

PHYTOREMEDIATION OF HEAVY METALS FROM WASTEWATER BY CONSTRUCTED WETLAND MICROCOSM PLANTED WITH *ALOCASIA PUBER*

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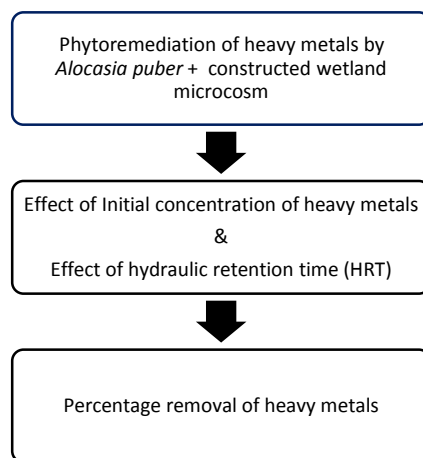
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Graphical abstract



Abstract

Water pollution by toxic heavy metals is a global environmental problem. It has led to the development of alternative technologies for heavy metals removal from contaminated sites. Constructed wetland microcosm by using *Alocasia puber* is a possible treatment method for wastewater containing heavy metals. Synthetic wastewater with heavy metals Cd, Cr, Cu, Ni, and Zn were used in this study. Several heavy metals concentrations (5 mg/L, 10 mg/L and 100 mg/L) were used in the systems. Six different hydraulic retention times (HRTs) (2, 4, 6, 8, 10 and 12 days) were tested in the present study. The results obtained showed removal efficiencies of heavy metals of >99% after day 12. The removal of Ni from 10 mg/L solutions (initial concentrations) recorded the best removal efficiency. Heavy metal translocation factor (TF) was found to be less than 1 for all metals tested, which confirmed the significance of roots as heavy metals accumulator compared to stems or leaves of *A. puber*. Therefore, this study concluded that *A. puber* has a great potential as an important component in constructed wetlands for water contaminated with heavy metals.

Keywords: Phytoremediation, *Alocasia puber*, constructed wetlands, heavy metals, waste water contamination

Abstrak

Pencemaran air oleh logam berat yang toksik merupakan masalah alam sekitar di seluruh dunia. Masalah ini telah membawa kepada pembangunan teknologi alternatif untuk menyingkirkan logam berat di kawasan yang tercemar. Tanah lembap rekaan mikrokosm yang ditanam dengan *Alocasia puber* telah dikaji sebagai perawat sisa air yang tercemar dengan logam berat. Sisa air sintetik yang mengandungi logam berat iaitu Cd, Cr, Cu, Ni, dan Zn telah digunakan dalam kajian ini. Kepekatan logam berat dalam air yang masuk ke dalam sistem telah ditetapkan pada 5 mg/L, 10 mg/L dan 100 mg/L. Enam ketetapan masa hidraulik (HRTs) yang berbeza telah digunakan untuk sistem rawatan dengan HRTs 2, 4, 6, 8, 10 dan 12 hari. Hasilnya, kecekapan penyingkiran logam berat adalah sehingga > 99% selepas hari ke-12. Penyingkiran Ni daripada 10 mg/L (kepekatan awal) larutan mencapai kecekapan penyingkiran tertinggi. Faktor traslokasi logam berat (TF) didapati kurang daripada 1 untuk semua logam berat yang dikaji, mengesahkan kepentingan akar sebagai pengakumulasi logam berat berbanding batang atau daun *Alocasia puber*. Hasil kajian ini menunjukkan potensi besar *Alocasia puber* di tanah lembap rekaan untuk fitoremediasi air yang tercemar dengan logam berat.

Kata kunci: Fitoremediasi, *Alocasia puber*, tanah lembap rekaan, logam berat, pencemaran sisa air

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1.0 INTRODUCTION

Various techniques are available for the purpose of heavy metals removal from the environment. Some of the most common methods used in wastewater include ion exchange, filtration, precipitation, reverse osmosis and adsorption. However, these methods are costly and have detrimental effects on the affected area [1]. Therefore, there is a need for new alternative treatments using low cost materials.

Constructed wetlands (CWs) have been widely used in wastewater for the removal of many contaminants, such as suspended solids, organic compounds, nutrients, pathogens, metals, and emerging contaminants [2]. CWs are gaining popularity in many countries as a wastewater treatment process due to its effectiveness [3]. It is not only cost-effective but also an operation-flexible solution compared to conventional wastewater treatment processes [4].

Núñez et al. (2011) tested the removal rates of heavy metals (Hg, Cu, Pb, Cd, and Zn) by CW with *Eichhornia crassipes*, *Ludwigia helminthoriza*, and *Polygonum punctatum* where removal rates were up to 100 % [5]. Collins et al. (2005) reported that acid mine drainage showed removal rates of 75-99 % cadmium, 26 % lead, 76 % silver, and 67 % for zinc [6].

Previous studies have also shown that metal removal processes using CWs are complex involve a combination of biotic and abiotic elements such as sedimentation, flocculation, adsorption, precipitation, co-precipitation, cation and anion exchange, complexation, oxidation and reduction, microbial activity and plant uptake [7]. The metals cannot be destroyed but their chemical and physical characteristics can be modified [8].

The variation in removal percentage among studies may be related to differences in macrophyte species and density, media, wastewater type, retention times, loading rates, climatic condition, temperature, design and size of the setups [9].

Based on previous studies, most of the plants used in effective CWs are either weeds or aquatic plants, possessing high growth rate which is an important criteria in phytoremediation. Examples of plants used in CWs are *Colocasia esculenta* [10], *Typha latifolia* [11], *Phragmites australis* [12], etc.

The main objective of this study is to explore the potential of metal removal by *A. puber* in vertical flow CW microcosm. This plant species has not been studied in the treatment of wastewaters containing heavy metals. This plant was selected, as other species under the Araceae family such as *Colocasia esculenta*, *Alocasia macrorrhiza* and *Pistia stratiotes* are proven of having the ability to treat the site contaminated with heavy metals. Furthermore, it is easily propagated with high survivability. *A. puber* also possess a high growth rate and extensive root system making it ideal for phytoremediation process. In this study, *A. puber* planted in CW microcosm was tested whether it could take up more than one metal when

exposed to multi-metal solutions (Cd, Cu, Cr, Ni, and Zn). Several hydraulic retention time (HRT) and initial concentration of heavy metals were used to identify which metal can be treated by CW microcosm system.

2.0 METHODOLOGY

2.1 Experimental Design

CWs microcosms were set in a transparent rectangular basin made up of polypropylene (30 cm x 18.5 cm x 18 cm). An outlet with a diameter of 3 cm on the reactor were constructed for effluent collection. The sampling process of *A. puber* was taken place at Bukit Bakar, Machang and the plants were transplanted into the system (each microcosm had individual plants, to have a significant plant root effect on the wastewater treatment). All microcosms were wrapped with aluminum foil to avoid sunlight penetration and photodegradation of the compounds.

Every reactor consisted of three layers as shown in Figure 1. The first layer contains 10 cm of gravel (10–20 mm), in the middle layer, 5 cm of gravel (1–5 mm), and the top layer, 10 cm of soil with gravel (0.2–0.5 mm) without the supplementation of nutrient or fertilizer. Healthy *A. puber* that possess similar characteristics such as (same plant size & number of leaves) were then transplanted in each reactor. Synthetic wastewater was added to the system while the water level was kept at the upper soil layer to stimulate sub-surface flow (SSF) system. *A. puber* was selected and planted separately in three experimental units. One control unit was developed with the same design as the experimental units but without the plant.

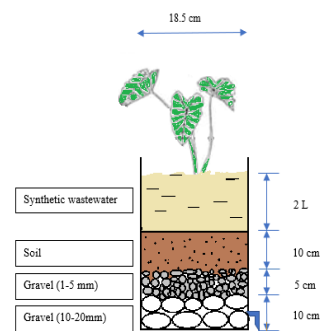


Figure 1 Experimental set up of CW reactor

Synthetic wastewater (2 L) was later added to each CWs microcosms on top of the substrate and the water levels were maintained at the upper soil layer to ensure sub-surface flow (SSF) and allowed to infiltrate each system [13]. The systems were designed to operate in batches, with the initial load of water and without any running flow during the tests, having only a tap at the base for sample collection.

2.2 Synthetic Wastewater Sample Preparation and Collection

Cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) were added at 5, 10 and 100 mg/L in water. All these metals were added as single metal solution, i.e. one experimental set contained a single metal concentration [14]. These heavy metals were added as CdSO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2$ and ZnCl_2 , dissolved in distilled water to obtain the desired concentrations.

Four water samples (100 ml) from each microcosm were collected every 2 days within 12 days wastewater sample were collected in glass bottles on each sampling day and stored at 4°C. Atomic Absorption Spectrometry (AAS) was used to determine the removal of heavy metals from synthetic wastewater [15].

2.3 Soil Sample Collection

Soil sample was collected at a depth of approximately 10 cm. It was assumed that at this depth, the soils sampled will cover the average root system of the plants. Soil samples (4 g) were digested in 15 ml of nitric acid (HNO_3 , 65% w/w) and 5 ml hydrogen peroxide (H_2O_2). AAS was used to determine the concentration of heavy metals in soil.

2.4 Plant Sample Collection

Plant samples that were involved in the phytoremediation process were collected. The content of metals before and after the treatment period were analysed. Roots, stems and leaves of the plants were dried at 105°C for 24 hours and were ground with a crushing machine. 0.5 g of dried plant materials were digested using 10 ml of HNO_3 , 65% (w/w). AAS was used to determine the concentration of heavy metals [15, 16].

2.5 Translocation Factor of Heavy Metals

Translocation factor (TF) was used to calculate the efficiency of phytoremediation. TF reflects the effectiveness of the plant when translocating the accumulated metal from roots to shoots using the following equation (1):

$$\text{TF} = (\text{C aerial}) / (\text{C root}) \quad (1)$$

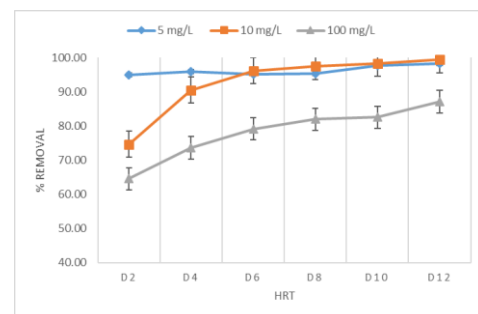
where C aerial is the metal concentration for above ground biomass (leaves + stem) and C root is the metal concentration in roots [18].

3.0 RESULTS AND DISCUSSION

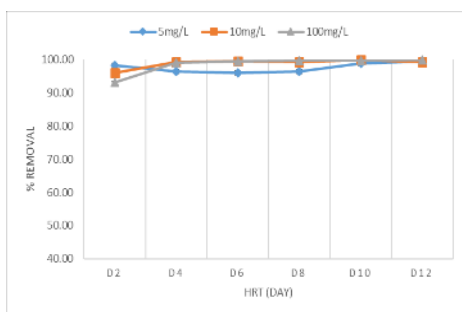
3.1 Heavy Metals in Wastewater

At the end of this experiment (after 12 days), it was observed that the concentrations (5 mg/L, 10 mg/L & 100 mg/L) of Cd, Cr, Cu, Ni and Zn in the synthetic wastewater samples exhibited a decreasing trend throughout the treatment period as shown in Figure 2. It was clear that the CW managed to reduce the concentrations of all metals tested to significantly lower levels within 12 days. The results in Figure 2 demonstrated that the highest removals of heavy metals from the systems are Ni, followed by Cr, Cu, Cd and Zn. The best removal rate for Ni was 99.92% (± 0.08) at 10 mg/L initial concentration and on day 12 HRT. The result with the highest removal of Ni in mix metal solution is in line with previous researcher, such as Ranieri [19] where he found out that CW planted with *Phragmites australis* removed Ni higher than Cr, with 82.7% for Ni and from 78.5% to 65.4% for Cr.

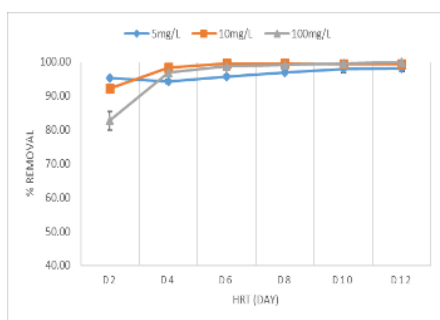
The results showed that 57.47% - 99.92% of initial metal concentrations were removed in CW with *A. puber* within the retention time of 2 to 12 days for different metals compared to control with only 42% - 97.87% removal level. The loss of heavy metals from the water in the control set up might be due to precipitation, adsorption to soil media particles and organic matter as well as co-precipitation with secondary minerals [20]. Other factors that may also contribute to the removal in the control treatment include environmental factors such as pH, water redox, presence of organic substances that enable the formation of chemical complexes, biodegradation by microorganisms and also climatic factors [21]. The lowering of heavy metals content in the wetland system was probably due to settling and sedimentation, precipitation as insoluble salts, binding to soil, sediments and particulate [22].



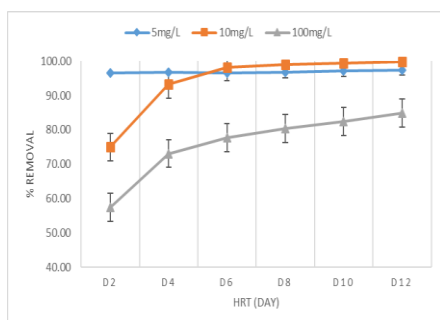
(a)



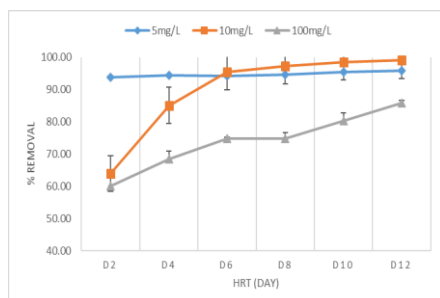
(b)



(c)



(d)



(e)

Figure 2 Percentage removal of heavy metals. (a) Cd; (b) Cr; (c) Cu; (d) Ni and (e) Zn

3.2 Effect of Concentration

Based on the results in Figure 2, the highest percentage removal for each heavy metal was at 10 mg/L concentration. This was shown by Cd, Ni and Zn. The lowest percentage removal was obtained for 100 mg/L for all heavy metals. This proved that the removal of most heavy metals tested decreased as the concentration increased. This may be a result of the

settling of suspended particles onto metals surfaces that are adsorbed and later taken up by the plant. Sedimentation is deemed as the main process in the removal of heavy metals from wastewater in CW. Nevertheless, other chemical processes like precipitation and co-precipitation must take place prior to above processes [23].

3.3 Effect of Hydraulic Retention Time (HRT)

The highest removal rate was recorded on day 12 for each metal tested while the lowest was observed on day 2. The hydraulic retention time is an important parameter that affects metal removal processes. Metal removal is generally better at higher retention times. Higher HRT may contribute to longer contact time between biomass in the reactor with wastewater, hence improving the degradation rates [24]. The HRT, including the length of time the water is in contact with the plant roots, influence the degree to which the importance of plants in the removal or breakdown of contaminants [25].

3.4 Heavy Metals in Plant's Tissue

Phytoremediation level of heavy metals (Cd, Cr, Cu, Ni and Zn) were analysed after exposure to the highest concentration tested (100 mg/L). The plant tissues were separated into stems, leaves and roots. Table 1 shows that accumulation of heavy metals occurred in the tissues of *A. puber*.

Table 1 Levels of Cd, Cr, Cu, Ni and Zn in mg/kg in the *A. puber* before and after exposure of heavy metals

	Cd		Cr		Cu		Ni		Zn	
	I	F	I	F	I	F	I	F	I	F
S	3.40	84.0	8.20	37.4	8.60	22.8	24.20	56.4	478.00	224.0
L	3.40	53.8	14.20	77.0	16.60	330.0	26.20	35.2	174.40	665.6
R	3.80	778.8	8.40	440.0	33.40	404.20	26.40	324.0	613.60	1193.8

I: Initial concentration of *A. puber*, F: Final concentration of *A. puber*, S: Stem, L: Leaves, R: Root

Results obtained from the beginning of the study (before phytoremediation) indicated that small amounts of Cd, Cr, Cu and Ni were naturally present into the tissues of *A. puber* (Table 1). The total heavy metals content in the plants that have been exposed to heavy metals was significantly higher than their initial contents. This proved that shows the macrophyte has the capacity to accumulate heavy metals in its tissues.

Based on Table 1, it was also observed that the accumulation of all heavy metals tested were higher in the roots of *A. puber* compared to the shoots (stems and leaves); Cd (778.8 mg/kg), Cr (440.2 mg/kg), Cu(404.2 mg/kg), Ni (324 mg/kg) and Zn(1193.8 mg/kg). Mishra & Tripathi [20] explained that a plant's capacity to accumulate each metal depend on their differential affinity towards the metal as well as the

competition between the heavy metal ions throughout the uptake process. In a different study, Demirezen et al. [26] determined that the accumulation ability of metal in plant is affected by plant density.

These findings demonstrated the positive significance of the macrophyte in pollutant removal, thus confirming its role as an essential component of CW.

The accumulations of heavy metals in plant tissues highlighted the plants contribution in the uptake of pollutants, apart from providing a large surface area for the growth of attached microbes, reducing carbons through root exudates and microaerobic environment, the release of root oxygen in the rhizosphere, as well as bed surface stability [27].

Besides that, the plant in CW provides mechanical resistance to the flow, improves retention time, and facilitate the settling of suspended particulates. They increase conductivity of the water through the soil as the roots grow and make spaces after their death. The plants add organic matter into the water and provide a large surface area for microbial growth [28].

3.5 Translocation Factor

The translocation factor (TF) of heavy metals in *A. puber* is illustrated in Table 2. The shoot/root metal concentration ratio was calculated for each metal. It demonstrated the translocation factor (TF) of heavy metals from roots to shoots (stem and leaves). Cu recorded the highest TF (0.87) whereas Cd had the lowest TF (0.18). Generally, the TF from roots to stems or leaves for metals is less than 1. The TF of heavy metals in this study is summarised in the following order: Cu> Zn> Ni> Cr> Cd.

Table 2 The translocation factor (TF) of heavy metals in *A. puber*

Element	Translocation Factor	
	Shoots/Roots	
Cd	0.18	
Cr	0.26	
Cu	0.87	
Ni	0.28	
Zn	0.75	

The TF for all heavy metals was found to be less than 1, thus verifying the role of roots as a heavy metals accumulator [29]. It was also found that the mobility of different metals were lower within the *A. puber* due to the TF values obtained. This study showed that the accumulation factor for Cd, Cr, Cu, Ni and Zn was higher in roots than stems or leaves of *A. puber*.

This finding is in line with previous studies from using different plants. Al-Farraj et al. [30] found that the TF for Cd, Zn, Cu, and Pb to be less than 1 by using *Ochradenus baccatus* while Al-Farraj et al. [31] stated

the TF for Cd, Cu and Pb TF were less than 1 when *Rhazya stricta* was used. In addition, Vandecasteele et al [32] also discovered that the highest accumulation of Cu, Cr, Pb, Fe, Mn and Ni was in the roots. Similarly, Gupta and Sinha [33] found that the accumulation of heavy metals (Fe, Zn, Cr, Mn, Cu, Pb, Ni, Cd) were greater in roots compared to shoots when treated with *Sesamum indicum*.

3.5 Heavy Metals in Soil Media

The concentration of heavy metals in soil before and after exposure of heavy metals are detailed in Table 3. The increased concentration of heavy metals in the soil samples collected after the exposure, indicated that heavy metals were also adsorbed to the soil. The wetland media is one of the most important components of CW, since it gives a practical condition for maximum removal of pollutant. Reduction can be achieved through various treatment mechanisms including sedimentation, filtration, chemical precipitation and adsorption, microbial interactions and uptake by vegetation, depending on the accurate selection of media type [34].

Table 3 Concentration of HM in soil (mg/L)

Element	Concentration in soil (mg/kg)	
	Initial	Final
Cd	0.78	136.7
Cr	8.83	188.3
Cu	13.03	148.4
Ni	5.25	200.0
Zn	52.30	144.0

4.0 CONCLUSION

The results of this study have shown that *A. puber* planted in CW are suitable alternatives for the treatment of wastewater contaminated with heavy metals. This plant was proven to have the abilities to take up more than one metal upon exposure to multi-metal solutions. Meanwhile, the soil media in CW system can act as a filter that contributes to the removal of heavy metals. The highest removal of heavy metals by phytoremediation process in CW system was recorded by Ni at 99.92%. The TF was found to be less than 1 for all heavy metals, which confirmed the significance of roots as a heavy metal accumulator. Therefore, it can be concluded that high removal in the system was obtained due to the higher HRT and low concentrations of heavy metals. The results in the present study highlighted the potential of *A. puber* application in CWs as heavy

metals treatment in wastewater thus offering new knowledge in terms of phytoremediation process.

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