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REMOVAL OF HEAVY METALS FROM WASTEWATER BY USING PHYTOREMEDIATION TECHNOLOGY

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Abstract — Contamination of soil and water by toxic metals is a major environmental hazard. The removal of heavy metals from wastewater by chemical methods is very costly and does not apply to the pretreatment process. Hence, phytoremediation process is one of the simplest methods to restore polluted environments. The present research paper investigates the potential of Mimosa Pudica for bio removal of heavy metals like Cd, Pb and Cu from wastewater by using phytoremediation or bioremediation technology. Heavy Metals were detected before and after the process using Atomic Adsorption Spectrometer (AAS). The synthetic wastewater contains Cd, Pb, and Cu at an initial concentration of 0.25mg/l, 0.5mg/l, and 2mg/l was introduced to the soil mass planted by Mimosa Pudica and treated for 16 days through their root. Collected samples were taken for laboratory analysis. The result showed that there was a reduction in Cd, Pb and Cu at a concentration of 0.02mg/l, 0.21mg/l, and 0.4mg/l level of heavy metals from the wastewater. After that, investigation of the potential of Mimosa Pudica accumulates up to 92% of Cd, 58 % of Pb, and 80% of Cu. The evidence presented by this study specified that Mimosa Pudica is an efficient accumulator plant for phytoremediation or bioremediation.

Keywords: Heavy metals, Mimosa pudica, phytoremediation, synthetic wastewater, xylem, phloem

1.0 INTRODUCTION

In recent years heavy metal pollution is a huge problem that may occur in soil and water which has harmful effects on plants, animals and humans. Heavy metals such as arsenic, mercury, cadmium, chromium, lead, thallium, etc. are categorized as hazardous contaminants to the environment [1,2]. Rapid population growth, deforestation, urbanization, development of industries, exploration, and exploitation of the environment have resulted in pollution of strong metals and metalloid to the world's environment. Problems caused by heavy-metal pollution pose a serious threat to human health and the entire ecosystem. For creating a world that can sustain environmental demand, strong industrial growth and technologies which lead to refining heavy metal pollution are increasing in demand [3]. Sources are industrial, agricultural and domestic effluents. Extreme release of heavy metals from industrial and urban areas into the environment is a great problem worldwide. Heavy metals do not easily degrade into harmless products. A large exploit of sewage residues, fertilizers, quarry wastes, and manufacturing development companies without control outputs creates consequential accretion of increase in the concentration of metals in agricultural lands that remained in the soil for many years. The increased practice of using heavy metals in manufacturing companies and farming activities have caused severe troubles of environmental deterioration [4].

Heavy metals such as Cadmium (Cd), Zinc (Zn), Chromium (Cr), Lead (Pb) and Copper (Cu) are examples of the toxic heavy metals which have been recognized for their negative effects on the ecosystem, where they can go up the food chain hence posing serious hazard to individual health. Heavy metal causes some diseases as a permanent effect on biological systems, which can gather in the human body [5]. The pollution of these metals affects the ecosystem and leads to global warming. This enforces measures to prevent metal pollution. The use of appropriate treatment technology is needed to solve the problem of water pollution [6]. Both human health and the environment are affected by the presence of heavy metals in wastewater. Heavy metals are showing serious threats to the living organisms and the surrounding environment in terms of the growth of plants, quality of crops and total yield. Cadmium, lead and copper among the heavy metals are found in higher percentages in wastewater and have the most serious effects on the environment and human beings. Among the heavy metals, cadmium causes the most toxic effects due to its non-biodegradability property and bioaccumulation when it is ingested even at very low concentrations. Cadmium is an ever-present non-essential element that possesses high toxicity and is easily accumulated from the environment by organisms and transferred to the food chain from the soil. The biological half time in humans for cadmium is 10 to 30 years and it accumulates in kidney cells and develops various chronic diseases [7]. In general, cadmium is introduced in water through the runoff from metal refineries, paints and waste batteries and also naturally from a volcanic eruption. Similarly, the source of lead which contaminates soil water is corrosive to the household plumbing systems and industrial effluents. The ability to synthesize the red blood cells and the central nervous system is affected by the lead [8]. It can also cause subtle abortions and miscarriages. Copper typically has a high concentration found in wastewater because it is considered the most valuable and generally used metal in many industries. The presence of copper in the human body causes damage to the liver and kidney and its effects include GI mucosal ulcerations and bleeding, cardiotoxicity with hypotension, etc. Therefore, the proper treatments should be developed essentially for the reduction of these heavy metal content in wastewater to avoid poisoning. However, extreme absorption of these heavy metals can develop into poisonous to vegetation and a possible risk to humans [9]. Copper is required for safe and reasonable methods for the exclusion of heavy metal from contaminated sources has necessitated research toward the production of cost-effective alternatives to commercially available materials. In the present research, three types of heavy metals named cadmium, lead, and copper has been selected because they are the primary concern to affect human health based on their toxicity [5] and recorded in soil and wastewater sample at higher concentration [10]. Several researchers had explained that bioaccumulation of above heavy metals in a higher percentage had observed in roots as compared to stem [11-13]. Remediation of heavy metal polluted water could be carried out through various processes like ion exchange, reverse osmosis, chemical precipitation, adsorption, membrane systems and electrodeposition. However, the measure requires external man-made resources and is more expensive. Considering the cost containment, in recent years, engineers started to generate low-cost technology to remove heavy metals from wastewater like phytoremediation.

Phytoremediation is an advanced and recently developed technique, where the usage of direct living green plants is used for the elimination of contaminants from soils, air, surface water and groundwater [14]. In this mechanism, pollutants are absorbed by the roots, accumulated in its body tissues and decompose the pollutants to a lower hazardous form. The roots of the plant reduce the contaminants present in soil by exudates. These roots demobilize, stabilize and bind to contaminants, which is termed as Phyto stabilization process. Again, the roots of certain species of plants adsorb, accumulate and precipitate the contaminants in water and soil through the immobilization process. Phytoremediation shows potential and economic technology that makes use of plants to remove, convert or stable pollutants from the environment. Phytoremediation methods are suggested for suitable techniques for de-contaminating polluted ecological surroundings like land, water, and air by trace metals as well as natural substances. The phytoremediation process consists of Rhizosphere biodegradation, Phyto stabilization, Phytoextraction, Rhizo filtration, Phyto volatilization, Phyto degradation and hydraulic control. In the rhizosphere biodegradation process, the plant releases natural substances through its roots and supplies nutrients to microorganisms. These microorganisms increase biological degradation. In the Phyto stabilization process, chemical substances are produced by the plant and stopped the contaminants from areas, before degrading them. In the process of Phytoextraction, plant tissue absorbs the contaminants along with other chemical constituents and water. The contaminant is not destroyed but ends up in the plant shoots and leaves. This method is adopted only for waste-containing metals. Rhizo filtration is similar to Phytoextraction, but the plants used for cleanup are raised in greenhouses with their roots in water. In Phyto volatilization, plants occupy water containing organic contaminants and release the contaminants into the air through their leaves. Plants metabolize and demolish contaminants within the plant tissues in the Phyto degradation process. In the process of Hydraulic Control, trees are indirectly remediated by controlling groundwater movement. Plants perform as a normal force when their roots reach down towards the water table and establish a dense root mass that takes up large quantities of water.

Several plants that have been identified in past years as highly effective in absorbing and accumulating various toxic metals are being evaluated for their role in the phytoremediation of soils and water polluted with trace elements. Normally, macrophytes (aquatic plant that grows in or near water and is either emergent, submergent, or floating) such as water mimosa, water hyacinth, water spinach, smartweed and lesser duckweed has been used in wastewater treatment as this plant grow rapidly, easy to harvest and can accumulate pollutant. Plants that can accumulate more metals to high concentration are called "Hyper accumulators".

Terrestrial plants are used for treating polluted soil and water by accumulating heavy metals in their tissues. Many previous studies have been used living plants as bio-absorbent metal removal, due to better growth and high tolerance of plants while they are exposed to heavy metals [15,16]. In the present research, Mimosa pudica has been selected for removal of heavy metals from waster based on the following selection criteria: (1) high vitality and productivity, (2) high abundance, (3) literature and local knowledge of this species. The plant used in the present research is shown in Figure 1. The roots of Mimosa pudica create carbon disulfide inhibiting certain mycorrhizal growth and pathogenic fungi within the plant rhizosphere. Due to these nodules on plant roots are formed which contain endosymbiotic diazotrophs. It has been found that heavy metals can accumulate in the root of Mimosa Pudica. This process itself indicates that the Mimosa Pudica plant is capable of the removal of heavy metals from wastewater by the phytoremediation process. This plant naturally grows in terrestrial land, therefore, providing certain benefits in its cultivation and use as a highly therapeutic, evergreen, high biomass production, and a good adaptation. Mimosa Pudica has toxic alkaloid mimosine, which was found to also have antiproliferative and apoptotic effects. It consists of both antioxidant and antibacterial properties. This plant has also been demonstrated to be non-toxic in brine shrimp lethality tests, which suggests that Mimosa Pudica has low levels of toxicity. Mimosa Pudica contains various chemical compounds like alkaloids, flavonoid C-glycosides, sterols, terpenoids, tannins, and fatty acids. The roots of the plant create carbon disulfide, which prevents pathogenic bacteria and fungi from water. Upon treating the wastewater, the plant takes heavy metal out. It means that microbes are living under the roots of the plant. Microbes are doing 90% of the work. In this study, the Investigator has adopted to choose the plant "Mimosa Pudica" for phytoremediation, because it has more tolerance to heavy metals compared with other terrestrial plants.

Figure 1 Mimosa Pudica Plant

The researchers worldwide have given their attention to Mimosa Pudica for its pharmacological activities such as antitoxin, antioxidant, antidiabetic, antihepatotoxic, wound healing, etc. [17]. The extraction of methanolic from the root of the Mimosa Pudica plant had been used as a very good healing activity probably due to the presence of phenols constituents [18]. Also, the methanolic extract from the root of this plant was tested for various antimicrobial activity such as Citrobacter divergence, Aspergillus fumigatus and Klebsiella pneumonia for different concentrations of 50, 100 and 200 μg/disc [19]. The ethanolic extract from the leaf of this plant was tested for analgesic activity and anti-inflammatory at the doses of 200 and 400 mg/kg. Successful results were found due to the presence of flavonoids in the ethanolic [20]. The ethanolic also had been evaluated for anti-diarrhoeal potential. The anti-diarrhoeal property of leaves may be related to the flavonoids and tannin present in the extract [21]. The decoction of leaves of Mimosa Pudica had protected mice against strychnine-induced seizures and pentylenetetrazol at the dose of 1000 to 4000 mg/kg [22]. The length of the estrous cycle had been prolonged and the duration of the diestrus phase increased significantly when the root of Mimosa Pudica was administrated orally at a dose of 300 mg/kg body weight/day [23]. Extraction of the methanol crude from the aerial part of Mimosa Pudica had been screened in vitro for antioxidant activity using the 1, 1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. Mimosa Pudica contains glycoside, flavonoid, tannins and alkaloid. It is also used for removing sexual weakness and blood coagulation. Every part of this plant possess medicinal properties and is used in the various treatment of dysentery, burning sensation, inflammations, vaginal and uterine complaints, biliousness, and etc. [24]. By seeing the above medicinal properties and pharmacological characteristics of Mimosa Pudica, it is decided that this plant will be used for the treatment of wastewater with biological applications. The present study aims to assess the potential of the phytoremediation ability of Mimosa Pudica for bio removal of heavy metals from wastewater.

2.0 RESEARCH METHODOLOGY

2.1 Sample Preparation

Metal solutions of Cadmium (Cd), Lead (Pb) and Copper (Cu) were prepared by dissolving Cd, Pb, and Cu separately in double-distilled water to result in known concentrations of the metal ions required and to make synthetic wastewater. A digital pH meter prepared with a combined glass electrode was used for the pH adjustments. The pH of the wastewater was adjusted by using HNO_3 and NaOH. Based on the literature review, the effect of pH on phytoremediation is 5.5 to 7.0. In this study pH of wastewater used was at a constant range of 6.

2.2 Phytoremediation of Heavy Metals

The setup of the phytoremediation process was placed in the laboratory and monitored regularly. In the laboratory, a tray of size 2.0 m length, 1.5 m breadth and a varied depth i.e. 15 cm at the inlet and 18 cm at the outlet was used for synthetic wastewater treatment. The base slope was kept as 1:66 for easy drainage of treated water through the outlet. The tray was filled with soil. Before putting the soil, it was confirmed that the soil mass did not contain any heavy metals. The bottom of the metallic tray was filled with a crushed aggregate of 10 mm size for 2cm of height to avoid the choke up at the outlet by the soil particles. The setup was similar to the rootzone filter system. The plants of Mimosa Pudica were planted at a rectangular pattern 15 cm apart from each other as shown in Figure 2. The solution of synthetic wastewater of 40 liters as described in section 2.1 was introduced to the soil for the 1st day through the inlet and it is allowed to pass in a horizontal direction through the entire soil length towards the outlet. From the 2nd day, 3 liters of synthetic wastewater of heavy metal were added to soil through the inlet on regular basis up to the 16th day because the water was lost daily from the soil through evaporation and transpiration process. The room temperature was maintained as $25^0 \pm 5^0$C and the relative humidity was 35. The microbes living at the root of the Mimosa Pudica plant can absorb heavy metals. The treated water was collected through an outlet on the 4th, 8th, 12th and 16th days as samples. The absorption of heavy metals was examined by using the AAS method.

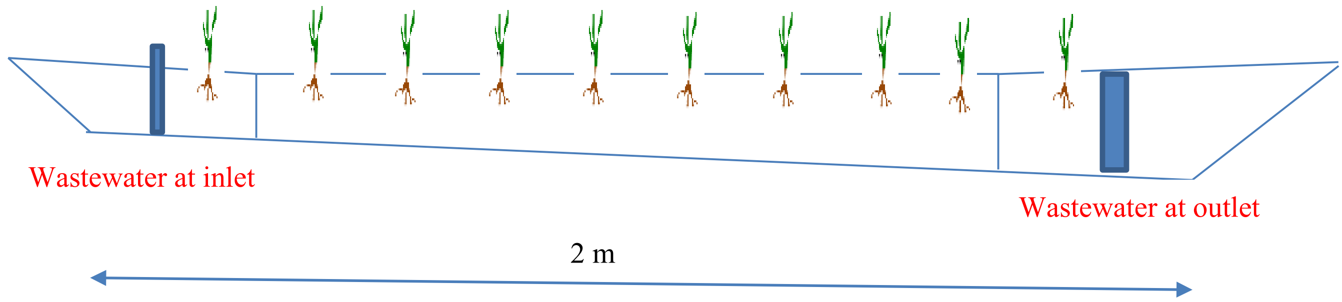

Figure 2 Lab setup for present research

3.0 EXPERIMENT AND RESULT

The study focused on evaluating the effectiveness of Mimosa Pudica as a phytoremediation agent in the removal of heavy metals from wastewater. The result of the study conducted for 16 days were presented. The absorption concentrations of Cd, Pb and Cu from collected synthetic wastewater samples at regular intervals before and after the phytoremediation process are noted.

Table 1 Initial concentration of heavy metals in wastewater

Heavy metal	pH	Initial Concentration mg / lit	Toxicity Limit as per WHO in mg/lit	References
Cd	6	0.25	0.003	[25]
Pb	6	0.5	0.01	[25]
Cu	6	2	1.3	[26]

Table 1 illustrated the initial concentration of trace metals in the synthetic wastewater. Cd level was 0.25mg/l, Pb was 0.5mg/l and Cu was 2mg/l with pH 6.

Table 2 Phytoremediation of Cd removal efficiency of wastewater at regular intervals

Heavy metal	pH	Initial Concentration mg / lit	Period Days	Final Concentration mg / lit	Removal efficiency (%)
Cd	6	0.25	4	0.15	40
	6	0.25	8	0.12	52
	6	0.25	12	0.08	68
	6	0.25	16	0.02	92

Table 2 showd the phytoremediation of Cd in synthetic wastewater at regular intervals. Cd level was 0.15mg/l on the 4th day, 0.12mg/l on the 8th day, 0.08mg/l on the 12th day and 0.02mg/l at the end of the 16th day. Compared to the 4th day, 0.15mg/l of Cd concentration in wastewater was reduced to 0.02mg/l at the end of the 16th day. It is evident from this table that the efficacy of Mimosa Pudica in the removal of Cd from wastewater improved from 40% on the 4th day to 92% at the end of the 16th day as shown in Figure 3. The heavy metal cadmium accumulated in the root of Mimosa Pudica due to the immobilization of cadmium from the vacuoles. Phloem most probably played a vital role in the accumulation of cadmium in the root of the Mimosa Pudica plant. It was found that the mean concentration of cadmium in phloem was 2 to 4 times higher than the other organs of the plant and it decreased by the other organs in the order of root, xylem and capsules [27]. The cadmium absorption reached up to 3.34 mg/kg, when soil content about 10 mg/kg [28]. The cadmium had transported by phloem and redistributed within the shoot for the accumulation in seeds and fruits. The penetration of metalloids like cadmium through the plant root was possible by passive process, most probably by cataions exchange process which occurred in the cell wall. As the treatment duration had increased the cataion exchange process has gradually increased and after the 12th day, the removal efficiency had recorded as highest. Hence it is recommended that the treatment process for removal of heavy metal by phytoremediation method must be carried out at least for two weeks.

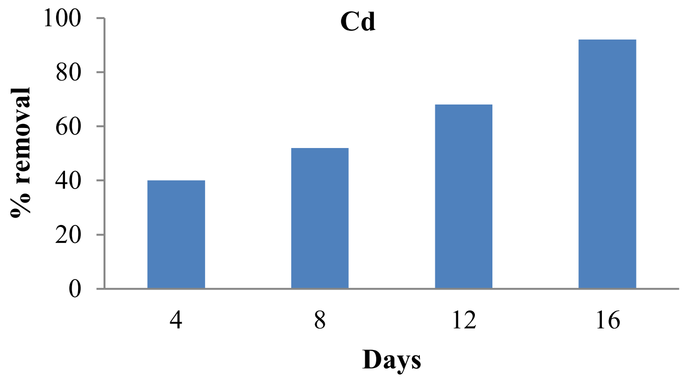

Figure 3 Percentage of removal of Cd using Mimosa Pudica

Table 3 Phytoremediation of Pb removal efficiency of wastewater at regular intervals

Heavy metal	pH	Initial Concentration mg / lit	Period Days	Final Concentration mg / lit	Removal efficacy (%)
Pb	6	0.5	4	0.38	24
	6	0.5	8	0.31	38
	6	0.5	12	0.25	50
	6	0.5	16	0.21	58

Table 3 shows the phytoremediation of Pb in synthetic wastewater at regular intervals. Pb level was 0.38mg/l at 4^{th} day, 0.31mg/l at 8^{th} day, 0.25mg/l at 12^{th} day and 0.21mg/l at the end of 16^{th} day. Compared to the 4^{th} day, 0.38mg/l of Pb concentration in wastewater was reduced to 0.21mg/l at the end of the 16^{th} day. It is evident from this table that the efficacy of Mimosa Pudica in the removal of Pb from wastewater improved from 24% on the 4^{th} day to 58% at the end of the 16^{th} day as shown in Figure 4. Previous studies have documented that lead is accumulated into leaf tissue in the phytoremediation process [29]. In general, the leaves of aquatic plants are bigger, hence a higher amount of lead can be stored in a larger space. But the leaves of terrestrial plant-like Mimosa Pudica are smaller in size as compared to aquatic plants like Nelumbo Nucifera. Hence, the percentage of removal of lead on the 16^{th} day was found to be very less in amount compared to the percentage of removal of cadmium and copper. Hence it is recommended that the aquatic plant should be used for the removal of lead from wastewater. The plants like Mimosa Pudica can grow in extremely hostile conditions of low nutrient, low acidity, waterlogged areas and elevated metal concentrations. It has been proposed that coatings of iron hydroxide on the roots of terrestrial or wetland plants may act as a good carrier to the uptake of phytotoxic metals into plant tissues by adsorption and immobilization process by iron plaque. The Mimosa Pudica plant might develop iron plaques at its roots. Hence the lead might be absorbed by iron oxide layers developed on roots not taken by the plant tissues. In the present research, the iron plaque may be a source of lead transport to the root and shoot of the Mimosa Pudica plant.

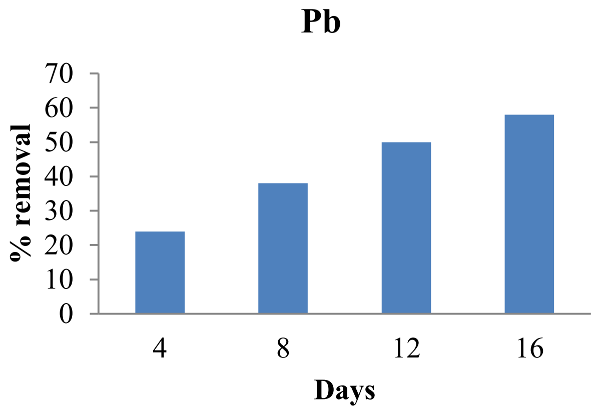

Figure 4 Percentage of removal of Pb using Mimosa Pudica

Table 4 Phytoremediation of Cu removal efficiency of wastewater at regular intervals

Heavy metal	pH	Initial Concentration mg / lit	Period Days	Final Concentration mg / lit	Removal efficacy (%)
Cu	6	2	4	1.2	40
	6	2	8	0.84	58
	6	2	12	0.75	63
	6	2	16	0.4	80

Table 4 shows the phytoremediation of Cu in wastewater at regular intervals. Cu level was 1.2mg/l on the 4th day, 0.84mg/l on the 8th day, 0.75mg/l on the 12th day and 0.4mg/l at the end of the 16th day. Compared to the 4th day, 1.2mg/l of Cu concentration in wastewater was reduced to 0.4mg/l at the end of the 16th day. It is evident from Table 4 that the efficacy of Mimosa Pudica in the removal of Cu from wastewater was improved from 40% on the 4th day to 80% at the end of the 16th day as shown in Figure 5.

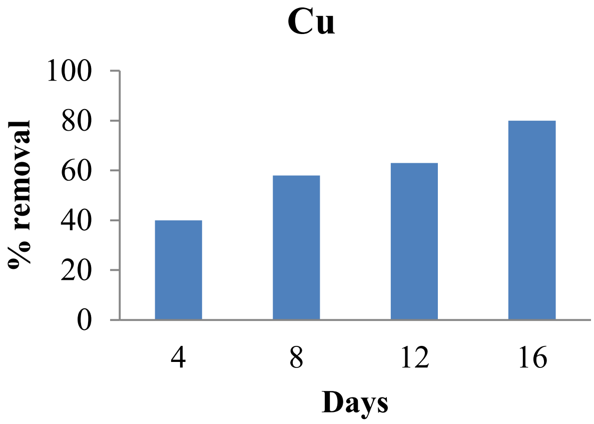

Figure 5 Percentage of removal of Cu using Mimosa Pudica

The root system of the plant plays an important role in the phytoremediation process because the initial contact with contaminants happened by itself. The study shows the result of heavy metal concentration found in wastewater samples at 4 days interval for 16 days. It is evident from the above tables that the concentration of Cd (0.25mg/l), Pb (0.5mg/l) and Cu (2mg/l) before phytoremediation were found to be reduced to Cd (0.02mg/l), Pb (0.21mg/l) and Cu (0.4mg/l) after 16 days of phytoremediation respectively. Hence, the cumulative concentration of copper uptake within the Mimosa Pudica plant has increased as time progressed. It is indicative of the phytostabilization potential for long days. But the rate of copper absorption was high for the first four days, and then the rate of absorption capacity slowly decreased with time. This type of behavior signifies that the translocation of contamination by other parts of the plant (i.e. culm and leaves) is starting at later ages of treatment of synthetic wastewater. It may be also possible that there is further restriction of roots for entering the copper into its cells for the prevention of further damage. From previous literature, it was found that translocation factor for copper concentration at 21 days of treatment process, the culm, branches and leaves were found to be 0.42, 0.063 and 0.17 respectively[30]. The uptake solution may be different depending upon the root system of plants. For smaller roots, the change of volume may be negligible. Hence it is assumed that the contaminant removal depends upon the concentration change in the case of phytoremediation of copper. It may also be possible that Mimosa Pudica can also play a role as erosion control agents near the contaminated bodies of water due to their large root biomass. It is also evident from the study that the percentage of removal efficacy of heavy metals from the samples improved from 40% to 92% for Cd, 24% to 58% for Pb and 40% to 80% for Cu respectively shown in Figure 6.

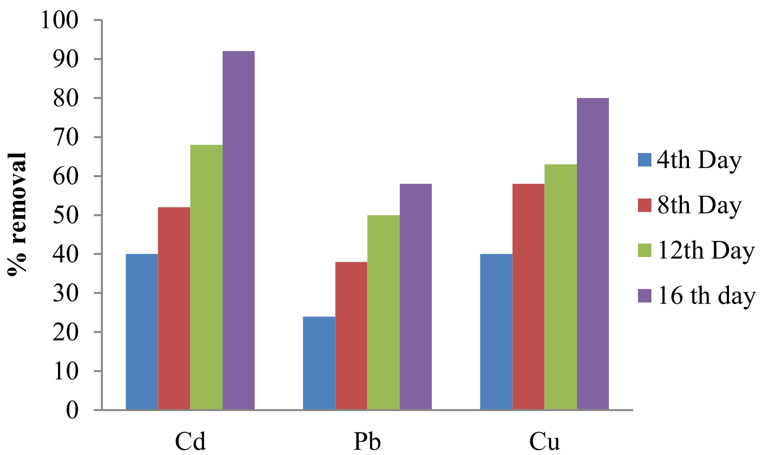

Figure 6 Removal effectiveness of various Heavy Metals using Phytoremediation

The study results proved that the Mimosa Pudica was found to be a good accumulator of Cd and Cu compared to Pb. These results explained that phytoremediation could be effective to reduce heavy metals from wastewater. The above three types of heavy metal taken by the Mimosa Pudica plant through translocation and absorption process and released by excretion. The accumulations of heavy metal in Mimosa Pudica were not directly transferred through the xylem. In this instance, these heavy metals were possibly transferred through the phloem after the transfer of heavy metals by xylem to the phloem at the plant nodes. From test results, it can be noticed that the root of Mimosa Pudica reached saturation at the end of the 16^{th} day of treatment and some mechanisms exist in its root that could detoxify the heavy metals of Cd, Pb and Cu or transfer them to aerial parts [31]. Since phloem is the mineral carrier as well as a productive part of the Mimosa Pudica contains bast fiber, the detection and safety evaluation for utilization of raw materials is required. Another thing noticed through the cumulative absorption of heavy metals increases as the curing period is increasing but, the rate of absorption by root is more for the first four days and the absorption rate gradually decreases as the time lapsed. The result of the present study is found to be similar to the study conducted by Aini Syuhaida (2014) [6], and Abdul Wahab (2014) [9] who assessed the phytoremediation ability of water mimosa and its protection for human consumption. The study confirmed that Mimosa Pudica may be used to rectify the contaminated wastewater with trace metals such as Cadmium (Cd), Lead (Pb) and Copper (Cu).

4.0 CONCLUSION

The wide usage of heavy metals in industrial and agricultural activities leads to a major concern of environmental pollution. Polluted wastewater contains a major biological and individual health problem, which may be partially resolved by the up-and-coming phytoremediation technology. The results obtained from the present research are as followd.

(i) It was deducted that there was a reduction of 0.02mg/l Cd, 0.21mg/l Pb and 0.4mg/l Cu in the final compared to the initial concentration from the obtained samples.
(ii) Mimosa Pudica accumulates upto 92% of Cd, 58 % of Pb, 80% of Cu. Hence it is concluded that Mimosa Pudica can be used as an effective method to remove the heavy metals from wastewater.
(iii) The rate of absorption of heavy metals is more in the early days of treatment is indicative that at the initial few days the accumulation process is conducted by the roots only and later the translocation of contamination is carried by the shoots of the plant.
(iv) The lower percentage of accumulation of Pb indicates that the root of the terrestrial plant with small leaf size is not suitable for the removal of Pb from wastewater. Aquatic plants with broadleaf should be used for removal of lead by phytoremediation process.
(v) From the present research, it is recommended that the wastewater should be treated by Mimosa Pudica for at least two weeks for removal of heavy metals more than 90%.
(vi) Treatment of wastewater by using the phytoremediation process with the terrestrial plant may be an alternative solution as a green technology.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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