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(IJIREV)www.ijirev.comTHE POTENTIAL OF *Catharanthus Roseus* AS A
PHYTOREMEDIATION AGENT FOR HEAVY METAL REMOVAL
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Abstract:

Heavy metals from human activities, such as industrial emissions and mining waste, contribute to environmental, nutritional, and health issues. *Catharanthus roseus*, a phytoremediation plant, was tested to determine the levels of heavy metals in soil and plants. The experiment involved preparing various amounts of heavy metals by combining four elements: Cadmium (Cd), Copper (Cu), Lead (Pb), and Zinc (Zn). Four treatment groups were tested, each with varying levels of heavy metal contamination. The initial group (T0) served as the positive control, while the next four groups (T1-T4) were subjected to escalating levels of heavy metals. T1 was given a 200ml solution containing 0.9 (Cd) + 4.0 (Cu) + 0.18 (Pb) + 6.9 (Zn). T2 was given a 200ml solution containing 11.90 (Cd) + 19.80 (Cu) + 36.00 (Pb) + 54.3 (Zn). T3 was given a 200ml solution containing 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn). T4 was given a 200ml solution containing 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn). The dataset included measurements of soil pH, heavy metal concentrations, plant height, and shoot and root length. The results showed that the T3 solution, consisting of 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn), had the most favourable plant growth performance based on soil and plant concentrations. This suggests that immediate action is needed to ensure safe food availability.

Keywords:

Catharanthus Roseus, Heavy Metals, Phytoremediation

Introduction

Heavy metals are major environmental contaminants, and their toxicity is an issue that is becoming more and more important for ecological, evolutionary, nutritional, and environmental reasons. Any metallic element with a relatively high density and is hazardous or

deadly even at low concentrations is called a “heavy metal” (Nagajyoti et al., 2010). If heavy metals accumulate in high quantities, they can damage plant physiology by lowering respiration and growth, interfering with photosynthetic activities, and disrupting essential enzyme functions (Malizia et al., 2012). Several plant responses are brought on by heavy metal stress. There are plant responses to avoid the impacts that a variety of heavy metals may have. Long-term accumulation of heavy metals in the soils close to industrial regions include iron (Fe), copper (Cu), nickel (Ni), cobalt (Co), cadmium (Cd), zinc (Zn), arsenic (Ar), and mercury (Hg). Most of these metals are crucial micronutrients that regulate several routine plant activities (Soumya et al., 2022).

Phytoremediation is the use of plants to remove, absorb, or stabilize possible pollutants such as heavy metals, metalloids, trace elements, organic, and radioactive chemicals from soil or water. Metallophytes have unique genetic and morphological traits that allow them to establish biological mechanisms to survive and reproduce in metalliferous soils while remaining poisonous (Yuan et al., 2019). Many engineering works and biological techniques have been devised to enhance the phytoremediation process. Phytoremediation has been used to confirm the viability of specific plants for environmental cleaning (Iqbal et al., 2019).

Madagascar periwinkle or Rosy Periwinkle are two popular names for *Catharanthus roseus*. Kemunting Cina is the Malay name for this species. It is genuinely native to Madagascar, but through time, it has been widely produced and naturalized in most tropical countries, where it occasionally turns into an unwanted plant (Siti, 2017). The pink, purple, and white flowers that these plants produce are grown for ornamental purposes.

This contaminated water and soil infiltrate our food chain further because the same natural resources are utilized for plant cultivation and drinking water. The use of these pollutants produces health problems, which impact both the environment and the economy. Contamination of soil and water is a huge issue that has implications for all living species, including people. Many natural and artificial causes contribute to soil and water contamination—sources from nature. Forest fires, volcano eruptions, radiation, and rock weathering are all examples of natural disasters (Iqbal et al., 2019).

Literature Review

Catharanthus Roseus

Some Apocynaceae members, especially *C. roseus*, are also showing positive outcomes for phytoremediation of different metal (Khan et al., 2017). According to Subhashini and Swamy (2017) previous studies, *Catharanthus roseus* was an excellent accumulator of Pb, Ni, Zn, and Cr. The species are suitable for phytoextraction of soils polluted with Pb, Ni, Zn, and Cr.

Ornamental plants are planted in gardens to provide beauty and to improve the scenery. Such plants are not notably part of the food chain and are necessary to accumulate metals in order to repair metal-contaminated soils (Qurban et al., 2021). Low phenolic and alkaloid content in *Catharanthus roseus*' sections makes isolation expensive. This plant has attracted much attention from researchers due to the high cost of the extracted components. Several studies show that metal treatments can speed up biosynthetic processes in cell cultures of *Catharanthus roseus*. Vanadyl sulphate treatment increased the levels of ajmalicine and catharanthine in the cell cultures (Soumya et al., 2021).



Figure 1: *Cataranthus Roseus* Flower

Phytoremediation

Phytoremediation is an environmentally favourable process that uses rapidly growing plants to remove, retain, or deliver harmless pollutants in soil or water. It is an attractive, economical, and environmentally beneficial method of detoxifying pollutants. Phytoremediation can be effective if the plant species employed can absorb and store significant concentrations of metal pollutants in their shoot sections. Phytoremediation techniques may be divided into five categories: phytostabilization, phytostimulation, phytotransformation, phytofiltration, and phytoextraction (Ashraf et al., 2019). Contaminated areas must be affected for long-term environmental and agricultural growth, and the entrance of contaminants into the food chain must be minimized. Considering this, the plant-based remediation technology known as phytoremediation has gained popularity during the last two decades. It is a simple, vital, cost-effective, low-labor-intensive, widely acceptable, compatible, eco-friendly, sustainable, reliable, and promising technology that is applicable in large areas, particularly when native, ecologically and socioeconomically valuable plants are used for polluted site remediation and income is generated from produced phytoproducts from contaminated lands.

Materials and Method

Experimental Site

This study was conducted in a greenhouse located at the Plantation Unit of the UiTM Perlis, Arau Campus, as shown in Figure 2. The experiment was conducted in a greenhouse to eliminate outside influences, such as rain. The research took place from September 2023 till December 2023. With a mean temperature range of 25.5°C to 35.7°C, and in a warm, humid tropical climate, the environment is ideal for cultivating the *Catharanthus roseus* plant.

Research carried out at the supplied by Agrotech facilities in Campus Arau's Star Complex UiTM and Soil Lab.



Figure 2: Location of UiTM Greenhouse at Unit Ladang

Experimental Design

The research was conducted in a greenhouse to eliminate the effects of weather conditions such as rain. To obtain accurate results, the study used a Complete Randomized Design (CRD) with five different treatments and six replicates for each treatment, resulting in a total of 30 plants. Table 1 shows the recommended concentration values for natural occurring metal concentrations in the soil.

Table 1: The Typical Range Of Natural Occurring Metals Concentrations

Element	Soil	
	Min	Max
	mg/L	mg/L
Cadmium, Cd	0.9	11.90
Lead, Pb	0.18	36.00
Copper, Cu	4.0	19.8
Zinc, Zn	6.9	54.3

Source: Department of Environment Malaysia (DOE, 2009)

Table 2: Treatment Range With Interaction Of Heavy Metal Concentrations. The Units Of Measurement For This Treatment Are Milligrams Per Liter (mg/L) (DOE, 2009)

Treatment	Concentration applied (mg/L)			
	Cd	Pb	Cu	Zn
T0 (control)	0	0	0	0
T1 (min)	0.9	0.18	4.0	6.9
T2 (max)	11.90	36.00	19.80	54.30
T3	23.8	72.00	39.6	108.6
T4	35.7	108.00	59.4	162.9

This research determined the treatment values by using the control value T0. Found that for each treatment, T1 represents the lowest value for the concentration applied, while T2 represents the highest value, indicating the amount of heavy metal. In addition, T3 represents twice the amount of T2, which clearly indicates an increase in the presence of heavy metals. Finally, T4 represents three times the amount of T2, indicating a substantial rise in the heavy metal concentration. The experiment designed with a total of 30 *Catharanthus roseus* plants,

with six duplicates for each of the five treatments. The Table 3 below clearly shows the experimental design details.

Table 3: Amount Concentration of 50mL

Treatments	Elements			
	Cu	Cd	Zn	Pb
T0	0	0	0	0
T1	0.2 mL	0.045 mL	0.35 mL	0.009 mL
T2	0.99 mL	0.595 mL	2.72 mL	1.8 mL
T3	1.98 mL	1.19 mL	5.43 mL	3.6 mL
T4	2.97 mL	1.785 mL	8.15 mL	5.4 mL

The solution must pour 200 mL to each polybag. The plant was initially treated with 50mL of the heavy metal one week before to planting. Following planting, 50 mL of the element was poured each of the next three weeks.

Table 4: Experimental Layout in CRD

T0R3	T3R2	T2R4	T4R6	T1R2	T4R4	T1R3	T3R3	T4R2	T0R2	T1R4	T0R6	T4R1	T1R6	T0R4
T2R6	T4R5	T1R1	T2R3	T3R5	T2R5	T0R1	T2R1	T3R6	T2R2	T3R4	T1R5	T3R1	T0R5	T4R3

* T represents the type of treatment that will be applied to each arrangement

* R represents the replication of each treatment

Data Collection

The experiment been run for 42 days (1 ¼ months), and the plant's height has been measured every 14 days to determine its viability under 5 different treatment concentrations. The first readings were taken 14 days after the transplanting. All data has been measured and recorded to gather information on heavy metal concentration and plant growth performance.

Statistical Analysis

The data have been analyzed using the Statistical Package for Social Science (SPSS) software version 26 by using one-way ANOVA, and the Tukey test have been performed. The mean evaluated at a 5% significance level.

Results And Discussion

Table 5: Output of One-Way ANOVA for the Concentration of Cd, Pb, Cu and Zn Content in soil and *Catharanthus roseus* Plant

Treatments	Heavy Metals					
	Soil			Plant		
	Cu	Zn	Pb	Cu	Zn	Pb
T0	0.18a	6.77a	3.24a	0.06c	1.58a	2.37ab
T1	0.14a	12.74a	4.52ab	0.02b	2.23a	0.31a
T2	0.15a	32.67a	8.01c	0.02a	8.15ab	0.61a
T3	0.15a	71.05b	6.93c	0.01ab	20.81b	4.04bc
T4	0.16a	69.87b	6.10bc	0.01ab	39.27c	6.89c

Note: Mean values within column followed by different letters are significantly different at the ($p \leq 0.05$) level of significance by Tukey's multiple comparison test $\pm$ standard deviation.

Soil and Plant Analysis

Copper (Cu)

Based on the result show there are significant difference ($P < 0.05$) in the concentration of copper in soil but it is only for the pre and T0. There are no differences for T1, T2, T3 and the T4 which Cu in soils were below the safe limit which range about 0.6 mg/kg which far above WHO standard. The highest Cu content in T0 the control which is 0.11mg/L. Meanwhile, the lowest is in post-analysis which is 0.14 mg/L. It has been reported that the presence of Cu(II) can have a dual effect on the uptake of Cd(II) in plants. As per studies, this led to a reduction in the accumulation of Cd(II) in the roots of *C. roseus* when exposed to Cu(II)+ Cd(II) treatment (Chen et al., 2018).

Copper toxicity and interaction with the biological movement of other elements such as phosphorus, zinc, and iron were seen as a result of the soil's extremely high copper concentration levels ("Effect of Some Ornamental Plants for Phytoremediation of Contaminated Soil with Some Heavy Metals," 2021).

Based on the figure, result show that have a significant different ($P < 0.05$) in the contaminated soil that content Cu element. From the graph, the highest concentration rate of Cu is in the T0. Meanwhile, for the lowest absorbed is T2 which 0.01.

Copper (Cu) in soil has cytotoxic effects. Elevated levels of Cu cause oxidative stress and the development of reactive oxygen species (ROS), which can disrupt metabolic pathways and damage macromolecules, leading to leaf chlorosis and plant growth retardation (Jhila et al., 2021).

Zinc (Zn)

The data presented in the graph indicates some significant differences ($P < 0.05$) in the levels of Zn element found in polluted soil. As depicted in the figure, the disparity had occurred between the soil's Zn content before and after day 42 of transplantation. Notably, the highest concentration of Zn was found in T4, with a recorded rate of 96.54 mg/L, while the lowest level existed before planting, with only 5.43 mg/L recorded after T0.

According to Chen et al. (2018), plants with adequate mineral nutrition may have lower concentrations of Cd(II). This might be because Cd(II) and other critical elements compete with one another. Examples of these elements include Zn(II) for plant absorption

Table 5 shows significant differences in heavy metal concentrations among the treatments. Plants in treatment 4 had the highest mean concentration with a recorded rate of 39.62 mg/L. Meanwhile the lowest is T0 the control which is 1.58 mg/L. Zn toxicity leads to accumulating other heavy metals, such as Cu and Mn, in both roots and shoots (Ghori et al., 2019).

When grown on zinc-supplemented soil, the concentration of metal in all plant organs increased significantly. Interestingly, the metal concentration was consistently higher in the plants than in the soil itself (Todeschini et al., 2011).

Lead (Pb)

According to the graph, the analysis revealed noteworthy distinctions ($P < 0.05$) in the presence of the Pb element in polluted soil. T2 exhibited the highest concentration rate of Pb after 42 days at 8.0 mg/L, while T0 indicated the lowest concentration of Pb at 2.84 mg/L as applied to the heavy metal.

Figure clearly indicates that the root system of *Catharanthus roseus* has effectively absorbed lead, as evidenced by the significant results observed in treatment 4. The p-value of less than 0.05 (0.001, respectively) reinforces the validity of these findings.

According to the graph, there is a significant difference in the levels of lead (Pb) found in the plant. The highest level was found in T4, which was 6.89mg/L, while the lowest level was found in T1, which was 0.31mg/L. Toxicity caused by lead in seedlings can lead to growth retardation and inhibit germination. The photosynthetic pathways of plants can be adversely affected by lead toxicity due to its ability to disrupt the ultrastructure of chloroplast and block the synthesis of essential pigments such as chlorophyll, carotenoids, and plastoquinone (Ghori et al., 2019).

According to a study by Subhashini & Swamy (2017), *Catharanthus roseus* absorbed high quantities of lead through its root system by the 20th day of experimentation. However, the lead was translocated slowly throughout the remaining period of the experiment. By the 60th day, only 50% of the absorbed lead had been translocated to the stem and leaves. The lowest accumulation of lead was found in the leaves (0.92 mg/kg) and the highest in the roots (67.33 mg/kg), resulting in a total accumulation of 77.05 mg/kg of lead in the entire plant. The study concluded that the translocation rate was meagre over the experimental period.

Cadmium (Cd)

It was intended to check the level of cadmium in the soil, but after conducting soil and plant analysis, no cadmium was found. It was discovered that the *C. roseus* plant is resistant to heavy metal contamination in soil, particularly cadmium. This is because the plant can tolerate up to 500 μM Cd^{2+} concentration during germination and up to 250 $\mu\text{g g}^{-1}$ soil when the plant is two months old. However, it is important to note that heavy metal contamination is highly dangerous as it reduces the percentage of plant germination, decreases biomass, induces sterility, and ultimately decreases total alkaloid content as well (Al-Khayri et al., 2023). The amount of Cd is not too high, it might gone evaporate to atmosphere.

Growth Development

Table 6: Output of One-way ANOVA for Examine the Growth of *Catharanthus roseus* Planted in Different Concentrations of Heavy Metal-contaminated Soils.

Treatments	Shoot & Root Length	Fresh Weight	Dry Weight
T0	S – 23.67b R – 8.67a	10.20b	1.84a
T1	S - 12.50ab R – 5.50a	3.62ab	0.74a
T2	S – 12.33ab R – 5.67a	1.25a	0.40a
T3	S – 11.00a R – 2.00a	0.73a	0.36a

T4 S – 16.67ab 1.80ab 0.66a
 R- 4.67a

Note: Mean values within column followed by different letters are significantly different at the ($p \leq 0.05$) level of significance by Tukey's multiple comparison test $\pm$ standard deviation.

Plant Height

Table 7: Plant Height 2 Weeks Interval Data

Treatment	Plant Height (cm)			
	Week 0	Week 2	Week 4	Week 6
T0	12.00 ^a	15.50 ^a	19.00 ^a	20.33 ^a
T1	8.33 ^a	10.50 ^a	11.67 ^a	12.33 ^a
T2	8.83 ^a	8.50 ^a	8.00 ^a	8.00 ^a
T3	8.17 ^a	8.00 ^a	8.00 ^a	8.00 ^a
T4	8.83 ^a	11.00 ^a	11.83 ^a	12.00 ^a

Note: Mean value within column followed by same letter is not significantly at $P \geq 0.05$ according to Tukey analysis.

T0 – positive control

T1 – 200ml solution of 0.9 (Cd) + 4.0 (Cu) + 0.18 (Pb) + 6.9 (Zn)

T2 – 200ml solution of 11.90 (Cd) + 19.80 (Cu) + 36.00 (Pb) + 54.30 (Zn)

T3 – 200ml solution of 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn)

T4 – 200ml solution of 35.70 (Cd) + 59.40 (Cu) + 108.0 (Pb) + 162.9 (Zn)

Table 7 shows no significant difference between T0 and the other treatments from week 0 to week 6. This study has found that the *Catharanthus roseus* is a good phytoremediation plant to absorb heavy metal from soil because the life span for the plant stable even though with the high concentration of heavy metals been applied. The current research indicates that the lifespan of *C. roseus* is finite.

State by Subhashini & Swamny (2013), in the present study *Catharanthus roseus*, a non-edible, shrub species aesthetically pleasant with beautiful flowers. Finally, it was concluded that the plant species highly accumulated lead than nickel. Based on the bioconcentration factor and translocation factor values the plant species was a good accumulator of these two metals (Pb and Ni).

Therefore, the morphological evaluations performed in the plants showed a significant increase in height, number of leaves, stem diameter and fresh mass promoted by the use of sewage sludge. The height of *C. roseus* continued to increase until the maturation period observed in the study, reaching an average of 36 cm in 108 days of evaluation (Barbosa et al., 2020).

Shoot and Root Length

The experiment measured the shoot length of *Catharanthus roseus* after 42 days under different concentrations of heavy metals. The results showed significant differences ($P < 0.05$), with the highest shoot length of 23.67cm found in T0. Surprisingly, even T4, which had the highest concentration of heavy metal, had a survival rate of 16.67cm. T0 serves as the control, indicating that the plant can survive in the area even without the addition of heavy metals concentration. T3 has the lowest height of shoot after 42 days (11cm) and the rate of heavy metal for T3 is 200ml solution of 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn) which is the second highest concentration of heavy metal behind T4.

Upon conducting a thorough analysis of the data showed the highest root length of 8.67cm found in T0. While T3, which contain of 200ml solution of 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn) has the lowest root length which is 2cm. The conclusion drawn is strongly supported by the data presented in figure, which demonstrates no significant differences in root length across all treatments.

According to Qurban et al. (2021), root lengths in Cd and Cu at 50 and 100 μM were 88% and 86% shorter than the control. After a week of exposure, no root was developed at 100 μM Cu. EDTA substantially increased root length at both Cu and Pb metal concentrations compared to the EDTA control and other metal treatments.

By 20th day Lead content was high in roots and low in leaves. There was no change in lead accumulation in leaf after 40th day. After the total experimental period it was concluded that root accumulation was higher compared to stem and leaves (Subhashini & Swamny, 2013).

Biomass

Fresh Weight

According to the figure, it can be observed that the control group (T0) had a higher survival rate compared to the treatment groups with heavy metal concentrations. The difference was found to be statistically significant ($P < 0.05$). The fresh weight of *Catharanthus roseus* was affected by different concentrations of heavy metals. The highest fresh weight was recorded in T0, which was 10.20g, while the lowest was in T3 with a value of 0.73g.

Dry Weight

Based on the evaluation of the oven dry weight of *Catharanthus roseus*, it was observed that there were no significant differences ($P > 0.05$) in the effect of different concentration rates of heavy metals on the oven weight of *Catharanthus roseus*. The graph showed that the highest value of oven weight was observed in T0, which was the control group and weighed 1.83 g. On the other hand, the lowest value of oven weight was observed in T3, which weighed 0.36 g.

Conclusion

Research indicates that using *Catharanthus roseus* in phytoremediation procedures to absorb certain heavy metals especially Pb (T4 - 6.89mg/kg in plants) and Zn (T4 - 39.27mg/kg in plants) shows positive impacts in this kind of soil. Furthermore, even after it dies, plants have the capacity to absorb and hold onto heavy metals from the soil.

As a result, the plant's growth performance and soil properties are not significantly improved. In addition, this plant is also not very effective in improving soil properties, such as pH and nutrient content. The results showed that the T3 solution - 200ml of 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 108.6 (Zn) - exhibited the best plant growth performance in terms of soil and plant concentrations reading.

The plant's growth performance has affected by the presence of heavy metals in the soil. Therefore, based on the research findings, using *Catharanthus roseus* in phytoremediation processes to absorb heavy metals can be approach and alternative methods should be explored.

Recommendation

For future research, I recommend using seedlings from seed and transplanting them at least two weeks after sowing, with a usage rate beginning with treatment 3 and higher. Other than that, recommend to planted other than silty clay loam texture because the *Catharanthus roseus* need wet soil but need to have good drains.

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