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SEM of *Aeromonas hydrophila*

# ***Ricinus communis: An Efficient Biological Tool for Heavy Metal Removal from Contaminated Soil***

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### **ABSTRACT**

Heavy metal contamination often occurs when there is an abnormal discharge of metal in the environment. This is caused mainly by human activities such as agriculture, mining and industrialization. When this heavy metal gets into the environment, it adversely affects plants, humans and aquatic biodiversity. Conventional treatment methods have been applied extensively, hence these methods are inefficient and expensive to carry out. This study was conducted to assess whether castor plants could be able to absorb heavy metal actively from the soil. Metal ions determined are lead and copper at different concentrations of 1.5, 2 and 2.5 g/dm<sup>3</sup>. The soil located at Gombe State University Botanical Garden was used for the cultivation of the plant. Acid digestion and Atomic Absorption Spectroscopy were used for metal determination. The results of the study showed that Copper and Lead were absorbed the highest in the leaves than in stem and root by the absorption efficiency of 100, 91 and 87% respectively. The plant leaves and roots accumulate high Lead content of 2.661, 2.43, in the root, 1.26, 1.52, and 0.031, 0.3 in the stem for both lead and copper respectively. Results of translocation factor revealed the highest absorption of metal from Root to stem of 1.83, 1.98, and the least was 0.36, 0.34, for copper and lead respectively.

### **INTRODUCTION**

The world is currently faced with serious environmental degradation resulting from an increase in the level of heavy metal ions in the environment. Farming activities involving the application of fertilizer are connected with high levels of metal. This thus calls for an immediate search for better and environmentally ways of removing the accumulated metal from the soil to protect the human, aquatic biota and the environment [1,2]. Some of the heavy metals identified to negatively affect the environment are lead, zinc, copper and nickel [2].

Several research studies have reported that treatment of soil polluted with heavy metals is expensive and sometimes requires different steps that may take time [3]. The available old methods of heavy metal sequestration from the soil are done unilaterally or in connection with one another to lessen the concentration of

metal to a level that will not affect the environment [4]. These methods generate a large number of secondary pollutants that deteriorate the environment and does not provide positive results at the end of treatment [3,5]. Moreover, higher plants are abundant and cheaply available. They provide a dual role of heavy metal uptake and removal of toxic carbon dioxide from the environment making them safer methods of treatment adopted by several researchers [6,7].

Castor plant, scientifically known as *Ricinus communis* fall under the dicotyledonous plant and family Euphorbiaceae. The plant is cosmopolitan occurring in a wide range of environments such as Ethiopia and Africa [6]. The plant can grow in complex environments and polluted regions. These features qualify it to be used as a suitable candidate for heavy metal sequestration [8]. Castor plant was reported to have the ability to survive in a water containing high amount of salt [9], and in other places with an

extreme lack of rainfall [10]. Furthermore, the plant was reported to neutralize the salinity level of seashore soil [11]. The plant is not edible making it unable to compete with food crops. Its seed was reported to produce a plentiful amount of glyceride which is a feedstock for biodiesel production [12,13]. These features are what differentiate castor plants from other plants for their use as bio-remediating agents and production of value-added substances.

The most common route by which heavy metals have access to the aquatic environment is soil especially that situated in industrial sites [14]. Accumulation of these metals in the soil has long been linked to the production of several diseases thereby making their phyto-removal necessary [1]. In Nigeria, it has become a common practice where farmers used fertilizer and sometimes industrial wastewater for watering [15]. These substances contain recalcitrant heavy metals that killed important soil microbes and lower the fertility strength of the soil [16]. Therefore, this research was designed to assess the potency of castor plants for heavy metal removal from the soil. Bio-concentration and translocation factors will be investigated to determine the plant's physiological response.

## MATERIALS AND METHODS

### Soil collection

A sample of soil used for this research was procured from the Botanical Garden located at Gombe State University at depth of 20cm. The site was located at coordinate 10° 15'N, 11° 10'E of Greenwich meridian. Gombe State is within the North-East region of Nigeria with an estimated population of 2,353,000. The collected soil was taken for analysis at the Biochemistry Laboratory

### Soil Pre-treatment

For the pre-treatment analysis, larger materials such as stalks were removed, followed by the crushing of the soil in a blender. After that, the crushed soil sample was sieved in a mesh of relatively 0.25 mm size. The justification for that is to get fine soil particles [17].

### Soil digestion

Soil digestion was done using nitric acid-perchloric acid as described by [17]. In this method, about 0.5g of the fine soil sample obtained from the pre-treatment stage was inserted into a beaker (50 ml) containing 20 ml of a mixture of acid (nitric acid and perchloric acid) in a molar ratio of 1:1. The mixture was refluxed on a hot plate at a low temperature. The heating was halted after the dense white fumes were observed indicating the formation of HClO<sub>3</sub>. The sample after digestion was kept aside for 15 minutes for cooling purposes. It was then filtered into a 50 ml flask. Top up was done using distilled water until it reaches the meniscus. The final sample was then analyzed using Atomic Absorption Spectrometer [17].

### Soil treatment

The soil was treated with lead and copper separately at different concentrations of 1.5, 2 and 2.5 g/dm<sup>3</sup> for each metal. One pot was kept containing no metals to serve as a control. The metal absorption capacity before plant and after were measured across all the concentrations of the heavy metals.

### Determination of Soil pH

The pH was determined with Hanna 420 pH meter; it was calibrated according to the instructional manual provided by the

manufacturer. The electrode of the pH meter was dipped into the water sample for 2-3 minutes and readings were recorded [7].

### Planting and harvest

Following the preparation of the soil, about Twelve (12) seeds of the plant (*R. communis*) were planted in the soil at about 3 cm depth. After 3 weeks of plant growth, the number of seedlings was reduced to 2 in a single pot. The plant was allowed to grow for six (6) weeks, after which it was harvested (Fig. 1). The rooted plant was inserted in water to remove soil particles and other impurities and it was then dried in an oven for three days at a temperature of 80°C. The purpose of this step is to get fully dried leaves, roots and stems of the plant.



Fig. 1. Parts of castor plant at the point of harvest.

### Analysis of plant tissues for metals

After successful drying of plants part (root, stem and leaf), they were then crushed into powder in mortar and pestle to obtain the powdered form. Accurately, 1g of the powdered sample was placed in a flask containing a mixture of Nitric acid and Perchloric acid at a molar ratio of 3:1. The mixture of the preparation was heated on a hot plate for 15 min under constant stirring to facilitate the dissolving of the powder. After 15 minutes, the sample was brought down for cooling purposes, it was then filtered to remove impurities and the final sample was placed in a beaker for subsequent analysis using an atomic absorption spectrometer for measurements of metals [17].

### Analysis of soil for metals

The sample of soil was dried in air for a period of 14 days and was sieved to obtain fine particles. The subsequent analysis was done following the method described under the analysis of plant tissue for metal.

### Bio-concentration and translocation factors

Computation of Bio-concentration Factor (BCF) alongside Translocation Factor (TF) was done in order to measure the potential of the plant to accumulate metals ions and transport them to the various part of the plant. The formulas were adopted from [18].

Microsoft excel was used for the computation and presentation of the available information regarding bio-concentration and translocation factors. The formula used to compute bio-concentration and translocation factors are presented in equations 1 and 2 respectively

$$BCF = \frac{\text{Concentration in Organ}}{\text{Concentration in sediment}} \quad \text{Eq. 1}$$

$$TF = \frac{\text{BCF of stem or leaf}}{\text{BCF of root}} \quad \text{Eq. 2}$$

## RESULTS AND DISCUSSION

The study of the heavy metal removal from soil by *Ricinus communis* was conducted over a period of 5 months. The justification for using 5 months is because it is the best period where castor absorbed heavy metal actively [20]. In order to ensure accuracy, the different parts of the plant as shown in Plate 1 were characterized and the preliminary result indicated that no heavy metal (copper and lead) has been detected (**Table 1**).

**Table 1:** Preliminary characterization of the different parts of the plant for the presence of heavy metal.

Samples	Concentrations (g/L)	
	Copper (Cu)	Lead (Pb)
leave	L1	n.d
	L2	n.d
	L3	n.d
stem	S1	n.d
	S2	n.d
	S3	n.d
root	R1	n.d
	R2	n.d
	R3	n.d

Note n.d. not detected

There were no heavy metal ions (below the detection limit) found in all the leaves, stems and roots of the plant before the analysis (**Table 1**). Results of heavy metal removal efficiency indicated that at 1.5 g concentration, copper removal concentration followed the trend of leave>stem>root. This is equivalent to the fact at copper absorption efficiency in leaves was found to be 98, 99 and 99% for 1.5, 2 and 2.5g concentrations. For the stem of the plant, the absorption efficiency at 1.5, 2 and 2.5g were found to be 93, 90 and 92% absorption respectively. Root absorption efficiency at 1.5g gives 87%, followed by 85 and 86% for 2 and 2.5 g concentrations (**Table 2**).

Results in **Table 2** also showed that there was a significant difference in copper absorption in the stem and root of the plant. A gradual decrease in copper absorption was observed in the leaf as the metal concentration increased. Moreover, Lead absorption in stems under 1.5 and 2 g/L concentrations was uniform just in the case of roots under the same condition. However, the absorption increases in both stem and root and roots were found to be lead concentrations reaching 2.5 g/L.

**Table 2.** Initial and final absorption of metal ions by the different parts of the plant.

Samples	Concentrations (mg/L)				
	Initial conc. of metal ions (Cu <sup>2+</sup> :AN D Pb <sup>2+</sup> )	Final conc. of Cu <sup>2+</sup>	Percentage removal of Cu <sup>2+</sup> (%)	Final conc. of lead (Pb <sup>2+</sup> )	Percentage removal of lead Pb <sup>2+</sup> (%)
Leaf	L1 1.5	0.002±0.11	98	0.000±0.00	100
	L2 2.0	0.004±0.16	99	0.004±0.13	99
	L3 2.5	0.004±0.01	99	0.007±0.03	97
Stem	S1 1.5	0.112±0.13	93	0.121±0.16	91
	S2 2.0	0.198±0.10	90	0.200±0.08	90
	S3 2.5	0.205±0.21	92	0.212±0.26	91
Root	R1 1.5	0.194±0.01	87	0.188±0.11	87
	R2 2.0	0.300±0.06	85	0.294±0.09	85
	R3 2.5	0.350±0.99	86	0.325±0.12	87
Soil	X1 1.5	0.264±0.07	82	0.224±0.29	85
	X2 2.0	0.314±0.05	84	0.320±0.10	84
	X3 2.5	0.440±0.18	82	0.360±0.22	87

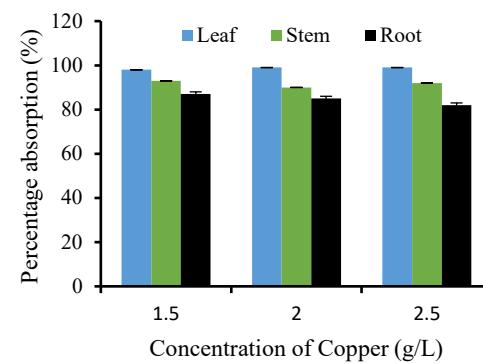
In the case of lead (Pb<sup>2+</sup>), the absorption efficiency of leaves of the plant at 1.5, 2 and 2.5g concentrations was found to be 100, 99 and 97% efficiency. Results in **Fig. 2** showed the stem

absorption capacity to reach 91, 90 and 91% respectively at similar concentrations. For root tissue, it indicated that 87, 85 and 97% of lead were absorbed in 1.5, 2 and 2.5 concentrations (**Fig. 3**).

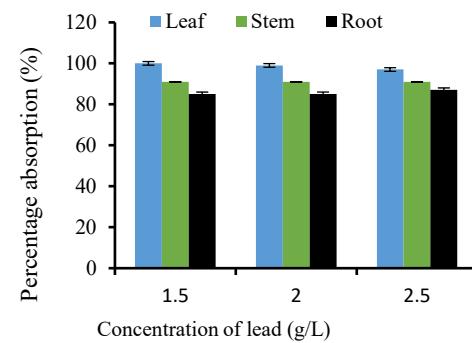
### Determination of bio-concentration factor

Literature reported that the Bio-concentration Factor (BCF) of nutrients can be described as the rate at which the nutrients or metal concentrations get accumulated in the part of the plant in relation to its concentration in the soil. The principle further stated that when BCF gets larger it implied that the plant has a strong ability to absorb metals from the soil [21].

The bio-concentration factors of heavy metals in *Ricinus communis* are shown in **Table 3**. Based on this, the bio-concentration factors of Cu in roots, stems, and leaves were found to be in the range of 0.01-0.828. Meanwhile, for Pb, mostly, the concentrations in the range of 0.013-0.887 have been absorbed from the sediments. It was observed that the castor plant absorbed more lead than copper.



**Fig. 2.** Comparison of heavy metal absorption in leaf, stem and root of *Ricinus communis* under different concentrations; **(a)** Copper



**Fig. 3.** Comparison of heavy metal absorption in leaf, stem and root of *Ricinus communis* under different concentrations; **(b)** Lead.

**Table 3:** Bio-concentration factor for heavy metal in a different part of the plant.

Replicate	Cu			Pb		
	Leaf	Stem	Root	Leaf	Stem	Root
1	0.008	0.424	0.734	0.00	0.054	0.839
2	0.013	0.631	0.955	0.012	0.625	0.919
3	0.009	0.466	0.795	0.019	0.589	0.903
Mean total	0.03	1.521	2.484	0.031	1.268	2.661

This absorption of Lead by the plant was found to be highest in the root (0.887), followed by the stem (0.585) and then leaves (0.013) (**Table 3**). While in the case of copper it follows the same trend as in the case of lead with the root being the highest absorber then followed by the stem and then leaves at a concentration factor of 0.828, 0.507 and 0.01 respectively (**Table 3**). More than 50% accumulation of copper and lead were found in only stem and root and the remaining concentrations were below 50%. This indicated that the *Ricinus communis* plant has high preferentially for lead absorption thus making it the best candidate for lead uptake from the environment. The results of this research were found to be slightly higher than the results obtained by the researcher when he tested the ability of *Avicennia* species the removal of copper and lead from solution. The result of their research shows that the copper removal was in a range of 0.29-0.42 and 1.14-1.59 for copper and lead respectively [19,1]. The variation in the results could be due to the difference in plant species used for heavy metal uptake.

Generally, plants have a different level of acclimatization in presence of heavy metals. The more soil accumulates a high amount of metals the more it will affect the physiological functioning of the plant (toxicity), which might have a negative effect on its ability to be used as a suitable phytoremediation agent. One of the easiest ways to lessen the toxic effect of metals on the plant is by the addition of organic substances for neutralization purposes [3]. This might be the reason why we encounter high absorption of heavy metals in lower concentrations than in higher concentrations the study shows.

#### Determination of translocation factor

In the case of translocation factor (TF), it described the movement of metal ions from the soil to the root, followed to the stem and then leaves [13]. Results of translocation factors of heavy metals in *Ricinus communis* were presented in **Table 4**. It was observed that the translocation factors of greater than 50% are mainly observed in root-stem of copper and lead whereas stem and stem-leaves values were relatively lower than 50%. The highest translocation factors for copper and lead were found in the root stem with values of 0.508 and 0.658 respectively. This finding indicates that the *Ricinus communis* is capable of translocation copper and lead actively from root to stem rather than from stem to leaves.

**Table 4.** Translocation factor for heavy metal in different parts of the plant.

Replicate	Cu		Pb	
	Root-stem	Root-leaf	Root-stem	Root-leaf
1	0.578	0.011	0.643	0.000
2	0.661	0.014	0.680	0.013
3	0.586	0.011	0.652	0.021
Mean total	1.825	0.036	1.975	0.034

Literature has explained that heavy metals are commonly found in roots and leaves [20,21]. Plant absorbed heavy metals via a binding mechanism. In some plants, the heavy metals are accumulated into the various plant tissues such as parenchyma and sclerenchyma while in some plant's metals are assimilated and used for growth especially photosynthesis process [24]. This thus suggested that the *Ricinus communis* plant can thus contribute to the prevention of heavy metal accumulation in the surrounding environment.

The translocation factors for heavy metals in plants should be more than one to be considered active bio-accumulators [22]. It can be considered moderate if the accumulation is greater than 50% and the lowest is accumulation below 50% [9]. The

moderate translocation factors of the metals observed in this study indicated that the *Ricinus communis* plant could use metals for both growth and absorption. While the mobility of the non-essential metal from roots to leaves is comparably higher. Root-leaf and root-stem translocation values of more than 1 showed that metal concentration in leaves or stems was higher than in roots. This further suggested that the roots of the plant have a greater ability to absorb rather than accumulate the heavy metal the study shows. The translocation of materials in plants implies that certain materials could be transported via xylems (water) and phloem (nutrient). Moreover, the transportation of water from the root to the stem by xylem could carry along the metal ions which are largely dependent on the capillary action of the plant.

#### Relationship between metal concentration and pH

The relationship between metal concentrations in soil and plant growth has long been established, however several plants have different absorption capacities for heavy metals. In this study, we give more priority to copper and lead as they affect the environment adversely. Other metal ions have not been considered because their absorption mechanism has not been established.

Generally, the rate of absorption of the copper by different parts of the plant follows the leave>stem>root pattern. There is a depression in the growth of the plant as the concentration of metal increases which consequently leads to the reduction of the soil pH to below 7 (**Table 5**). This is because metal ions reduce the neutral state of soil to relatively less acidic [25,26]. The pH of control samples was found to be in the neutral range of 7.2-7.3. Moreover, the soil sample contaminated with copper was observed to be moderately acidic in the range 6.1-6.6 whereas the soil sample contaminated with lead was more acidic (range 5.8-6.7) than the copper sample.

**Table 5.** Value of the soil pH in the presence of heavy metals.

soil sample	pH Reading
control (c1)	7.2
control (c2)	7.2
control (c3)	7.3
copper (1.5g)	6.1
copper (2.0g)	6.3
copper (2.5g)	6.6
lead (1.5g)	6.7
lead (2.0g)	5.8
lead (2.5g)	5.8

Element accumulation by higher plants depends upon the binding and solubility of particles deposited on the leaf surface and pH is one of these critical factors that determined the solubility of heavy metal ions [8]. High accumulation of copper was observed in the leaves of the plant than in that of the stem and root. This is evident that roots absorb soluble material from the soil and transport it to the stem and then to the leave of the plant [27]. Going by this trend, the concentration of copper could be high in leaves. A similar scenario was also observed in lead.

#### CONCLUSION

Conclusively, the different parts of the castor plant were found to absorb heavy metals at different concentrations. Castor plant's leave and roots were observed to remove more lead than copper while the stem was able to remove more copper than lead at the same concentration. Heavy metal levels in plants depend on the physicochemical parameters of soil such as pH, turbidity, conductivity, salinity and TDS. It is a fact that the solubility of toxic metals increases with decreased pH (from surface to depth, from alkaline to acidic). With the development of proteomic or

gene engineering, the capabilities of hyper-accumulators may be greatly enhanced through specific metal gene identification and its transfer to certain promising species.

## CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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