil treated with 0 (control), 125 and 250 µM CdCl2 for 10 weeks (mean ± SD). ns: not significant

auriculiformis (4.74-7.68 g) had similar and comparable fresh weights. The effect of Cd on organ development during the seedling phase was also examined. *A. mangium* seedlings under Cd treatment and control conditions had more true leaves compared with *A. crassicarpa* and *A. auriculiformis* at 6 weeks after germination (Figure 4(A), 4(B), 4(C)). After 8 weeks of Cd exposure, the true leaf count in all tested Acacia was relatively steady. At weeks 8 and 10, *A. mangium* seedlings treated with 125 µM Cd (9.25 at week 8 and 9.06 at week 10) had significantly higher true leaf numbers than those in the 250 µM Cd treatment (7.75 at week 8 and 7.5 at week 10) and control (7.8 at week 8 and 7.82 at week 10) (Figure 4(A)), indicating that true leaf development remains relatively sustained in *A. mangium* seedlings under Cd-induced stress compared to *A. crassicarpa* and *A. auriculiformis* at that level of Cd concentration.

In Acacia species, compound true leaves develop soon after germination, but are later succeeded by phyllodes, which are modified petioles with leaf-like functions. At 6 weeks after sowing, phyllodes had formed in *A. crassicarpa* (Figure 4(E)) and *A. auriculiformis* (Figure 4(F)), but not in *A. mangium* (Figure 4(D)). However, there were no significant differences in the number of phyllodes formed in Acacia seedlings treated with or without Cd. This result suggests that the onset of phyllode development, transitioning from true leaves, in *A. mangium* appears to be delayed compared to the other two Acacia species, regardless of the presence of Cd stress.

PHOTOSYNTHETIC-RELATED RESPONSES OF ACACIA SEEDLINGS TO Cd STRESS

To understand the physiological effects of Cd, photosynthesis parameters were investigated. *A. mangium* seedlings had significantly higher photosynthetic rates (*A*) and stomatal conductance compared to *A. crassicarpa* and *A. auriculiformis* (Figure 5(A)). Within each Acacia species, the photosynthesis rates (*A*) were not significantly different between control and Cd treated seedlings (Figure 5(A)). *A. mangium* seedlings showed reduced stomatal

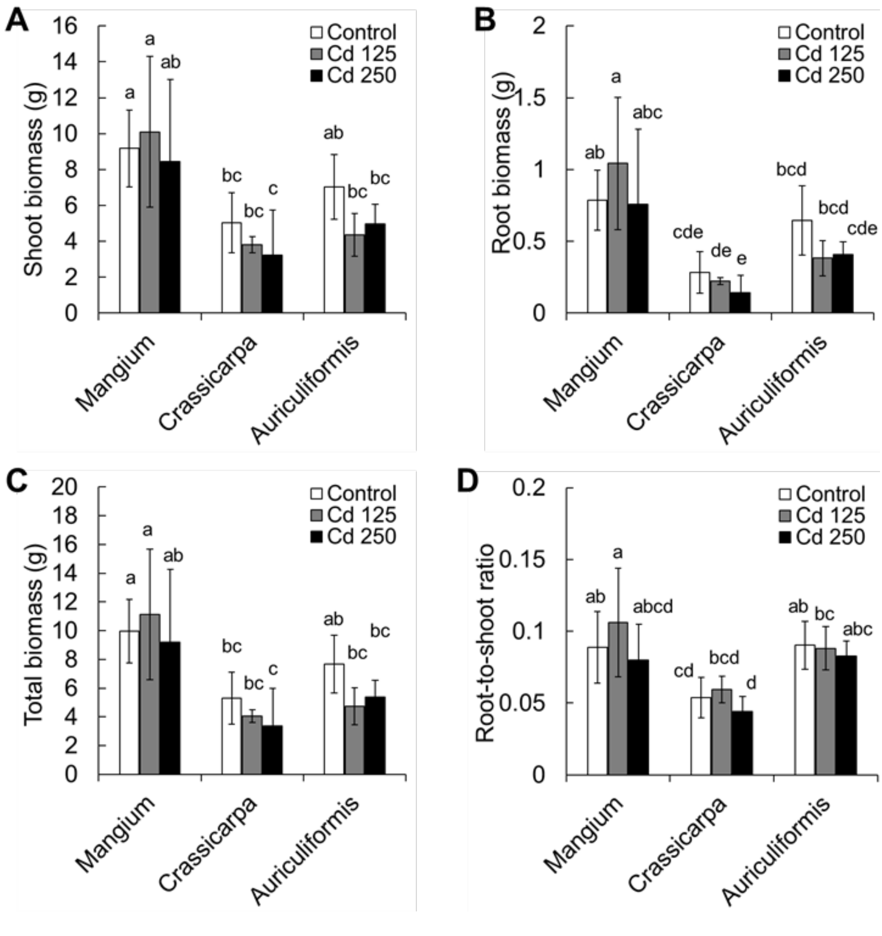

FIGURE 3. Acacia seedling biomass was not significantly affected by Cd stress during early vegetative growth. (A) Shoot, (B) root, (C) total fresh weight and (D) the root-to-shoot ratio of seedling fresh biomass of *A. mangium, A. crassicarpa* and *A. auriculiformis* seedlings grown in soils treated with 0 (control), 125, and 250 µM CdCl2 for 10 weeks. The data is presented as mean ± SD. Different letters indicate significant differences as determined by ANOVA and Tukey test at a significance level of 5%

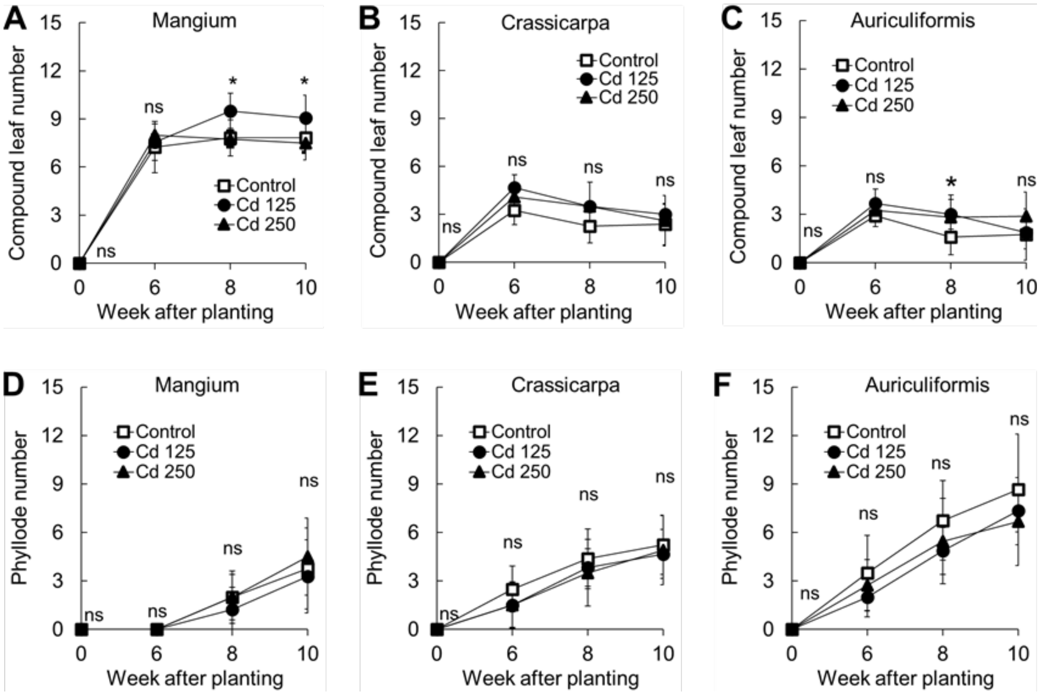

FIGURE 4. Compound leaf and phyllode number of (A and D) *A. mangium*, (B and E) *A. crassicarpa* and (C and F) *A. auriculiformis* seedlings grown in soil treated with 0 (control), 125 and 250 μM CdCl2 for 10 weeks (mean ± SD). Asterisks indicate a significant difference between Cd-treated seedlings and control at the corresponding time point as determined by ANOVA and Tukey test at a significance level of 5%; ns, not significant

conductance (g_s) in response to Cd, while *A. crassicarpa* and *A. auriculiformis* did not exhibit such reduction (Figure 5(B)). Leaf transpiration rates (*E*) did not differ significantly among the three acacia species under Cd treatments or control conditions (Figure 5(C)).

Under control conditions, intrinsic water use efficiency (WUE), calculated as A/g_s, was comparable between *A. crassicarpa* and *A. auriculiformis* and significantly higher than that of *A. mangium*. Intrinsic WUE did not significantly differ between Acacia species under Cd treatments (Figure 5(D)). Instantaneous WUE, calculated as A/E, remained stable across the three acacias in response to Cd (Figure 5(E)). Leaf chlorophyll SPAD values showed no significant differences between control and Cd-treated groups (Figure 5(F)).

Cd CONTENT IN ACACIA SEEDLINGS GROWN ON Cd-CONTAMINATED SOIL

The application of Cd solutions in soil resulted in high concentrations (140.2 and 389.4 mg kg^{-1} soil for 125 and 250 uM Cd treatments, respectively) compared to the average abundance of Cd in uncontaminated soil (0.36 mg kg^{-1}) (Table 1). This concentration is considered high because the average abundance of Cd in uncontaminated soil worldwide is 0.36 mg kg^{-1} and Cd level above 3 mg kg^{-1} is generally considered contamination (Kubier, Wilkin & Pichler 2019). Cd accumulation was only observed in *A. mangium* seedlings (2.8 mg kg^{-1}), particularly in the 250 μM Cd treatment group. Cd accumulation in the other Acacias was below the detection threshold of the AAS

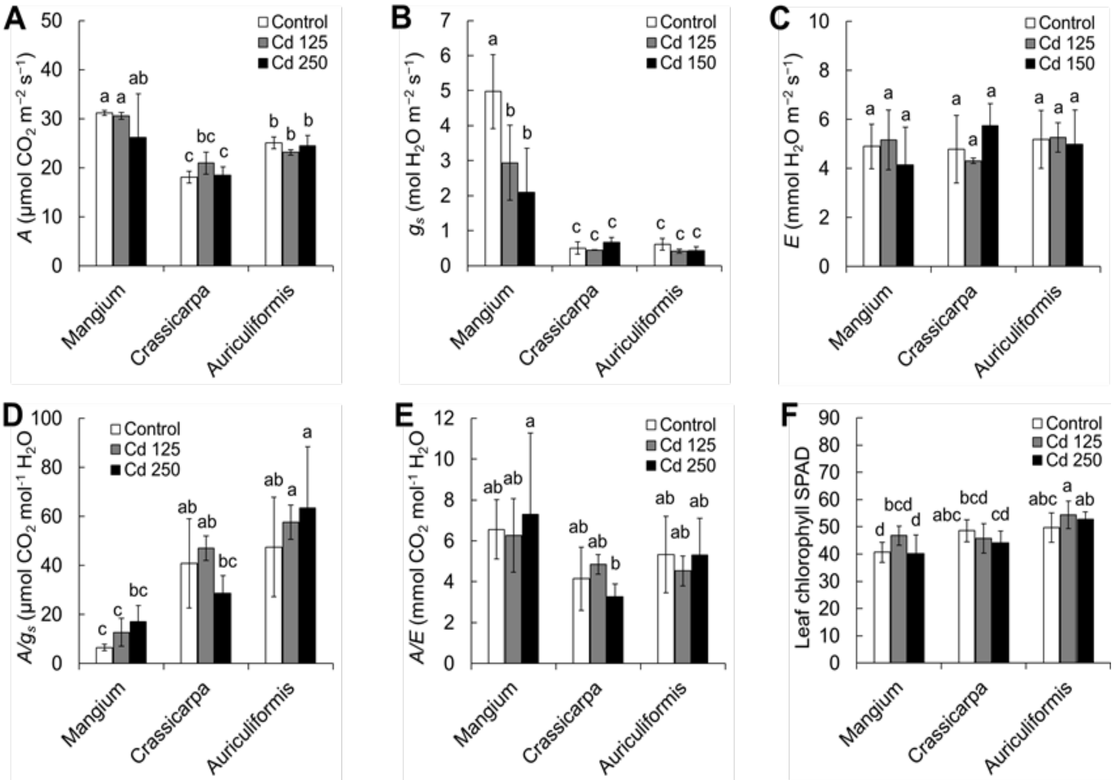

FIGURE 5. Comparison of (A) the photosynthetic rate, *A*, (B) stomatal conductance, gs, (C) transpiration, *E,* (D) intrinsic WUE, A/gs, (E) instantaneous WUE, *A/E,* and (F) leaf chlorophyll of *A. mangium, A. crassicarpa, A. auriculiformis* seedlings grown in soils treated with 0 (control), 125, and 250 µM CdCl2 for 10 weeks (or 9 weeks for SPAD data). The data is presented as mean ± SD. Different letters indicate significant differences as determined by ANOVA and Tukey test at a significance level of 5%

TABLE 1. Cd accumulation in soil media and acacia seedling biomass after 10 weeks of Cd treatment of Cd

Treatments	Cd concentration (mg kg^{-1})			
	Soil media	*A. mangium*	*A. crassicarpa*	*A. auriculiformis*
Cd 0 µM (control)	BDL	BDL	BDL	BDL
Cd 125 µM	140.2	BDL	BDL	BDL
Cd 250 µM	389.4	2.8	BDL	BDL

BDL, below the instrument detection limit of 2.68 mg kg^{-1} DW instrument used (2.68 mg kg^{-1}). The bioconcentration factor (BCF), which indicates a plant's ability to accumulate metals, was approximately 0.0072 for Cd in *A. mangium* seedlings, suggesting a metal exclusion phenomenon.

DISCUSSIONS

Cadmium (Cd) is a hazardous heavy metal that adversely affects plant growth and productivity. Acacia species have shown promise as phytoremediation agents for Cd-contaminated soil, given their tolerance to abiotic stresses and high biomass production. Previous studies have highlighted the ability of Acacia species, including *A. auriculiformis*, *A. mangium*, and *A. pycnantha*, to thrive in Cd-polluted mine sites (Ang et al. 2010; Majid, Islam & Mathew 2012; Nirola et al. 2015; Sari, Giyanto & Sudadi 2016). However, most research has focused on plant responses during later vegetative phases, such as old seedlings, saplings, and mature trees, leaving a knowledge gap regarding the responses of Acacia species during early germination and seedling growth stages to heavy metal stress (Hossain, Huda & Hossain 2009; Majid, Islam & Mathew 2012; Mok et al. 2012; Ng et al. 2018; Shabir et al. 2018; Zhang et al. 2022).

Selecting the appropriate growth stage for introducing plants into Cd-contaminated sites is crucial for successful phytoremediation. Different growth stages may vary in sensitivity to heavy metal stress, necessitating a comprehensive understanding of Acacia species' stage-specific responses to Cd stress. To address this issue, the effects of Cd on seed germination and early seedling growth in three significant Acacia species: *A. mangium*, *A. crassicarpa*, and *A. auriculiformis* were investigated. These species, native to Indonesia, hold economic importance for timber, pulpwood, biofuel, and land rehabilitation (Griffin et al. 2011). This current study found that seed germination, primary growth, and above-ground organ formation in young Acacia seedlings were tolerant to Cd stress. While leaf gas exchange parameters and chlorophyll are typically affected by Cd toxicity in plants (Ekmekçi, Tanyolaç & Ayhan 2008; Krantev et al. 2008; Küpper et al. 2007; Muradoglu et al. 2015), the photosynthesis rate, transpiration, and leaf chlorophyll content in the seedlings of the three Acacia species were relatively unaffected. Other pivotal metrics that are derived from the main photosynthetic parameters are intrinsic WUE and instantaneous WUE. Intrinsic WUE refers to the amount of net photosynthesis (A) compared to the conductance for water vapor (g_s), which ultimately shows how much assimilation occurs per unit of water used. Instantaneous WUE, on the other hand, is the ratio of net photosynthetic rate (A) to transpiration rate (E) (Hatfield & Dold 2019). Intrinsic and instantaneous WUEs did not significantly differ between the Cd-treated and control groups for each Acacia species. These findings suggest that photosynthesis and leaf-level WUE in the early stage of seedling growth in these Acacia species are tolerant to Cd stress.

Mature *A. mangium* trees thrived in Cd and Pb-contaminated ex-tin mines, with concentrations up to 1.5 mg kg^{-1} and 11 mg kg^{-1}, respectively (Ang et al. 2010). A study on *A. mangium* saplings in sludge contaminated with Cd, Cr, Pb, Cu, and Zn showed minimal reduction in plant biomass (Majid, Islam & Mathew 2012). Furthermore, *A. mangium* saplings effectively accumulated Cd in their leaves, stems and roots, with uptake increasing in higher soil Cd concentrations (Majid, Islam & Mathew 2012). Accordingly, *A. auriculiformis* has demonstrated tolerance to Cd and potential for soil phytoremediation. Zhang et al. (2022) observed that 1-year-old *A. auriculiformis* seedlings maintained growth, leaf characteristics, and gas exchange parameters under Cd stress. *A. auriculiformis* exhibited higher germination in soil mixed with industrial sludge containing toxic levels of Cd and other heavy metals (Hossain, Huda & Hossain 2009). These previous reports align with the result of the current study, highlighting the extensive tolerance of *A. mangium* and *A. auriculiformis* to Cd contamination across various growth stages, from germination to maturity.

Seed germination sensitivity varies depending on species/genotypes, Cd concentration, and exposure duration (Haider et al. 2021; Huybrechts et al. 2019; Lin & Aarts 2012). The seed dormancy in many Acacia species is attributed to a hard and impermeable seed coat, necessitating scarification through chemical, mechanical, or heat treatments for dormancy breaking and improved germination (Ghassali et al. 2012; Riveiro et al. 2020). In natural settings, ecological cues such as fires and heat positively impact seed dormancy breaking (Riveiro et al. 2020). In a previous study by Ghassali et al. (2012), the mean seed germination percentage for 12 Acacia species at 2 weeks after sowing was only 3%, and dormancy-breaking treatments using strong acid solutions, boiling water, and mechanical abrasion improved germination rates to 45%, 31%, and 23%, respectively. In the current experiment, the germination percentage in three tested Acacia species at 4 weeks after germination was around 15.3-53.25% in soil experiments, and 14.67-32% in petri dishes, and it was not reduced by Cd exposure (Figure 1).

Previous studies on the effect of Cd or other heavy metals on acacia seed germination are scarce. *A. auriculiformis* germination has been reported to be improved in response to industrial and residential sludge containing high concentrations of Cd and other pollutants compared to the control (Hossain, Huda & Hossain 2009). However, it is difficult to separate the specific effect of Cd to seed germination, since the sludge may contain substance or nutrients that may contribute to enhanced germination (Hossain, Huda & Hossain 2009). *A. mangium* and *A. auriculiformis* seed are tolerant to soil contaminated with up to 100 mg/kg chromated copper arsenate substance, and above that threshold, both species show significant germination percentage reduction (Kumari & Nagaraja 2023). In other species, Cd concentrations of 50 mg/L or mg/kg, equivalent to ~250 µM Cd(II) solutions, significantly reduce germination in soybean, alfalfa, wheat, rice, lettuce, pea, and tomato (Ahsan et al. 2007; Baruah et al. 2019; Bautista, Fischer & Cárdenas 2013; Guilherme, de Oliveira & da Silva 2015; Li et al. 2013; Peralta et al. 2001; Titov, Talanova & Boeva 1996). In contrast, common bean, barley and certain genotypes of wheat and rice exhibited higher germination tolerance to exceptionally higher Cd concentrations of 0.4-5.0 mM Cd(II) (Ahmad et al. 2012; Cheng et al. 2008; Munzuroglu & Zengin 2006; El Rasafi et al. 2016).

During the 10-week experimental period, Cd accumulation was not detected in *A. crassicarpa* and *A. auriculiformis*, while only *A. mangium* had a very low Cd concentration (Table 1). The low bioavailability of Cd may be responsible for this observation (Kirkham 2006). Unfortunately, important variables such as soil pH, organic matter, and cation exchange capacity, which contribute to overall Cd bioavailability, were not considered in the current experiment. The duration of Cd exposure and inherent species characteristics, including biomass production and bioconcentration factors (BCF), also influence Cd bioaccumulation in Acacia (Mok et al. 2012).

In early seedlings, *A. mangium*, *A. crassicarpa*, and *A. auriculiformis* exhibited very low BCF for Cd, indicating their non-accumulator nature. However, as the plants reached advanced vegetative stages and increased in biomass, the accumulated Cd in the plant also increased, resulting in higher BCF values. Indeed, Majid, Islam and Mathew (2012) found BCF values of 0.44-0.88 in 5-month-old *A. mangium* seedlings grown in Cd-contaminated sewage sludge. *A. mangium* seedlings grown in Cd-contaminated soils with organic amendments accumulated up to 5.1 mg kg^{-1} of Cd over three months, with root BCF ranging from 1.1 to 3.8 (Taeprayoon, Homyog & Meeinkuirt 2022). Shabir et al. (2018) observed that *A. nilotica* accumulated 5.8 mg kg^{-1} Cd in the root and 9.3 mg kg^{-1} Cd in the shoot when grown in soil with 15 mg kg^{-1} Cd, resulting in a BCF between 1 and 1.43. Overall, BCF in Acacia varies depending on the growth stage, interactions with soil amendments, and the combined effects of multiple stressors in the substrate mixture. Despite their low BCF, the high biomass production of Acacia species may still contribute to a significant accumulation of Cd.

The mechanisms underlying the plant response to Cd stress are still elusive. Cd is thought to disrupt water uptake and metabolic reactivation during germination (Huybrechts et al. 2019). It induces a burst of reactive oxygen species (ROS) and oxidative stress, activating antioxidant responses. In Acacia species, Cd-induced ROS may promote germination, with antioxidant machinery restoring cellular balance, resulting in a tolerant outcome for germination. ROS signaling also regulates Cd-responsive gene transcription, activating antioxidant machinery, Cd chelation, and compartmentation to vacuoles (Huybrechts et al. 2019; Lin & Aarts 2012). Although Cd stress does not affect chlorophyll and carbon assimilation in *A. mangium*, *A. crassicarpa*, and *A. auriculiformis* seedlings, the cellular and molecular mechanisms behind these responses require further investigation.

The current understanding of the Cd tolerance mechanism primarily comes from hyperaccumulator species such as *Noccaea caerulescens, Arabidopsis halleri,* and *Sedum alfredii*, which accumulate high Cd levels in their biomass (Krämer 2010; McGrath & Zhao 2003). Duplication of the metal transporter gene, *HMA4*, amplifies the expression of the encoded transporter protein in *A. halleri* which, in turn, enhances Cd uptake, detoxification, and compartmentation into vacuoles, resulting in Cd hyperaccumulation (Krämer 2010; Merlot, de la Torre & Hanikenne 2021; Verbruggen, Hermans & Schat 2009). Metal chelator synthesis and metal-induced oxidative stress response genes are also crucial for Cd tolerance and hyperaccumulation in these species (Merlot, de la Torre & Hanikenne 2021). Complexation of Cd with metal chelators like histidine, malate, citrate, glutathione, phytochelatin, and metallothionein is essential for Cd transport and detoxification in leaf cell vacuoles (Verbruggen, Hermans & Schat 2009).

Many Acacia species exhibit high Cd tolerance without hyperaccumulation tendencies, suggesting the presence of Cd avoidance mechanisms commonly observed in non-hyperaccumulator plants. Non-hyperaccumulators typically prevent Cd uptake and translocation to metabolically active leaf cells, thus protecting them from Cd-induced damage. In the root tissue, ionic Cd^{2+} is sequestered in the root apoplast and bound to organic acids or anionic groups in cell walls to prevent entry into the cytoplasm (Rascio & Navari-Izzo 2011). Cd that enters the root symplast is detoxified through complexation with amino acids, organic acids, or metal-binding peptides and/or sequestered in root cell vacuoles. Additionally, plants exposed to heavy metals often enhance their antioxidant systems as a defense mechanism against Cd-induced oxidative stress (Rascio & Navari-Izzo 2011). Taken together, one speculation for this phenomenon is that Acacia employs different strategies at various growth stages to respond to Cd stress. Upon germination, young Acacia seedlings may primarily utilize metal exclusion mechanisms to mitigate toxicity and promote normal growth. However, as the seedlings mature and increase in biomass, their strategy shifts towards metal accumulation while enhancing sequestration and compartmentation of Cd in vacuoles or specialized cells or tissues to protect their physiology. Further investigations on the developmental stage-dependent responses of Acacia to Cd stress will enhance our understanding of its mechanisms in heavy metal stress.

CONCLUSIONS

A. mangium, A. crassicarpa, and *A. auriculiformis* demonstrate clear tolerance to Cd-contaminated soil during germination and early seedling stages, as indicated by the overall non significant changes in growth, biomass, leaf gas exchange, and chlorophyll content parameters upon Cd exposure compared to normal conditions. The treatment of the soil with 250 µM Cd resulted in a small increase in *A. auriculiformis* germination when compared to the control. Acacia seedling biomass and height after ten weeks of Cd treatment did not differ substantially from the control. True leaf fornation remained essentially unaffected by Cd stress except for the true leaf number in A. mangium. Regardless of the presence of Cd, the transition from the true leaf to phyllodes in *A. mangium* was delayed than the other two Acacias. Interestingly, the tested Acacia species exhibit minimal bioaccumulation of Cd in their seedling biomass (BCFs below 0.0072), suggesting a mechanism of metal exclusion. The avoidance of stress through enhanced Cd exclusion may be crucial in mitigating toxic effects and maintaining proper growth during early seedling establishment. These research findings offer empirical evidence supporting the concept of direct introduction of Acacia seeds into Cd-contaminated land as an approach for low-cost, labor-efficient phytoremediation revegetation.

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