Effect of Adding Banana Peel Biochar on the Growth of *Vignaradiata* in Heavy Metal Contaminated Soil

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Abstract: ***We investigated the potential application of banana peel biochar (BPB) for the adsorption of heavy metals (Ba, Cd, Cu, Pb) in contaminated soil. The study proved that banana peel biochar is effective in neutralizing lead and copper toxicity in contaminated soil. The percentage increase in height with BPB was Copper (28.56%) > Lead (15.51%) > No Chemical (13.70%) > Barium (5.24%) > Cadmium (3.87%). This study revealed that when copper and lead - treated V. radiata plants are supplemented with banana peel biochar, they show better growth, yield and survival percentage as compared to untreated, metal ion - contaminated plants. Therefore, banana peel biochar can serve as an eco - friendly, low - cost, and efficient adsorbent to immobilize heavy metals in contaminated soils.***

Keywords: heavy metal, Barium, Cadmium, Copper, adsorbent, banana peel, biochar

1. Introduction

Unrestrained anthropogenic activities have resulted in an alarming global increase in heavy metal contaminants leading to the pollution of terrestrial and aquatic ecosystems (Tóth et al., 2016, Tiller, 1989, Alengebawy et al., 2021). This in turn, effects primary productivity as pivotal functional processes in the plants are impaired.

The growing population and limited land availability, food security is a primary concern in the current geographical and economic climate (Farooq et al., 2019). With the increasing probability of crop loss in such contaminated soils, remediation and ensuring plant survival is of utmost importance. Additionally, heavy metals may be retained in the crop produce causing adverse effects in the food chain (Tutic et al., 2015). It is also important to study the many inhibitory effects of heavy metals on the physiology, biochemistry, structural integrity, growth, and functionality of plants (Cheng, 2003), in order to take appropriate remediation measures.

The uptake of heavy metals such as copper and cadmium can impair functional and structural components of the plant through the induction of oxidative stress (Valko et al., 2016)

The propensity for heavy metals to undergo redox cycling can also lead to lipid peroxidation, DNA and protein denaturation (Valko et al., 2005). By disrupting intracellular pathways, these ions can hinder the thylakoid network, induce iron deficiency causing adecrease in chlorophyll concentration (Pätsikkä et al., 2002), inhibit seed germination, impede electron transport and disrupt membrane permeability maintenance (Pourrut et al., 2011).

In view of the imminent food shortage, remediation of contaminated soilsis increasingly necessary in order to reclaim and increase soil arability. In this regard, different methods have been tried with varied success.

The use of specific plants like *P. americana*, *E. acicularis* and *S. alfredii* for soil decontamination process is referred to as phytoremediation and these plants are referred to as hyperaccumulator plants (Xiao et al., 2017). This method is sustainable, affordable and biodegradable as plants that adsorb heavy metals at higher concentrations can be cultivated across large spans of land with relative ease (Yan et al., 2020).

Phyto - stabilization, phytovolatilization and phytofiltration all involve either the absorbance and translocation of heavy metal ions, formation of stable heavy metal ligand complexes through root exudates or the formation and release of volatile compounds from heavy metals by plants. Whilst all these means of soil reclamation are efficient, they demand excessive resources as the low biomass of hyperaccumulators necessitate several harvests (Nikolić et al., 2008). This in turn requires practices such as frequent disposal which enables the reintroduction of contaminants into the environment, posing an ecological and financial burden (Römkens et al., 2002, Munn et al., 2008).

In cognizance of this excessive resource consumption, organic adsorbents can be used to mitigate the effects of heavy metals in soil. In India alone, approximately 33 million tonnes of fruit and vegetable waste is generated annually. These wastes are indigestible but are rich in a variety of bioactive compounds – cellulose, phenolic compounds, vitamins, antioxidants, and other phytochemicals (Kumar et al., 2020). Peels from dragon fruit, avocado, Hami melon and pineapple all exhibit heavy metal ion sorption capabilities (Suleria et al., 2020, Bulai et al., 2021, Xu et al., 2022). The abundance of cellulose determines the peel's efficiency as a biosorbent (Annadurai et al., 2003, Jayanbakht et al., 2014).

Banana peels are highly abundant (Putra et al., 2022) and contain high concentrations of cellulose, hemicellulose, lignin and other compounds, banana peels can act as sustainable and efficient biosorbents as compared to other peels (Orozco et al., 2014). Therefore, this research aims to study the effect of banana peel biochar on the growth of *V. radiata* in heavy metal - contaminated soil.

2. Materials and Method

Banana Peel Biochar Preparation:
This method was adapted from Putra et al., 2022
4 - 6 cm sections of *Musa acuminata* peels was heated for an hour at 100°C. The dried peels were homogenized into a fine powder. The banana peel powder was then soaked in distilled water for 30 minutes, strained and dried at 80°. C for 2 hours.

Heavy Metal Solution Preparation and application:
0.75M solutions of barium chloride, cadmium chloride, copper sulphate, and lead nitrate were prepared. The experiment was conducted in pots under natural setting. Each pot was filled with 7 kilograms of soil and supplemented with 20 ml of 0.75 M Ba, Cd, Cu and Pb respectively. Additionally, the experimental pots were treated with 40gms of banana peel biochar. Control pots were not supplemented with biochar. All pots were watered with freshwater and planting was done a week after the treatment.30 seeds were planted in each pot.

Plant analysis:
Plant height and leaf number was measured at 7, 14, 21, 28, 35 and 42 days after sowing (DAS). After 42 DAS (6 weeks) plants were uprooted to measure root length and biomass. Chlorophyll concentration was measured using a spectrophotometer according to the method described by Sumanta et al., 2014. The concentrations of chlorophyll - a, chlorophyll - b and carotenoids was calculated using the formulae given by (Sumanta et al., 2014)

1) Chlorophyll - a=12.47A665.1 – 3.62A649.1
2) Chlorophyll - b=25.06A649.1 – 6.5A665.1
3) Carotenoids= (1000A480 – 1.29Ca - 53.78Cb) /220.

Statistical Analysis: All recorded data was expressed in mean. A two - way analysis of variance was used to analyse the effect of heavy metals and banana peel biochar on the growth of V. radiata. Two - way ANOVA was done using MS Excel and level of significance was determined at $p \leq 0.05$.

Ethical Consent: Since the experiment did not involve any human subject no ethical consent was required.

3. Results and Discussion

Figure 1.1: Barium, lead and Cadmium- Treated Plants: A-28 DAS B- 35 DAS

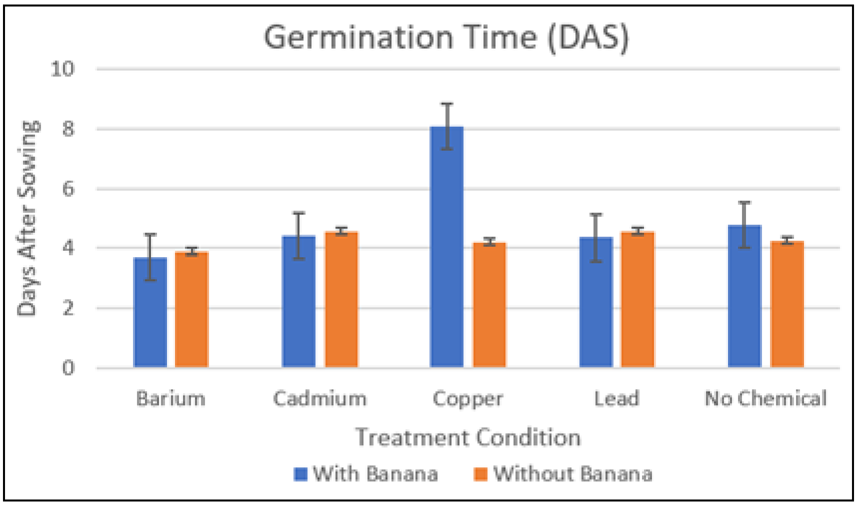

Figure 1.2: Germination time (DAS)

**Error bars represent standard deviation*

The time taken for each seed to germinate was closely observed and denoted in terms of days after sowing (DAS). The Banana - treated Barium group was the fastest to germinate at an average of 3.7 days, while the Banana - treated Copper group took the most days to germinate (8.08 days).

Stem height and Number of Leaves:

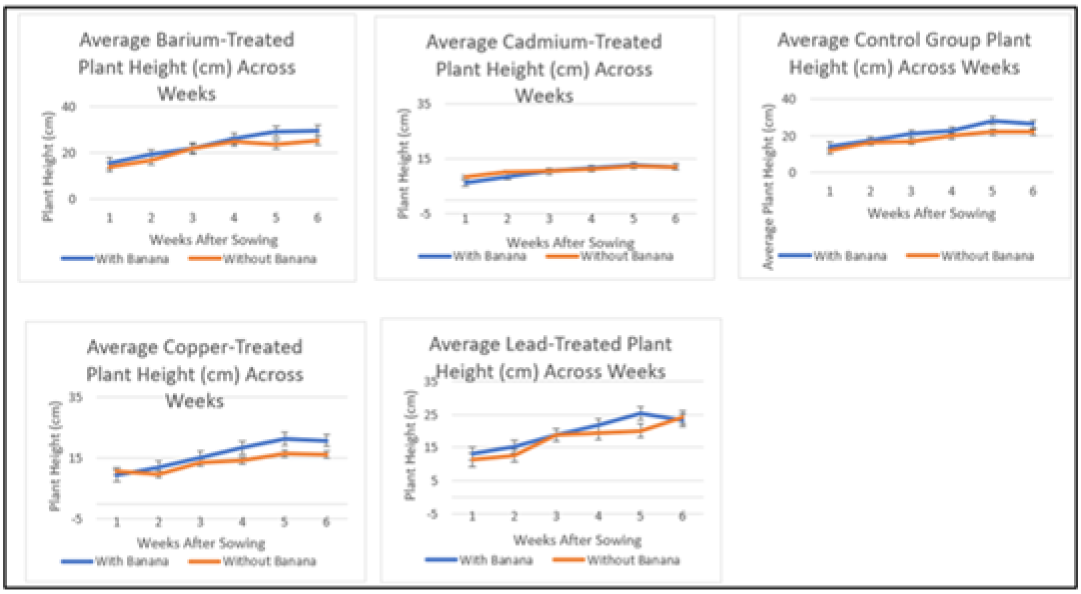

Figure 1.3: Average Stem Height (cm), 0- 6 weeks

As seen in **fig 1.3**, Barium + Banana group exhibited the tallest average height at 26.13 centimeters, while the untreated Cadmium group displayed the least average height at 11.07cm at 4th week (28 DAS). Banana biochar had the highest effect on the copper - treated plants with a 28.56% difference between the banana - treated and untreated plants. The percentage increase in height was Copper (28.56%) > Lead (15.51%) > No Chemical (13.70%) > Barium (5.24%) > Cadmium (3.87%). The 'F' calculated values (**60.40** and **12.17**) were greater than their respective critical values (**6.40 and 7.71**) at $p \leq 0.05$, indicating a statistically significant difference between the heights of bananabiochar treated vs. untreated plants.

Barium had the least impact on the height of the plant while copper had the greatest stunting effect. Addition of banana biocharled to an increase in plant height. It can be inferred from the data that banana peel biochar can adsorb copper and lead from the soil as there is a 28.6% and 15.51% percent difference in height of plants treated with copper + banana and lead + banana respectively, as compared to only copper and lead treated plants. The reduction in height in heavy metal plants may indicate a tendency for *V. radiata* to accumulate heavy metals in its shoots resulting in oxidative stress. Similar results have also been observed in cucumber (Sleimi et al., 2021).

Banana peel biochar had the least effect on cadmium - treated plants. This may be because of insufficient biochar or the failure to adsorb Cadmium ions. Relative to other heavy metal ions, cadmium is readily taken up by many plants and can cause cytotoxicity and general metabolic harm at relatively low concentrations as well (Gill & Tuteja, 2011). Cadmium not only causes oxidative stress as other heavy metals do, but also impacts photosynthesis by inhibiting Rubisco (Wahid et al., 2007). Salim et. al reported copper and cadmium had the greatest impact on shoot growth, but the above data shows that the effect of copper can be alleviated by banana peel biochar. They also reported that lead had a minimal negative effect on plant height. The no - chemical, banana - treated plants showed a significant positive response to banana peel.

Leaf Number:

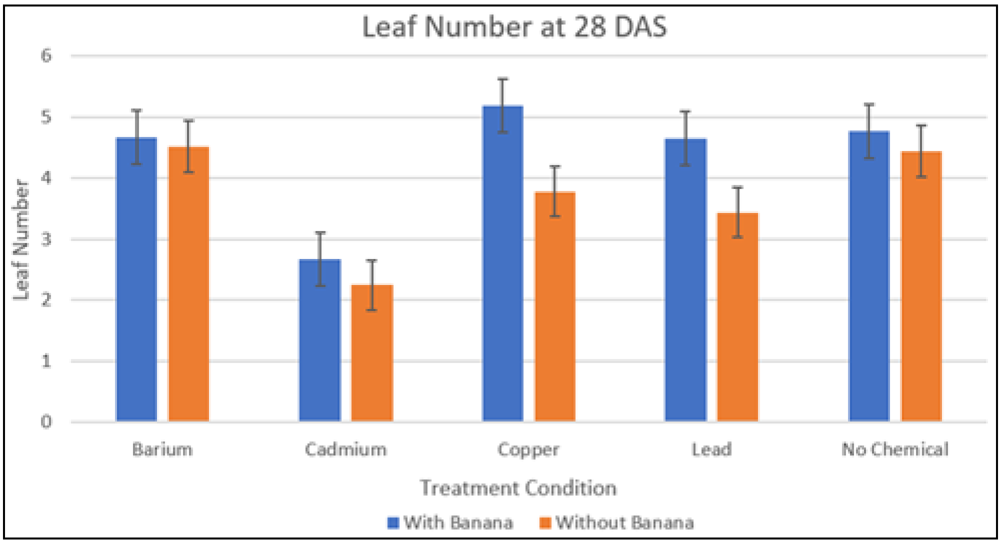

Figure 1.4: Average leaf number at 28 DAS

Error bars represent standard deviation

The banana - treated copper group displayed the highest average leaf number at **5.2**. The addition of the banana peel effected the greatest percent increase **(37.037%)** in the copper - treated groups, showing that banana peels effectively adsorbed copper and increased the leaf number. The no - banana cadmium group exhibited the minimum value for average leaf number at **2.25.** The banana - treated cadmium group was also significantly damaged, possibly indicating an ineffectiveness of banana peels in cadmium adsorption.

This may be due to the potency of cadmium (Hani et al., 2020) in plant growth inhibition where small concentrations can elicit greater response (Hatamian et al., 2020).

Lead ions were found to inhibit leaf number as seen in fig **1.4**. The addition of the banana peel biochar had a positive effect on the Lead - treated plants with a percentage change of 35.17%.

The decrease in leaf number in the metal ion - treated groups may be ascribed to the powdery mildew attack after week 4, because of which the statistical analyses indicated non - significance – supporting the null hypothesis for leaf number. However, a t - test was done to ascertain the effect of the banana peel on copper and lead treated plants. The t - calculated (**3.28**) was greater than t - critical (**1.72**) for both copper and lead - treated plants, proving that the addition of banana peel had a significant effect on the leaf number in both copper and lead groups.

Flowering and Fruiting:

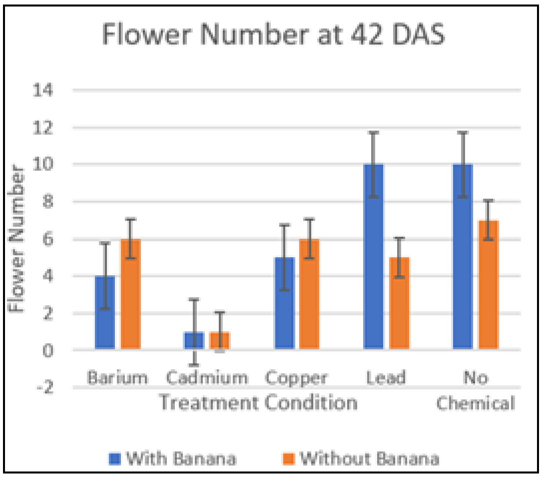

Figure 1.5: Number of flowers at 42 DAS

Error bars represent standard deviation

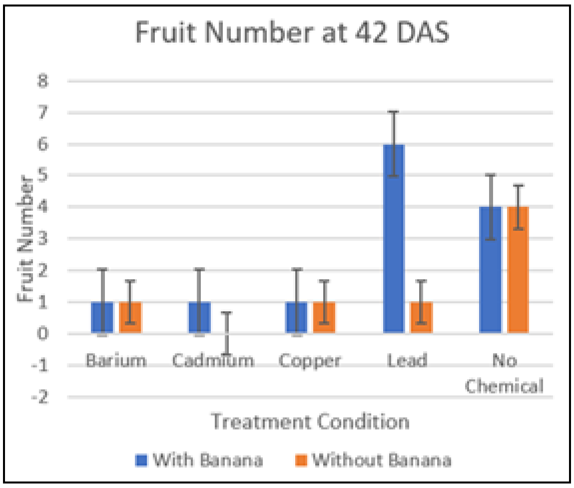

Figure 1.6: Number of fruits at 42 DAS

Error bars represent standard deviation

Addition of banana peel biochar increased the number of flowers and fruits only in plants treated with lead. It did not have a significant effect on the other groups. In the cadmium - treated groups, the “no - banana” group had flowers, but did not bear fruit as the flowers shed.

Biomass and Root Length:

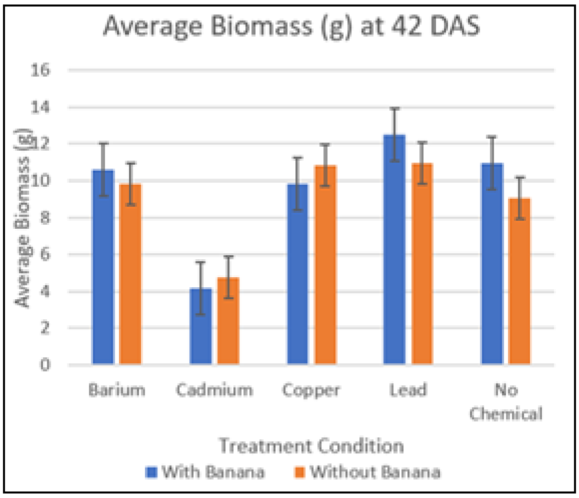

Figure 1.7: Biomass (g) at 42 DAS*
Error bars represent standard deviation

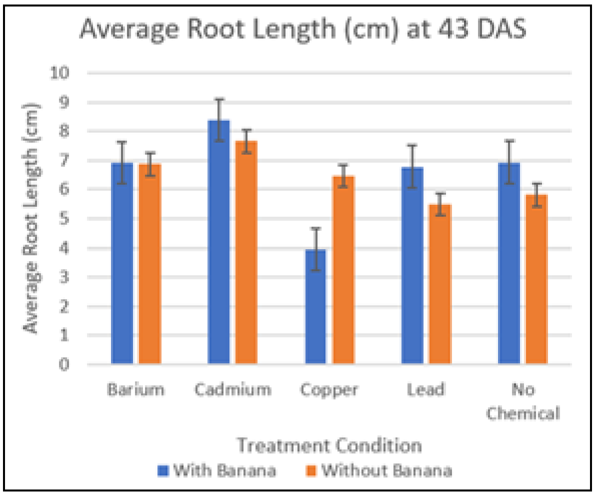

Figure 1.8: Root Length (cm) at 42 DAS
Error bars represent standard deviation

As shown in figures **1.7**and **1.8** addition of banana peel had a significant positive impact on the biomass and root length only in the lead - treated plants. The t - calculated (**2.68**) was greater than t - critical (**1.86**) for lead - treated plants, proving that the addition of banana peel had a significant effect on the root length for lead - treated groups.

Copper - treated plants displayed a marginal decrease in biomass with the addition of banana peels. This can be attributed to the greater extent of powdery mildew infection in the untreated group. The greater extent of infection may have been a result of an induced virulence within the powdery mildew infection by superoxide dismutase synthesis (Dashti et al., 1995) (Cox et al., 2003). Additionally, the powdery mildew was likely to have developed a resistance to copper toxicity due to the soil environment containing large quantities of nitrate ions - isolating the positive benefit of copper ion presence in the synthesis of superoxide dismutase (Sazanova et al., 2015). For this reason, the powdery mildew infection may have confounded the biomass as a result of an increase in water retention with the infected copper and cadmium groups (Ayres, 1997).

Carotenoid and Chlorophyll Content:

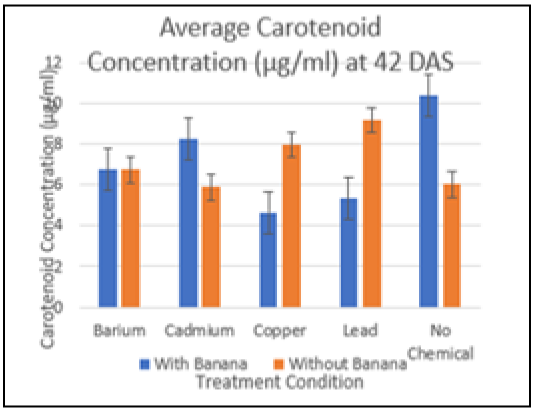

Figure 1.9: Carotenoid concentration
Error bars represent standard deviation

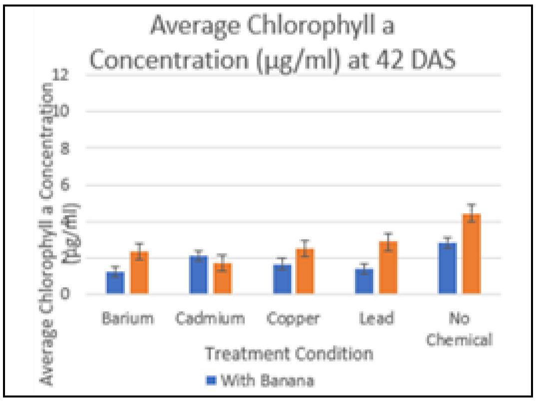

Figure 2.1: Chlorophyll- a concentration
Error bars represent standard deviation

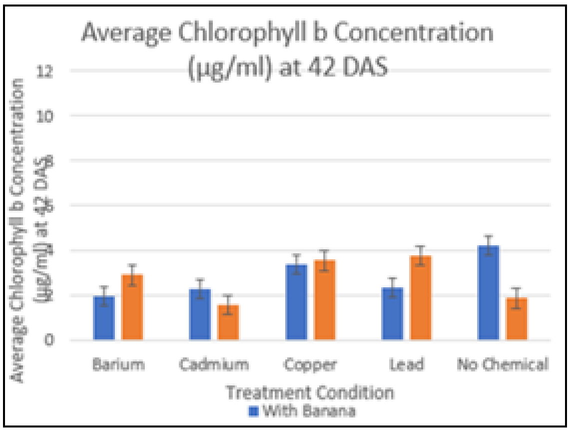

Figure 2.2: Chlorophyll- b concentration
Error bars represent standard deviation

Chlorophyll content increased in plants that were not treated with banana peel (Figure **2.7** and **2.8**,). These plants had severe fungal attacks; this might be because metal ions in nitrate media provide conducive environments for fungal growth (Gajewska et al., 2022). Due to the low - light penetration in mildewed leaves, it is possible that the chlorophyll concentration increased as an adaptive mechanism in response to low light (Ferreira et al., 2016).

Carotenoid concentration was also observed to be higher among mildewed plants. Groups contaminated with copper, lead and barium saw no difference in carotenoid content in the absence or presence of the banana peel biochar and in the case of copper and lead, the opposite effect was observed. Saja et. al., 2020reported that powdery mildew is capable of inducing carotenoid production which explains the greater concentrations of carotenoids in plants not treated with banana biochar.

Addition of banana peel did not show a positive response in any of the other treatment conditions. From the above discussion, it can be inferred that the addition of banana peels reduced the extent of fungal attack on the copper and lead - treated groups, as carotenoid and chlorophyll concentrations were low.

4. Conclusion

The present study proved that banana peel biocharis effective in neutralizing lead and copper toxicity in contaminated soil. The study revealed that copper and lead contaminated *V. radiata* plants responded positively to banana peel biochar as shown by growth, yield and survival rate.

The efficacy of banana peel biochar can be ascribed to its irregular porous surface and the presence of adsorbent C - H, =CH AND C - Cl functional groups in the cell walls which electrostatically interact with metal ions (Shenwari et al., 2019, Mallampati et al., 2015).

Contrary to earlier studies (Xu et al., 2022), this study showed that banana peel biochar was more effective against copper and lead than cadmium. A possible reason for this may be the use of soil as the medium of contamination rather than an aqueous solution.

From the present study, it can be concluded that banana peel biochar is an effective adsorbent of copper and lead in contaminated soil. The effect of banana peel biochar on other heavy metals needs to be further investigated by increasing the amount of biochar and by effective treatment against fungal infections. Further studies can done to test the efficacy of other fruit peel biochar such as citrus and watermelon peels and alsoother unused plant wastes as biosorbents.

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