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Research Article

Phytoremediation of cadmium-contaminated soil using terrestrial kale (*Ipomoea reptans* Poir) and corncob biochar

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Abstract: This study aimed to examine the potential of terrestrial kale (*Ipomoea reptans* Poir) combined with corncob biochar for phytoremediation of cadmium-contaminated soil. The experiment design was a completely randomized block design with two factors. The first factor was the population density of *Ipomoea reptans* (0, 2, and 4 plants/polybag). The second factor was the dose of corncob biochar (0, 5, and 10 t corncob biochar/ha). The variables observed were plant height, leaf number, leaf area, chlorophyll content, wet shoot weight, dry shoot weight, wet root weight, dry root weight, effectiveness of plant in Cd absorption and removal efficiency of Cd. The results showed that *Ipomoea reptans* could adsorb 73.59% of Cd without application of corncob biochar. *Ipomoea reptans* planted with a population density of 4 plants/polybag reduced Cd content in the soil by 57.70% Application of 10 t corncob biochar/ha reduced Cd content in the soil by 43.42%. There was an interaction between *Ipomoea reptans* panted with a population density of 4 plants/polybag with the application of 10 t corncob biochar that reduced Cd content in the soil by 62.42%.

Keywords: *cadmium, corncob biochar, metal accumulator plant, terrestrial kale*

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Introduction

Environmental pollution caused by heavy metals is a very serious problem. One of the main factors of heavy metal pollution in the soil is the result of excessive input applications in the agricultural sector (Hindarwati et al., 2018). Heavy metal pollution comes from industrial waste, the application of pesticides and synthetic chemical fertilizers continuously with excessive doses, and the disposal of household waste in streams (Sutrisno and Kuntyastuti, 2015). One of the heavy metals that cause environmental pollution is cadmium (Cd). Cadmium is often found on agricultural land (Erfandy and Ishaq, 2014). On agricultural land, Cd mainly comes from inorganic phosphate fertilizer (Chien et al., 2003). Cadmium is one of the heavy metals which has a high toxic effect on living things (Resmaya et al., 2014). One of the techniques that can be developed to reduce or remove Cd from Cd-contaminated soils is phytoremediation. It is widely known that phytoremediation is a remediation method by relying on the role of plants to absorb, degrade, transform and immobilize heavy metal pollutants from soil and water (Morel et al., 2006; Hardiani). Terrestrial kale (*Ipomoea reptans* Poir) is a fast-growing species that is often used as a metal accumulator plant for phytoremediation of Cd-contaminated soil (Suhaeni and Rida; 2016; Herlina et al., 2018). Suhaeni and Rida (2016) reported that *Ipomoea reptans* could accumulate

Cd in roots, shoots, and leaves by 0.4303 ppm, 0.1513 ppm and 0.1667 ppm, respectively, within 18 days. These values, however, were relatively small compared to the initial content of Cd in the soil studied. Another technique that can be developed to reduce or remove Cd from Cd-contaminated soil is the use of biochar. According to Hidayat (2015), biochar has the ability physically and chemically to eliminate the activity of heavy metals. Biochar can also stabilize heavy metals in polluted soils by significantly reducing the absorption of heavy metals by plants, and can improve their quality by improving soil physical, chemical and biological properties (Ippolito et al., 2012).

This study was aimed to examine the potential of terrestrial kale (*Ipomoea reptans* Poir) combined with corncob biochar, for phytoremediation of cadmium-contaminated soil.

Materials and Methods

Preparation of Cd-contaminated soil

The main solution of 5 ppm Cd that was made by weighing 0.6846 $CdSO_4.8H_2O$ was transferred into a 1000 mL baker glass. The distilled water then was added until the boundary mark to obtain the main solution of Cd with a concentration of 1000 ppm. From the main solution of 1000 ppm Cd, 5 mL was diluted to get Cd solution with a concentration of 5 ppm (Liong et al., 2009). The Cd solution was then injected to the soil. Cd concentration in the initial soil that measured using an atomic absorption spectrophotometer was 0.89 ppm. The initial soil used for this experiment had a pH value of 7.0.

Preparation of corncob biochar

Corncob biochar was prepared according to the procedure developed by the Indonesian Agricultural Environment Research Institute of Pati. Corncob biomass was put into a furnace and then tightly closed with a combustion temperature between 100-300^{0}C for 3-5 hours. After 3-5 hours, the combustion fire was turned off, and the furnace was allowed to cool for about 12 hours. The biochar was then subjected to refinement process.

Experimental design

The experimental design used was a completely randomized block design with two factors and three replications. The first factor was the density of *Ipomoea reptans* consisting of K0 (without *Ipomoea reptans*), K1 (2 *Ipomoea reptans* plants/polybag) and K2 (4 *Ipomoea reptans*/polybag). The second factor was the corncob biochar consisting of B0 (without application of corncob biochar), B1 (5 t/ha) and B2 (10 t/ha). One week old of *Ipomoea reptans* seedlings were planted in polybag with a density of 2 and 4 plants. Each polybag was applied with two days old corncob biochar with the rates equivalent to 5 and 10 t /ha. The plant was harvested at the age of 5 weeks after planting. The variables observed at harvest were plant height, leave number, leave area, shoot weight, and root weight.

Plant sample and analysis

The harvested plant roots, shoots, and leaves that have been separated, washed, and cleaned were then stored in plastic bags, weighed, oven-dried for 48 hours at 70^{o}C, and ground for laboratory analysis (Liong et al., 2009). For the analysis of Cd concentration, a 0.5 g dried-ground plant sample was dissolved in a mixture of 5 mL of HNO_3 6 M and 5 mL of 30% H_2O_2, heated to a perfect soluble sample. The soluble sample was cooled, and distilled water was added, heated and filtered in a hot state into a 50 mL. The pH of the sample solution was adjusted to about pH 3 with nitric acid and or sodium hydroxide. The sample solution was mixed with distilled water until the boundary mark, and shaken until homogeneous. Cadmium concentration was measured with Atomic Absorption Spectrophotometer using the calibration curve (Liong et al., 2009).

Effectiveness of plant absorption and removal efficiency

The effectiveness of plant absorption (EPA) in metal absorption illustrates the ability of plants to absorb heavy metals. The EPA value was calculated using the following formula (Prayudi et al., 2015),

$$\text{EPA (\%)} = \frac{\text{CMAP}}{\text{DCMS}} \text{ x } 100\%$$

where :

EPA = effectiveness of plant in metal absorption

CMAP = concentration of metal in plant

DCMS = decreased concentration of metal in soil

Removal efficiency (RE) of Cd from the soil by the plant was calculated using the following formula (Hardiani, 2008),

$$\text{RE (\%)} = \frac{\text{IMC} - \text{FMC}}{\text{IMC}} \text{ x } 100\%$$

where :

RE = removal efficiency

IMC = initial metal concentration in soil

FMC = final metal concentration in soil

Results and Discussion

Soil pH and cadmium

After one-week incubation of Cd-contaminated soil, the soil pH decreased from 7.0 to 6.2. Resmaya et al. (2014) reported that high concentration Cd in soil decreased soil pH because of the increased mobility of Cd (Widyatmoto, 2011). The decrease of soil pH indicated the high heavy metal toxicity. The decline in pH was due to the release of heavy metals in the soil that increases their mobility (Widiyatmoko, 2011)

Effectiveness of plant absorption

Based on the results of the analysis of variance (Table 1), plant density did not significantly affect the effectiveness of plant in Cd absorption. This is probably because the absorption of water by the metal accumulator plant was not the same in every polybag even though the amount of water supplied was the same (200 mL/polybag), thus affecting the effectiveness of plant in Cd absorption. According to Sari et al. (2015), each plant has the ability to accumulate heavy metals differently. The more water content in the soil media, the more Cd that can be easily dissolved and absorbed by plant roots.

Table 1. Effect of population density on the effectiveness of plant in Cd absorption.

Treatment (plant density)	EPA (%)
K1	54.61
K2	58.74
F test	0.24
F table	4.75

Remarks: EPA= effectiveness of plant in Cd absorption. K1 = 2 plants/polybag, K2 = 4 plants/polybag.

Based on the results of the analysis of variance (Table 2), the application of corncob biochar had a significant effect on the effectiveness of plant in Cd absorption. The efficiency of plant absorption in the B0 treatment was 73.59%, while that in the B1 treatment (application of 5 t corncob biochar/ha was 51.3%. The lowest effectiveness of plant absorption (45.15%) was observed in the B2 treatment (application of biochar 10 t corncob biochar/ha). The higher value of the effectiveness of plant absorption in the B0 treatment (no application of corncob biochar) than that in treatments with the application of 5 t and 10 t corncob biochar/ha was because corncob biochar could minimize the plant absorption of Cd. This is in accordance with results of a study conducted by Prayudi et al. (2015) that application of corncob biochar can reduce the mobility of heavy metals in polluted soils that makes the metals unavailable to plants.

Table 2. Effect of biochar on the effectiveness of plant in Cd absorption.

Treatment (biochar dose)	EPA(%)
B0	73.59 a
B1	51.30 a
B2	45.15 ab
F test	4.23*
F table	3.89

Remarks: EPA = effectiveness of plant in Cd absorption. The average numbers followed by different letters in the same column show significant different effect based on the 5% error level of DMRT. B0 = without corncob biochar, B1 = 5 t corncob biochar/ha, B2 = 10 t corncob biochar/ha.

The combination of treatment of plant density and different biochar doses did not significantly affect the effectiveness of plant in Cd absorption (Table 3). According to Sari et al. (2015), this was because plants have the ability to absorb different heavy metals, so the density of plants combined with the administration of biochar also could absorb different heavy metals following the accumulation of Cd in the plants.

Table 3. Effect of combination of plant density and corncob biochar on the effectiveness of Cd absorption by the plan.

Treatment combinations	EPA (%)
K1B0	69.35
K1B1	48.10
K1B2	46.39
K2B0	77.82
K2B1	54.30
K2B2	45.15
F test	0.16
F table	3.89

Remarks: EPA = effectiveness of plant in Cd absorption. K1 = 2 plants/polybag, K2 = 4 plants/polybag, B0 = without corncob biochar, B1 = 5 t corncob biochar/ha, B2 = 10 t corncob biochar/ha.

The removal efficiency of Cd

Data presented in Table 4 show that the treatment of plant population density significantly affected the efficiency of absorption of heavy metals in the soil. The best plant density was 4 plants/polybags (K2 treatment) of 57.70%, followed by 2 plants/polybag (K1 treatment) of 55.46%. The lowest removal efficiency (45.53%) was observed in the treatment without metal accumulator plant (K0 treatment). The higher plant populations, the higher the removal of Cd in the soil. According to Prayudi et al. (2015), the more plants there are, the more metal is absorbed in plants. The K0 treatment or without metal accumulator plant had low efficiency due to the nature of the Cd metal which is very difficult to decompose if no absorption facilities such as metal accumulator plant or some materials are used to reduce the levels of Cd in the soil.

Table 4. Effect of plant density on the removal efficiency of Cd in the soil.

Treatment (plant density)	RE (%)
K0	45.53 b
K1	55.46 ab
K2	57.70 a
F test	11.08**
F table	6.01
CV (%) = 11.029	

Remarks: RE = removal efficiency. The average numbers followed by different letters in the same column show significant different effect based on the 5% error level of DMRT. K1 = 2 plants/polybag, K2 = 4 plants/polybag.

The application of corncob biochar significantly affected the efficiency of Cd removal in the soil. The removal efficiency of Cd due to the application of 10 t corncob biochar/ha (B2 treatment) was 59.15%, while that due to the application of 5 t corncob biochar/ha (B1 treatment) was 56.02%. These values were significantly different from that observed in the B0 treatment (without biochar application) of 43.32% (Table 5).

Table 5. Effect of corncobs biochar application on the removal efficiency of Cd in the soil.

Treatment (biochar dose)	RE (%)
B0	43.32 b
B1	56.02 a
B2	59.15 a
F test	18.10**
F table	6.01
CV (%) = 11.029	

Remarks: RE = removal efficiency. The average numbers followed by different letters on the column show significant different effect based on the 5% error level of DMRT. B1 = 5 t corncob biochar/ha, B2 = 10 t corncob biochar/ha.

Besides the ability of biochar to minimize heavy metals uptake by a plant (Komarek et al., 2013), biochar has the ability to stabilize heavy metals in polluted soils (Ippolito et al., 2012). According to Lu et al. (2012), the decrease in the concentration of heavy metals in soils due to application of biochar is by reducing the toxicity of heavy metals through several mechanisms. Zhou et al. (2008) reported that the removal of Cd in soil and the reduction of Cd absorption by plants due to the application of cotton stalk biochar cotton stalk is through the adsorption process. The combination of plant density and biochar doses had a very significant effect on the removal efficiency of Cd in the soil (Table 6). The combination of plant population density of 4 plants/polybags and application of 10 t corncob biochar/ha had the highest removal efficiency of 62.42% which was very significantly different from K1B2 of 59.87%, K0B1 of 57.72%, K2B0 of 56.08%, K1B1 of 55.74%, K0B2 of 55.17%, K2B1 of 54.61%, and K1B0 of 50.76%. The removal efficiency of Cd in the K0B0 treatment was only 23.71%.

Tabel 6. Efficiency removal of Cd in the soil.

Treatment combination	RE (%)
K0B0	23.71 c
K0B1	57.72 b
K0B2	55.17 ab
K1B0	50.76 b
K1B1	55.74 ab
K1B2	59.87 ab
K2B0	56.08 ab
K2B1	54.61 ab
K2B2	62.42 a
F test	8.44**
F table	4.58
CV (%) = 11.029	

Remarks: RE = removal efficiency. The average numbers followed by different letters in the same column show significant different effect based on the 5% error level of DMRT. K1 = 2 plants/polybag, K2 = 4 plants/polybag, B0 = without corncob biochar, B1 = 5 t corncob biochar/ha, B2 = 10 t corncob biochar/ha.

The treatment combination of plant density of 4 plants/polybag with 10 t corncob biochar/ha had the best removal efficiency of Cd. The results of the analysis of variance with the Annova test presented in Table 7 show that plant density had no significant effect on all plant growth variables in Cd. This is because each plant has a different response, one of which is the adaptation of each plant under Cd stress conditions. The ability of plants to adapt to the environmental stress by heavy metals varies. According to Irwan et al. (2004), plant growth is not significantly affected by density because each plant has a different response. Data presented in Table 7 also show that the application of corncob biochar had a very significant effect on plant height and number of leaves. The application of corncob biochar also significantly affected fresh plant weight, dry plant weight, and leaf width. It is thought that corncob biochar reduced the toxicity of Cd in the soil so that there was only a small amount of Cd taken up by plants, and plants could grow well. According to Skjemstad et al. (2002), plants absorb heavy metals in the form of free radicals that have high mobility, the application of biochar can reduce the mobility of heavy metals in polluted soils so they cannot be absorbed by plants because they are no longer available to plants. The application of 10 t corncob biochar/ha (K2 treatment) gave the best plant height, number of leaves, fresh crown weight, dry crown weight, and the leaf width, whereas the B0 treatment (without application of corncob biochar) gave the lowest yields. It is suspected that the soil was not treated with biochar still have a significant amount of heavy metals that are easy to accumulate to plants, so that plant growth was inhibited.

Table 7. Analysis of the various effects of treatment on plant growth.

Treatments (plant density)	PH (cm)	LN (leaves)	WSW (g)	DSW (g)	WRW (g)	DRW (g)	LA (cm)
K1	51.01	11.00	13.17	0.93	1.49	0.32	1.50
K2	45.47	10.11	12.49	0.94	1.48	0.31	1.48
F test	2.47	2.28	0.15	0.02	0.01	0.004	0.54
F table	4.75	4.75	6.16	4.75	4.75	4.75	4.75
CV (%)	15.45	11.81	14.45	6.14	17.13	69.49	4.95
Biochar dose							
B0	40.48 b	9.50 b	9.05 a	0.89 a	1.40	0.32	1.42 b
B1	47.34 ab	10.00 ab	11.58 ab	0.93 a	1.55	0.34	1.48 ab
B2	56.89 a	12.16 a	17.41 a	0.99 a	1.50	0.28	1.56 a
F test	7.33**	7.75**	6.16*	4.55*	0.56	0.12	5.69*
F table	6.93	6.93	3.89	3.89	3.89	3.89	3.89
CV (%)	15.45	11.81	14.45	6.14	17.13	69.49	4.95
Combination of plant density and biochar dose							
K1B0	42.59	10.00	10.18	0.89	1.45	0.40	1.45
K1B1	48.47	10.00	11.30	0.91	1.58	0.32	1.47
K1B2	61.95	13.00	18.045	0.99	1.45	0.22	1.58
K2B0	38.37	9.00	8.83	0.89	1.35	0.23	1.39
K2B1	46.21	10.00	11.86	0.94	1.53	0.36	1.49
K2B2	51.84	11.33	16.78	0.98	1.56	0.33	1.55
F test	0.45	0.67	0.14	0.19	0.27	0.03	0.46
F table	3.89	3.89	3.89	3.89	3.89	3.89	3.89
CV (%)	15.45	11.81	14.45	6.14	17.13	69.49	4.95

Remarks: PH = plant height, LN=leave number, WSW= shoot fresh weight, DSW= dry shoot weight, WRW= root fresh weight, DRW= dry root weight, LA = leave area. The average numbers followed by different lowercase letters in the same column show significant different effect based on the 5% error level of DMRT. B1 = 5 t corncob biochar/ha, B2 = 10 t corncob biochar/ha.

Symptoms that appeared on metal accumulator plant without application of corncob biochar was chlorosis on the leaves (below), leave fall, and the decreased number of leaves. One of the negative effects of heavy metals on plants is the decrease in the number of leaves due to disruption of physiological processes related to photosynthesis and plant growth (Astrini, 2004). Liong et al. (2009) who studied the growth of groundwater spinach plant in media contaminated with heavy metals, reported that the plant experienced physical abnormalities such as chlorosis and small size. The application of corncob biochar did not significantly affect the fresh root weight and dry root weight of the metal accumulator plant. This is thought to be the response of plant roots after the application of corncob biochar has the ability to adsorb different Cd. Hamzah et al. (2012) who studied the use of *Vetiver* plant and biochar for remediation of gold mine tailing contaminated soil reported that heavy metals absorbed by plants were mostly distributed to the plant roots.

Conclusion

The density of 4 *Ipomoea reptans* plants/polybag effectively reduced Cd to 57.70%. Application of 10 t corncob biochar/ha effectively reduced Cd by 43.42% There were interactions of *Ipomoea reptans* planted with a population density of 4 plants/polybag with 10 t corncob biochar/ha that reduced Cd to 62.42%.

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