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# Phytoremediation of chromium(VI) using *Colocasia esculenta* in laboratory scale constructed wetlands

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## Abstract

The existing water is becoming polluted nowadays due to high anthropogenic emission of water. The phytoremediation technology is used to treat the contaminated soil and water which containing higher amount of pollutants. The water that contains heavy metals will cause water scarcity and affect on human health when human drink the water. In this study, *Colocasia esculenta* was chosen for the removal of Cr(VI) in water. The efficiency of *C.esculenta* in accumulates Cr(VI) in synthetic wastewater was evaluated. The effect of time and concentration of Cr(VI) were identified in this study. Cr(VI) concentration was determined using diphenylcarbazide method (DPC). Experiment were set up in Laboratory Scale Constructed Wetlands with varies the concentration of synthetic wastewater of 1, 2, 5, 10, 50, 500 and 1000 mg/L and was continued for 36 days. The result of removal percentage for the 1, 2, 5 and 10 mg/L reach 100% while for 50, 500 and 1000 mg/L only remove 99.99%, 94.79% and 55.84% respectively. Roots of *C.esculenta* are able to accumulate Cr(VI) in wastewater which with BCF value was 1.557 mg/L. The BCF value of roots were more than 1, represents the higher accumulation of metal in plant. Therefore, *C.esculenta* has potential to use in removing Cr(VI) in water.

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### 1. INTRODUCTION

Water is the source of life and is the most essential natural resources. There are approximately 98% of water is seawater which is not suitable for drinking and only 2% are fresh water. 1.6% of 2% fresh water are locked up in polar ice cap, 0.36% of fresh water is underground water. There are only 0.036% of fresh water are water supply for rivers (The International Polar Foundation, 2003). The existing water is becoming polluted due to high anthropogenic emission of water. The most common contaminants in the environment are heavy metals (Wuana & Okieimen, 2011). The heavy metals and metalloids that pollute the water are rapidly expand from industrial areas and release to rivers (Khan et al., 2008). Researchers reported that there are sources of toxic heavy metals in the environment such as geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He et al., 2005). The non-biodegradable heavy metals in the water may affect animals, plants and human health (Ong et al., 2007).

Heavy metals are ubiquitous in the environment and also known as elements that occur naturally throughout the earth's crust. There are many heavy metals pollutants in the environment for example arsenic, cadmium, chromium, copper, nickel, lead and mercury. Chromium available in all phase of environment includes air, water and soil. In Malaysia, steelmakings are mostly produced using electric arc furnace (EAF). One of the heavy metals that included in EAF steel slag is chromium. The use of scrap metal with high chromium content in steelmaking process causes the present of chromium in the slag. The most common form of chromium exist in environment is trivalent chromium, Cr(III), and hexavalent chromium, Cr(VI). Cr(VI) can cause health effect such as lung cancer, weaken immune system and can cause kidney and liver damage (Mackenzie et al., 1958).

Phytoremediation is a technology that used plant to accumulate the contaminant from the waste water. Metal accumulating plant use to treat contaminated water which toxic metals is the environmental-friendly with technologies (Raskin et al., 1997). Phytoremediation encompass four different subgroups with different mechanisms that are rhizofiltration which where plants adsorb the heavy metals from wastewater and concentrate the heavy metals in roots and stems (Abubakar et al., 2014), phytostabilization is the plants reduces the mobility of contaminants in the environment either by immobilization or by prevention of migration. phytovolatization is involve the use of plants to remove metals from soil and release to atmosphere through volatization, and phytoextraction is the plants remove metals from soil and concentrate in the harvestable parts (Pulford & Watson, 2003).

*C. esculenta* is herbaceous perennial plant belonging to the Araceae family. The leaves of *Colocasia* 

*esculenta* are also used as a leafy vegetable (Subhash et al., 2012). The root of *C. esculenta* commonly is known as taro. It has tiny flowers which crowded on upper part of fleshy stalk. The tiny flowers are with female flowers below and male flowers above. Besides, the productivity of Taro plants is very high such like weeds.

There were many type of plant species have been studied for phytoremediation. Aquatic species are very efficiency to uptake pollutants from wastewaters through the degree of potential for removal varies from species to species. The research of using aquatic species to treat heavy metals was reported by (Swain, Adhikari, & Mohanty, 2014). The species was Eichhornia crassipes common name is water hyacinth, showed removal efficiency in removing Cadmium and Copper. Besides, tropical plant species also use in phytoremediation to remove contaminants in soils or waters. There was previous research that used Gynerium sagittatum and Heliconia psittacorum to accumulate landfill leachate that contains heavy metals. According to the research result, the experimental constructed wetlands were showed that they have good pollutant removal capacity. The removal of heavy metals reached average values from 92% to 98%. At the end of the experiment, species that valued in this research did not become hyper-accumulators, but it proved a good performance in accumulating heavy metals. G.sagittatum in the study has the best performance on accumulating metals (Madera-Parra et al., 2015).

## 2. MATERIALS AND METHODS

### 2.1. Sampling of Colocasia esculenta

*C.esculenta* plant samples were collected at Jeli, Kelantan. The samples were collected by digging the soil around the plant with a scoop. After that, the plant sample was pulled out carefully from the soil medium so that the roots of the plant are not damaged (Chavan & Dhulap, 2012). The plants samples were collected and acclimatized for one week into the nursery tank. For the whole period of research, the healthier plant samples and with the almost same plant size and number of leaves were selected and used for research purpose to make sure the accuracy experimental result.

## 2.2. Preparation of chromium(VI) synthetic wastewater

Synthetic Cr(VI) wastewater was used in this study. Analytical grade of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O7) was used as synthetic wastewater samples in the experiment. A stock solution of Cr(VI) was prepared by dissolving 2.829 g of potassium dichromate salt, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in 1000 ml of distilled water. The stock solution underwent the serial dilution and diluted to the required concentrations i.e. 1, 2, 5, 10, 50, 500 and 1000 mg/L.

## 2.3. Laboratory scale constructed wetland preparation

Three sets of tanks were prepared in the process of wetland preparation for each synthetic water concentration. Two sets of reactor tanks that with plant were prepared for duplication of the experiment to get accurate result. The water storing capacity of the tank was around 13 L. Two layers of the medium which were pebbles and garden soil is used in this experiment (Figure 1). The pebbles were put on the most bottom layer of the tank. The sample plants were transplanted into the tanks. 5 L of synthetic wastewater was added into the tanks. One control tank was prepared without transplant the sample plant so that it can correct experimental result caused by the environment factor.

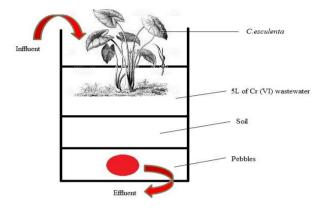


Figure 1: Laboratory Scale Constructed Wetland

### 2.4. The diphenylcarbazide (DPC) method

Cr(VI) concentration was determined using the diphenylcarbazide (DPC) method with a detection limit of 5  $\mu$ g/L (Greenberg et al., 1985). The procedure were carried out using 10 mL of volumetric flasks, where 1 ml of sample was mixed with 9ml of H<sub>2</sub>SO<sub>4</sub> (0.2 M) and added with 0.2 ml of freshly prepared 0.25% (w/v) DPC in acetone. The mixture was vortexed for 10 – 15 seconds and left the solution to stand between 10-15 minutes for full color development. The purple color mixture and the blank which content with1 ml of distilled water mixed with 9 ml of H<sub>2</sub>SO<sub>4</sub> (0.2 M) and added with 0.2 ml of 0.25% (w/v) DPC in acetone were measured at 540 nm (HACH DR 6000). A standard calibration curve was prepared using Cr(VI) concentration ranging from 0.4 – 2.0 mg/L.

## 2.5. Plant analysis preparation

The plant samples were washed under flowing tap water followed by rinsed with distilled water to remove the impurities. The excess water was removed by using filter paper. The sample plants before treatment and after treatment were being separated into three parts according to leaves, stems and roots. The sample plants were chopped into small pieces using knife and blender. The sample plants were then oven dried in 80°C for 12 hours until completely dried. The dried plant samples were ground into powder form by using mortar and pestle. Oven-dried and powdered plant samples were digested with concentrated HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> solution and analyzed by Atomic Absorption Spectrophotometer (AAS) to determine the Cr(VI) accumulation.

## 2.6. Soil analysis preparation

The soil samples before and after the treatment were collected. The soil samples were oven dried at 65°C for 48 hours (Pavinato et al., 2008). The oven dried soil samples were ground into powder by using mortar and pestle. Soil samples were digested with concentrated HNO<sub>3</sub> and  $H_2O_2$  solution and analyzed by Atomic Absorption Spectrophotometer (AAS) to determine the Cr(VI) accumulation.

## 2.7. Effect of contact time with different concentrations of Cr (VI)

The optimum contact time for phytoremediation using constructed wetland was studied for this experiment with different concentrations of Cr(VI) wastewater i.e. 1, 2, 5, 10, 50, 500 and 1000 mg/L. The effluents were collected every day from day 1 until day 10. The final readings of Cr(VI) were taken until the removal percentage become constant. The initial and final readings of Cr(VI) wastewater concentration were analyzed using DPC method.

#### 2.8. Data analysis

The percentage of removal (%) was calculated using the following equation to determine the efficiency of the plants:

Percentage of removal =  $\frac{Cf - Co}{Co} \times 100 \%$ 

 $C_o$  is the initial concentration and  $C_f$  is the final concentration of water.

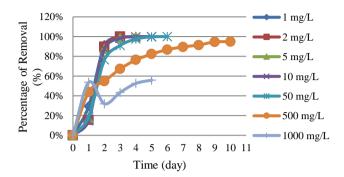
The initial and final concentrations of Cr(VI) in plant sample (*C.esculenta*) in the whole experiment were recorded. The Bioconcentration Factor (BCF) defined as the ratio of metal concentration in plant roots, stems and leaves to metal concentration in the growth media was calculated. BCF indicates the efficiency of a plant species in accumulating of elements into its tissues from the surrounding environment (Ladislas et al., 2012). The BCF is calculated by using:

 $BCF = \frac{Element accumulate in plant (ppm)}{Average initial element in wastewater (ppm)}$ 

## 3. **RESULT AND DISCUSSION**

## 3.1. Effect of contact time with different concentrations of Cr(VI)

The contact time of the experiment may influence the removal efficiency of Cr(VI) (Asgari et al., 2008). The Figure 2 showed the effect of time with different concentrations of Cr(VI) on removal efficiency.



**Figure 2:** The effect of time with different concentrations of Cr (VI)on removal efficiency.

The effects of contact time affect the adsorption of Cr (VI) by using *C.esculenta*. The longer the contact time conducted in this experiment, the higher the removal percentage of the Cr(VI) (Asgari et al., 2008). According to Figure 2, removal percentages of 1 mg/L and 2 mg/L of Cr (VI) wastewater only need 3 days to reach 100%. While for 5 mg/L of Cr (VI) wastewater, the *C.esculenta* used 4 days to reach 100% and 10 mg/L need 5 days to reach 100%. This can be explained that the higher the concentration of Cr(VI) being loaded, the longer the contact time needed for the removal process.

However, from 50, 500 and 1000 mg/L, the removal percentage does not achieve 100% but the removal becomes constant. In the last batch of experiment, the plant begins to wilt. This showed that the capacity of accumulation of C.esculenta was reaching the limit of adsorption (Mahmoud et al., 2012). Based on the result, while the concentration of Cr(VI) wastewater become higher, the plant will need longer time to accumulate the heavy metal. However, while concentration of Cr(VI) water was 1000 mg/L, the plant was not able to adsorb the Cr(VI) completely within 10 days due to the capacity of plant had reached to its limit. Based on the result, the plant sample only willing to remove the Cr(VI) until the removal percentage becomes constant. In previous research, Mahmoud et al. (2012) conclude that the rate of accumulation is higher at the beginning of the experiment due to the availability of capacity in plant to accumulate the heavy metal. While the capacity of plant to adsorb Cr(VI) is full, the removal will slow down due to the contact time between adsorbent and adsorbate has a significant role to reach equilibrium in experiment.

According to the result, the efficiency of an accumulation process is not only depending on the properties and ability of the accumulator, however concentration of elements in the wastewater also plays an important role.

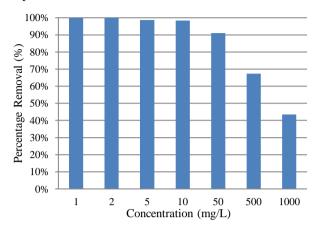


Figure 3: Percentage removal according to the concentration.

Based on Figure 3, the effect of concentrations were affected the accumulation of the *C.esculenta*. The effect of initial concentration had been studied for seven different concentrations of 1, 2, 5, 10, 50, 500 and 1000 mg/L. Based on the result, at lower concentration of influent, *C.esculenta* was more efficient in accumulating Cr(VI) compared to the higher concentrations of Cr(VI) wastewater. According to Bindu et al. (2009) in previous research, the effluent analysis discovered that as the concentration of metal in the influent wastewater increased, the removal percentage of metal decreased due to the accumulation rate decreased.

From this study, *C.esculenta* showed its efficiency in removing the Cr(VI) wastewater in lower concentration. Based on Figure 3, the result indicated that the percentage of removal decreases while the concentration had increased. This can be proved that the uptake efficiency is higher at low external concentration. This is probably due to low metal concentration per adsorption area, giving low competition between ions at the uptake sites while the opposite occurs at high concentration (Prasad & Hagemeyer, 1999).

#### **3.2.** Analysis of plants and soils

The concentration of Cr(VI) accumulated by different part of *C.esculenta* was shown in Table 1.

**Table 1:** Concentration of Cr(VI) accumulated by different part of *C.esculenta*

Plant	ant Concentration of Chromium Co		
parts	<b>Before Treatment</b>	After Treatment	
Leaves	1.045 mg/L	23.01 mg/L	
Stems	0.180 mg/L	61.63 mg/L	
Roots	1.112 mg/L	361.85 mg/L	

The leaves part of C.esculenta showed the lowest concentration among the other parts in the plant. Stems showed the second highest concentration of chromium content in the plants. For roots part of the plants showed the best efficiency of chromium accumulation. The chromium content in the roots part showed the highest concentration due to it exposed more to Cr(VI) wastewater and act as the conduit for transfer of metal to the stems and leaves, their response to the high chromium concentration is important (Parmar et al., 2012). Based on the result, C.esculenta is capable to remove chromium from the water. According to previous study, chromium uptake by plants and accumulate at higher concentration in roots than the stem and leaves (Seregin & Ivanov, 2001). The distribution of Cr within plant followed the trend roots>stems>leaves. This weak translocation of Cr(VI) to the leaves and stems due to sequestration of chromium in the vacuoles of the root cells to render it non-toxic, which may be a natural toxicity response of the plants. It must be noted that chromium is a toxic and nonessential element to plants. Hence, the plants may not possess any specific chromium transport mechanisms (Shanker et al., 2005).

Chromium content in soil sample in different of tank was shown in Table 2. According to Table 2, it can be proved that the soil also contributes to the remediation.

Table 2: Chromium content in soil sample

Chromium content in soil sample			
Initial	Final		
1.087 mg/L	102.95 mg/L		

According to this result, the soil contain chromium is due to the soil were able to accumulate some of the chromium. Heavy metal ions are the most toxic inorganic pollutants which occur in soils and can be of natural or of anthropogenic origin. The disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, most soils of rural and urban environments may accumulate one or more of the heavy metals (Wuana & Okieimen, 2011).

### 3.3. Bioconcentration factor (BCF) of C.esculenta

Bioconcentration Factor (BCF) is a more important measure than the actual concentration of a heavy metal in a plant sample, when considering the potential of a given biological species for phytoextraction (Sakakibara et al., 2013).

Based on the result in Table 3, the roots of sample plant showed the higher capacity compared with stems and leaves. The accumulation capacity of stems was higher than the leaves part. The ration bigger than 1 means, higher accumulation of metals in plant part (Bahemuka & Mubofu, 1999). According to the result in Table 3, the roots of plant samples were more than 1 which is 1.557 mg/L. The BCF values of roots part is the highest compared to the leaves and stems part which are only 0.099 mg/L and 0.265 mg/L respectively. This indicated that the uptake of Cr(VI) in roots part were better than the other part. From the view of phytoremediation, a good accumulator should have the capability to concentrate the elements in its tissue (Swain et al., 2014).

**Table 3:** BCF for the plant leaves, stems and roots growing in wastewater.

Plant Sample	Bioconcentration (BCF)	Factor
Leaves	0.099 mg/L	
Stems	0.265 mg/L	
Roots	1.557 mg/L	

## 4. CONCLUSION

Based on the result, it can be conclude that the *C.esculenta* has the ability to accumulate Cr(VI) in the synthetic waste water. *C.esculenta* tend to remove Cr(VI) from the waste water and able to survive in higher concentration of Cr(VI) within 36 days of whole experiment. The accumulation of Cr(VI) was highest in the roots part compared to the other parts. The chromium content in the roots after treatment was 361.85 mg/L. This can be concluded that this plant has the potential to use in Cr(VI) remediation.

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