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Phytoremediation of Cadmium by Selected Leguminous Plants in Hydroponics Culture

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ABSTRACT

Attempts to use the plants for remediation of heavy metal contamination have been made. This study was conducted to determine the phytoremediation of Cadmium (Cd) by three leguminous plants (*Acacia mangium* Willd, *Pterocarpus indicus* Willd and *Cassia fistula* Linn.) where assessment was done for the Cd accumulation, relative growth, biomass productivity and bio concentration factor. The plants used for testing were obtained from Ratchaburi Nursery Center, Royal Forest Department, Ministry of Natural Resources and Environment, Thailand. Plants were hydroponically cultured in 10% modified Hoagland solution and treated with various Cd concentrations under the laboratory condition for 15 days. Obtained results indicated that *P. indicus* at 8 mg/L of Cd concentration had the highest Cd accumulation (522.91 ug/g dry wt.). The relative growth and biomass productivity in plants were significantly decreased when the test concentrations were increased (P<0.05). The highest relative growth and biomass productivity was observed in *P. indicus* followed by *A. mangium* and *C. fistula* (P<0.05). The highest bio concentration factor (BCF) was found in *P. indicus* when exposed to 8.0 mg/L of Cd concentration. A higher Cd accumulation was also detected in roots compared to the shoots. The results suggests that *P. indicus* has the capability of phytoremediation in case of Cd contamination.

INTRODUCTION

Traditional technologies of cleaning, remediating approaches for areas contaminated with heavy metals, such as, cadmium, relies heavily on ‘dig-and-dump’ or encapsulation method. None of these methods addresses the issue of decontamination of the soil. Immobilization or extraction by physicochemical techniques can be expensive and is often appropriate only for small areas, where, rapid and complete decontamination is required [1,2]. Some methods, such as, soil washing, have an adverse effect on biological activity, soil structure and fertility. Some methods require significant higher engineering cost as well. In situ approach of phytoremediation is attractive as it offers site restoration, partial decontamination, and maintenance of the biological activity and physical structure of soil. This methodology is potentially cheap, visually unobtrusive, and there is the possibility of bio recovery of metal.

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to convert them to a harmless product [3,4,5]. The development of phytoremediation is primarily being driven by the cost factor of the available soil remediation methods as well as by the desire to use a ‘green’, sustainable process [6]. The efficacy of phytoremediation as a viable remediation technology is still being explored, yet, the results are positive so far [7]. Phytoremediation has recently become a tangible alternative to traditional methodologies [8,9].It has been established that certain wild and crop plant species have ability to accumulate elevated amounts of toxic heavy metal[10]. The harvested plant tissue, rich in accumulated contaminant, is easily and safely processed by drying, ashing or composting. The volume of toxic waste produced in this process is generally a fraction of other invasive remediations technologies used. Similarly, the associated cost is much less than other available techniques. In this very process, some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenue [11].

However, researchers all over the world are searching new plant species suitable to use in phytoremediation. Considering such need, the present study was performed to determine the cadmium accumulation capability of three legumes of the Leguminosae family: *Acacia mangium* Willd., *Pterocarpus indicus* Willd. and *Cassia fistula* Linn. with the specific focus on cadmium accumulation in plants. In addition, in this study, the author tried to study cadmium accumulations in different plant parts at different exposure times.

MATERIALS AND METHODS

Plants for experiment

Three leguminous plant species were used in the study, namely *Acacia mangium* Willd., *Pterocarpus indicus* Willd and *Cassia fistula* Linn. Seedlings were obtained from Ratchaburi Nursery Center, Royal Forest Department, Ministry of Natural Resources and Environment, Thailand.

Initially, the plants were separated from soil. An effort was made to keep the root structure intact as much as possible. Plant roots were thoroughly washed with gentle running tap water to remove all the dirt and dead plant biomass that could contain trace elements and rinsed with deionized water. This was done to remove the metal compounds adsorbed on plant root surfaces. After that, they were acclimatized and hydroponically grown in modified 10% Hoagland's solution [12] under a light regime L:D 12:12 (1,000 watt cool fluorescent light) [13] at the room temperature for 2 weeks. Prior to the experiments, each plant species were considered for estimating the fresh weight and dry weight followed by analysis for total cadmium concentration as background data.

Preparation of cadmium and stock solution

The stock solution of cadmium (1,000 mg/l) was prepared by dissolving analytical grade of cadmium chloride ($CdCl_2.2.5H_2O$) 2.032 g in 1000 ml deionized water. The concentrations were expressed in terms of cadmium ion (Cd^{2+}) in milligrams per litter (mg/L) of solution.

Selection of capability and optimum of cadmium accumulation among leguminous plants

Selection of three plants species of Leguminosae family to understand the accumulation of cadmium, plants were taken from the acclimatized container and rinsed with deionized water. Each plant species was treated with 2, 4, 8, and 16 mg/L of cadmium concentration in respective container with a volume of o.4 L. The container without cadmium remained as the control. Each treatment for the plant species considered was carried out in triplicate. Plants were grown under controlled conditions with the light regime L:D 12:12 (1,000 watt cool fluorescent light) at the room temperature. The pH was adjusted to 5.6 using 1 N NaOH and 1 N HNO_3 daily and the test solution was strictly maintained at 0.4L.

Plants were harvested after 15 days, rinsed with deionized water and dried at 103°C for 24 h or until their weight become constant. Later on they were cooled down in desiccator for 30 minutes and dry weight for each plant was measured. Then, dried plant samples were homogenized with an electric tissue grinder. Later, 0.5 g of plant tissue was used into a 15 ml test tube containing 3-5 ml concentrated nitric acid.

Each plant species was digested using the nitric acid (HNO_3) digestion method according to the Standard Method of Water and Wastewater Analysis [14]. After digestion, the metal concentration was determined by using the frame atomic absorption spectrophotometer (FAAS) applying flameless method at wavelength of 228.8 nm for cadmium. The cadmium accumulation in plant tissue was then determined and relative growth, biomass productivity and bio concentration factor were calculated.

Relative growth

Each treated plant and the control were weighed after harvesting. Relative growth of treated plant and the control were calculated as follows:

$$\text{Relative growth} = \frac{\text{Final fresh weight } (\text{FFW})}{\text{Initial fresh weight } (\text{IFW})}$$

Biomass productivity

The biomass productivity of each plant was determined by drying the samples to a constant weight in an oven at 103^{0}C for 24 h. The dry weight for each plant species exposed to metal concentration was expressed as the percentage of decrease of biomass productivity with respect to the control.

Bio concentration factor

The bio concentration factor (BCF) provides an index of ability of the plant to accumulate the trace element with respect to

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the trace element concentration in the substrate. This factor was defined as the ratio of metal concentration in the biomass to the initial concentration of metal ion in the biomass to the initial concentration of metal ion in the tested solution. It was calculated by the following equation:

$$\text{Bio concentration factor }(\text{BCF}) = \frac{\text{Concentration of metal in dried plants}}{\text{Initial concentration of metal in solution}}$$

Data analysis

Cadmium accumulation and growth of each leguminous plants and cadmium concentrations were determined as a range, mean (¯), and standard deviation (SD). ANOVA and Tukey-HSD were used to determine the difference of plants effect on cadmium accumulation and growth in different plant parts (roots and shoots) at the different cadmium concentrations. The significant level was determined at α level of 0.05.

RESULTS

Capability of leguminous plants for cadmium accumulation and estimating the optimum cadmium concentration

Total cadmium accumulation of *A. mangium, P. indicus* and *C. fistula* at 2, 4, 8 and 16 mg/L Cd at 15 days is shown in Table 1. Among leguminous plants, the total cadmium accumulation were significantly increased in plant (P<0.05).

Maximum amount of cadmium accumulation for the plants were observed at 8.0 mg/L whereas significant decrease of Cd concentration accumulation was observed at 16.0 mg/L concentration (P<0.05).

For *A. mangium*, the maximum Cd accumulation was observed at Cd concentration of 8.0 mg/L (444.51 2.55 µug/g dry wt.) and significant decrease was observed at concentration of 16.0 mg/L (294.36 ± 5.95 ug/g dry wt.) (P<0.05). Similar pattern of Cd accumulation was found in *P. indicus*. It was found that at Cd concentration of 8 mg/L, significantly maximum Cd accumulations were observed (522.91 ± 4.77 ug/g) whereas decrease occurred at concentration of 16.0 mg/L (374.12 ± 8.76 ug/g dry wt.) (P<0.05). For *C. fistula*, the maximum Cd accumulations were observed (359.55 ± 2.95ug/g) and the significant decrease appeared at concentration of 16.0 mg/L (225.45 ± 5.27 ug/g dry wt.) (P<0.05).

Among the plants in this study, it was found that the greatest Cd accumulations were found in *P. indicus*, followed by *A. mangium* and *C. fistula*. **(Table 1).** Regarding the capability of Cd accumulation in plant species, it was found that Cd accumulation in root was more rather than the shoot **(Figure 1 and Figure 2).**

Table 1. Mean (±SD) of total cadmium accumulation of *A. mangium*, *C. fistula* and *P. indicus* for 15 days.

Plant species	Cadmium concentration (mg/L)	Mean(±SD)of cadmium accumulation ug/g)
A. mangium	0	0
	2	72.85 ± 2.19[a]
	4	167.74 ± 3.69[b]
	8	444.51 ± 2.55[c]
	16	294.36 ± 5.95[d]
P. indicus	0	0
	2	91.05 ± 2.25[a]
	4	215.40 ± 7.39[b]
	8	522.91 ± 4.77[c]
	16	374.12 ± 8.76[d]
C. fistula	0	0
	2	57.07 ± 3.24[a]
	4	130.08 ± 9.59[b]
	8	359.55 ± 2.95[c]
	16	225.45 ± 5.27[d]

Remark: Different letters in superscript show significant difference in pair (P<0.05).

The utmost Cd accumulation was noted at 8 mg/L Cd of *P. indicus* and it gave the total Cd accumulation up to 522.91 ± 4.77ug/g dry wt., further observation revealed that amount of Cd accumulation was 144.55 ± 2.63 ug/g dry wt. in shoots and 378.36 ± 6.32 ug/g dry wt. in roots. Therefore, the screening of leguminous plant species for Cd accumulation resulted in choosing *P. indicus* as the best one **(Figure 1 and Figure 2).**

Effect of cadmium on relative growth of *A. Mangium, P. Indicus* ***and*** *C. Fistula*

Effect of cadmium on relative growth of *A. mangium, P. indicus* and *C. fistula* at 2, 4, 8 and 16 mg/L of Cd concentration and the exposure time of 15 days is illustrated in **Table 2.** All of three leguminous plants exposed to Cd concentrations of 2, 4, 8 and

16 mg/L, the significant decrease of the relative growth was observed (P<0.05). For *A. mangium*, at Cd concentrations of 2, 4, 8 and 16 mg/L, the relative growths were 1.05 ± 0.00, 1.02 ± 0.01, 0.97 ± 0.01 and 0.83 ± 0.01, respectively. Similar pattern of effect of Cd on percentage of biomass productivity was observed in *P. indicus*. At Cd concentrations of 2, 4, 8 and 16 mg/L, the relative growths were 1.07 ± 0.00, 1.05 ± 0.00, 1.01 ± 0.00 and 0.94 ± 0.01, respectively. Moreover, at Cd concentrations of 2, 4, 8 and 16 mg/L, the relative growths for *C. fistula* were 1.02 ± 0.00, 0.98 ± 0.00, 0.94 ± 0.01 and 0.80 ± 0.01, respectively.

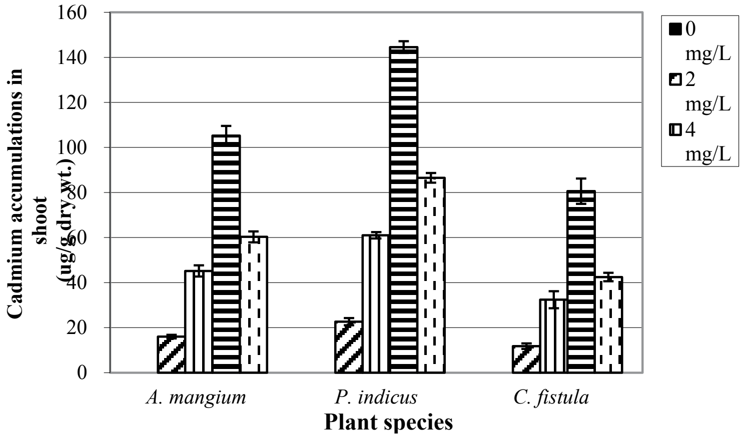

Figure 1. Cadmium accumulations in shoots of *A. mangium*, *C. fistula* and *P. indicus* for 15 days.

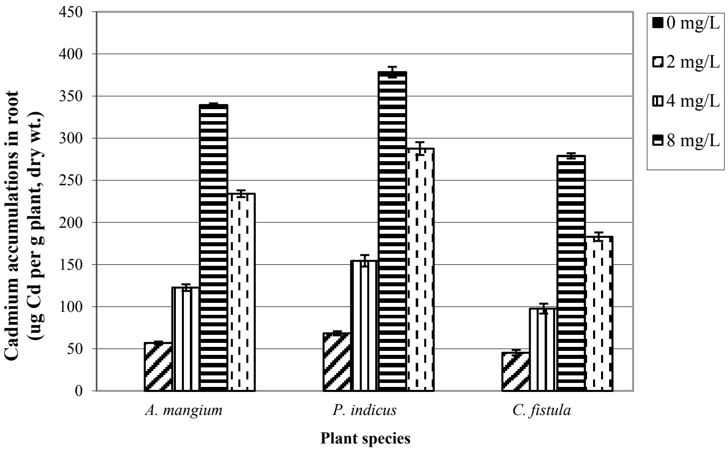

Figure 2. Cadmium accumulations in roots of *A. mangium*, *C. fistula* and *P. indicus* for 15 days.

Table 2. Mean (± SD) of relative growth of *A. mangium, C. fistula* and *P. indicus* for 15 days.

Plant species	Cadmium concentration (mg/L)	Mean (± SD) of relative growth
A. mangium	0	1.06 ± 0.01[a]
	2	1.05 ± 0.00[b]
	4	1.02 ± 0.01[c]
	8	0.97 ± 0.01[d]
	16	0.83 ± 0.01[e]
P. indicus	0	1.08 ± 0.01[a]
	2	1.07 ± 0.00[b]
	4	1.05 ± 0.00[c]
	8	1.01 ± 0.00[d]
	16	0.94 ± 0.01[e]
C. fistula	0	1.03 ± 0.00[a]
	2	1.02 ± 0.00[b]
	4	0.98 ± 0.00[c]
	8	0.94 ± 0.01[d]
	16	0.80 ± 0.01[e]

Remark: Different letters in superscript show significant difference in pair (P<0.05).

Considering relative growth of all three leguminous plants, the plant species that had better relative growth in every concentration was found in *P. indicus*, followed by *A. mangium* and *C. fistula.* In addition, during analysis of variance for relative growth between different plant species and Cd concentrations it was found that different species of plants and Cd concentrations had significant difference in relative growth (P <0.05).

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Effect of cadmium on biomass productivity of *A. mangium, P. indicus and C. fistula*

Effect of Cd on biomass productivity of *A. mangium, P. indicus* and *C. fistula* at 2, 4, 8 and 16 mg/L Cd with an exposure time of 15 days is presented in **Table 3.**

Table 3. Mean(± SD) percentage of biomass productivity of *A. mangium*, *C. fistula* and *P. indicus* after 15 days.

Plants species	Cadmium concentration (mg/L)	Mean(± SD) % of biomass productivity
A. mangium	0	100.00 ± 0.00[a]
	2	93.03 ± 1.19[b]
	4	83.10 ± 1.07[c]
	8	74.02 ± 1.84[d]
	16	60.59 ± 0.99[e]
P. indicus	0	100.00 ± 0.00[a]
	2	95.77 ± 2.27[b]
	4	86.80 ± 1.01[c]
	8	81.01 ± 0.34[d]
	16	71.76 ± 2.02[e]
C. fistula	0	100.00 ± 0.00[a]
	2	86.69 ± 2.28[b]
	4	78.04 ± 1.82[c]
	8	68.17 ± 1.34[d]
	16	51.74 ± 1.53[e]

Remark: Different letters in superscript show significant difference in pair (P<0.05).

All of three leguminous plants exposed to Cd concentrations of 2, 4, 8 and 16 mg/L, the percentage of biomass productivity were significantly decreased (P<0.05). For *A. mangium*, at Cd concentration of 2, 4, 8 and 16 mg/L, the biomass productivity were 93.03±1.19%, 83.10 ± 1.07%, 74.02 ± 1.84% and 60.59 ± 0.99% respectively. Similarly, *P. indicus* at Cd concentrations of 2, 4, 8 and 16 mg/L displayed the percentage of biomass productivity of 95.77 ± 2.27%, 86.80 ± 1.01%, 81.01 ± 0.34% and 71.76 ± 2.02% respectively. In case of *C. fistula*, for the Cd concentrations of 2, 4, 8 and 16 mg/L, the observed biomass productivity were 86.69 ± 2.28%, 78.04 1.82%, 68.17 ± 1.34% and 51.74 ± 1.53%, respectively.

Following this analysis the plant species that showed the highest percentage of biomass productivity in every concentration was *P. indicus. A. mangium* and *C. fistula* showed comparatively less biomass productivity. Analysis of variance calculation for percentage of biomass productivity between different plant species and Cd concentrations showed that different species of plants and Cd concentrations had significant difference in percentage of biomass productivity (P <0.05).

Bioconcentration Factor (BCF) of Cadmium in *A. Mangium*, *P. Indicus* and *C. Fistula*:

The BCFs of cadmium in *A. mangium, P. indicus* and *C. fistula* at 2, 4, 8 and 16 mg/L of Cd contamination while maintaining an exposure time of 15 days are shown in **Table 4.** The BCFs were significantly increased in all of the three leguminous plants, especially in shoots and roots (P<0.05). A significant decreased BCFs of cadmium was observed at 16 mg/L of Cd concentration in both shoots and roots (P<0.05). In *A. mangium*, the BCFs at 2, 4, 8 and 16 mg/L of Cd concentration were 8.00 ± 0.40, 11.29 ± 0.62, 13.15 0.54 and 3.77±0.15 in shoots, and 28.43 ± 0.91, 30.65 ± 1.00, 42.41 ± 0.26 and 14.63 ± 0.25 in roots, respectively. For *P. indicus*, the BCFs of Cd concentration were 11.32 ± 0.79, 15.25 0.35, 18.07 ± 0.33 and 5.41 ± 0.13 in shoots and 34.21 ± 1.23, 38.60 ± 1.70, 47.30 ± 0.79 and 17.98 ± 0.48 in roots, respectively under similar experimental conditions. In *C. fistula*, the BCFs at 2, 4, 8 and 16 mg/L Cd were 5.88 ± 0.62, 8.10 ± 0.94, 10.07 ± 0.70 and 2.65 0.12 in shoots, and 22.66 ± 1.59, 24.42 ±1.46, 34.87 ± 0.40 and 11.44 ± 0.32 in roots, respectively.

Table 4. Mean (±SD) of the BCFs (BCF) of cadmium in root of *A. mangium*, *C. fistula* and *P. indicus* for 15 days.

Plants species	Cadmium concentration (mg/L)	Mean(± SD) of BCF of cadmium in shoot	Mean(± SD) of BCF of cadmium in root
A. mangium	0	0	0
	2	8.00 ± 0.40[a]	28.43 ± 0.91[a]
	4	11.29 ± 0.62[b]	30.65 ± 1.00[b]
	8	13.15 ± 0.54[c]	42.41 ± 0.26[c]
	16	3.77 ± 0.15[d]	14.63 ± 0.25[d]
P. indicus	0	0	0
	2	11.32 ± 0.18[a]	34.21 ± 1.23[a]
	4	15.25 ± 0.35[b]	38.60 ± 1.70[b]
	8	18.07 ± 0.33[c]	47.30 ± 0.79[c]
	16	5.41 ± 0.13[d]	17.98 ± 0.48[d]

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C. fistula	0	0	0
	2	5.88 ± 0.62^{a}	22.66 ± 1.59^{a}
	4	8.10 ± 0.94^{b}	24.42 ± 1.46^{b}
	8	10.07 ± 0.70^{c}	34.87 ± 0.40^{c}
	16	2.65 ± 0.12^{d}	11.44 ± 0.32^{d}

Remark: Different letters in superscript show significant difference in pair (P<0.05).

Comparison of the BCFs of all three leguminous plants showed that *P. indicus* was having maximum BCFs in all the concentrations used in this study. This was followed by *A. mangium* and *C. fistula.* The greatest BCFs was observed at 16 mg/L Cd of *P. indicus* and it gave the BCFs up to 18.07 ± 0.33 in shoots and 47.30 ± 0.79 in roots. Analysis of variance for bio concentration estimation of cadmium for different plant species and Cd concentrations showed significant difference in BCFs (BCF) of cadmium (P <0.05).

DISCUSSION

Even though Cadmium is a non-essential heavy metal and is not toxic to plant at low concentrations but cadmium accumulation in soil and water now posing a major environmental and human health problem [15] . Due to the industrial revolution Cadmium pollution has accelerated dramatically [16]. Several investigators proposed the use of metal accumulating plants, such as, *Brassica jancea*, *Thlaspi caerulescens* to remove toxic metals, including cadmium. Studies of tree establishment or contaminated land have considered a number of different species, e.g. *Salix* (Willow), *Betula* (Birch), *Alnus* (Alder) and *Acer* (Sycamore) [17]. While many of these studies were interested primarily in metal uptake, distribution and accumulation and toxicity symptoms with the purpose of phytoremediation related analysis where most attention has been paid to fast growing species[18].

In this study, the researcher focused on the native species such as the leguminous plants, which are found throughout the country beside the tropical forest and used in the experiment for the Cd accumulation. All the three leguminous plants,i.e.., *A. mangium, P. indicus* and *C. fistula* possesses the potential to accumulate cadmium. Results indicated that the Cd accumulation were significantly increased (P<0.05) in plant parts like roots and shoots when exposed to cadmium concentrations of 2, 4 and 8 mg/L. However, a significant decrease of cadmium accumulation in plants occurred at 16 mg/L(P<0.05) cadmium concentration. *P. indicus* showed better Cd accumulation both in root and shoot of plants (395.81 ± 4.59 ug/g dry wt. and 144.55 ± 2.63 ug/g dry wt, respectively).

Cd accumulation in plants is related to their uptake capability. In *P. indicus*, the increased accumulation of cadmium in both stems and roots corresponded with metal concentration in the external media. However, the uptake was not linearly incremental in relation to the increasing external concentration. This may be due to the fact that plants exposed to high concentration of cadmium (16 mg/L) were not as healthy as those treated with the lower concentrations (2, 4 and 8 mg/L).

In addition, it may be due to the abilities of plants to translocate cadmium from root to shoot, which were different in 2, 4, 5 and 16 mg/L Cd treatments. From the study, the better translocation of Cd in plants treated with 8 mg/L of Cd concentration was clearly observed in *P. indicus* whereas the translocations of Cd in plants at 16 mg/L of Cd concentration were significantly decreased in all the plant species.

As a result, it was found that among three leguminous plants, *P. indicus* had the highest cadmium accumulation capability when exposed to the cadmium concentrations under the experimental setup. Therefore, the results suggest that *P. indicus* species should be selected as a suitable leguminous plant species for further study where the optimum concentration of Cadmium might be maintained as 8 mg/L Cd.

In addition, growth changes in plant are often the first and most obvious reactions under heavy metal stress [19]. In this study, it is evident that relative growth and biomasss productivity of all the three leguminous plants, i.e., *A. mangium, P. indicus* and *C. fistula* significantly decreased (P<0.05) when the cadmium concentration was increased. Among three leguminous plants, *P. indicus* showed the comparatively better relative growth and biomass productivity than other species when exposed to the various cadmium concentrations during the experimentation.

Finally, the result of the leguminous plants indicated that the BCFs were significantly increased (P<0.05) in plants, specifically in roots and shoots when the treated solutions were increased during the 15 day experimentation. However, at cadmium concentration of 16 mg/L, the BCFs significantly decreased (P<0.05) while compared with the lower cadmium concentrations. The plant species that had highest BCFs for each individual concentration was *P. indicus*, followed by *A. mangium* and *C. fistula.* As a result, this can be confirmed that *P. indicus* species should be selected for the further remediation of Cd contamination.

CONCLUSION

The fortnight long study on cadmium accumulation of *A. mangium, P. indicus* and *C. fistula* revealed that cadmium accumulation significantly increased (P<0.05) in shoots and roots of the plants. However, at experimental concentration of 16 mg/L, the cadmium accumulation significantly decreased (P<0.05) when compared with the lower concentrations applied for this study.

P. indicus had highest cadmium accumulation in all experimental concentrations. The second one was *A. mangium* and the lowest cadmium accumulation was found in *C. fistula*. With regards to the capability of accumulation of cadmium in plant roots were more efficient than shoots. . The greatest cadmium accumulation occurred at 8.0 mg/L Cd of *P. indicus* and the cadmium accumulation went up to 105.24 ± 4.29 ug/g dry wt. in shoots and 359.14 ± 5.25 ug/g dry wt. in roots.

The study on the effect of cadmium on relative growth and biomass productivity of *A. mangium, P. indicus* and *C. fistula* continued for 15 days indicated that they were significant decrease in biomass productivity when the cadmium concentration was increased ($P<0.05$). In addition, the bioconcentration factor (BCF) of Cd in *A. mangium, P. indicus* and *C. fistula* were significantly increased ($P<0.05$) in plants parts (roots and shoots) when the experimental concentrations were increased ($P<0.05$) . The greatest bioconcentration factors found at 16 mg/L Cd of *P. indicus* were 18.07 ± 0.33 in shoots and 47.30 ± 0.79 in roots.

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