

Research Article

Phytobioremediation of cadmium-contaminated soil using combination of *Ipomoea reptans* Poir and *Trichoderma sp.* and its effect on spinach growth and yield

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Abstract: This research aimed to study the potential role of *Ipomoea reptans* and *Trichoderma sp.* on the absorption of cadmium from cadmium-contaminated soil and its effect on and growth rate and yield of spinach. The research was arranged in a completely randomized blok design with two factors. The first factor was the density of *Ipomoea reptans* consisting of 0, 2, and 4 plants/polybag. The second factor was the dosage of *Trichoderma sp.* consisting of 0, 50 and 100% dose of *Trichoderma sp.* Results of the research showed that *Ipomoea reptans* with 2 plants/polybag effectively reduced Cd to 66.31% and increased the growth of spinach by 20% on plant height, shoot dry weight of spinach by 35%. Application of 100% dose of *Trichoderma sp.* effectively reduced Cd by 63.81% and increased spinach plant growth by 18% on plant height and increased shoot dry weight of spinach by 23%). There were interactions of phytoremediator of 2 *Ipomoea reptans* plants/polybag with bioremediator of 50% *Trichoderma sp.* that effectively reduced Cd to 71.19% and improved 43% of plant height and leaf number, 31% of leaf area, and 63% of shoot dry weight of spinach plant compared with plants without application of *Ipomoea reptans* and *Trichoderma sp.*

Keywords: bioremediation, cadmium, *Ipomoea reptans*, phytoremediation, *Trichoderma sp.*

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Introduction

Agricultural and non-agricultural wastes can cause pollution of water and cultivated land. One of the hazardous environmental pollutants is heavy metals, which will have a negative impact on the plant and cause production to decline as well as the food chain contaminated by heavy metals. Heavy metals are often found in the agricultural area, one of them is Cd (Cadmium) (Erfandy and Ishaq, 2014). Cadmium is one of heavy metal that has a toxic effect on human, animal and plant (Wulandari, 2014). According to Fang and Zhu (2014), Cd metal contamination on agricultural land comes from the use of inorganic

pesticides and phosphate fertilizers. An effort that can be made to reduce the content of Cd in the soil is phytobioremediation, which is the combined method of phytoremediation and bioremediation simultaneously. Phytoremediation is a method of transferring or immobilizing metals in contaminated soil using plants (Sidauruk and Sipayung, 2015). But not all plants can be used as phytoremediator because some plants cannot perform metabolism, volatilization and accumulation of all pollutants. *Ipomoea reptans* Poir is one of phytoremediator plants. According to research done by Suhaeni and Rida (2016), *Ipomoea reptans* can absorb heavy metal Cd in the soil in root organs, stems and leaves. In the

root organ as much as 0.4303 ppm, stems as much as 0.1513 ppm and leaves as much as 0.1667 ppm within 18 days after planting. Bioremediation is a method of pollutant removal by involving the role of microbes in the reduction of natural contaminants so that environmental conditions can be reused optimally, safely, healthily and sustainably (Kurniawan and Ekowati, 2016). Application of the secondary metabolite *Trichoderma* sp. can be utilized to assist the growth of plants in the environment containing heavy metals (Priyambada, 2014). The study results of Andini et al. (2015) showed that the addition of rice straw resulting from *Trichoderma viride* fermentation enhances the accumulated ability of Pb and Cd metal in rooting areas of sweet corn crop (*Zea mays*). The purpose of this research was to explore the potential role of *Ipomoea reptans* and *Trichoderma* sp. on the absorption of cadmium from cadmium-contaminated soil and its effect on and growth rate and yield of spinach plant.

Materials and Methods

Place and time

The research was conducted at Agroecology Laboratory of Agriculture Faculty and greenhouse of Universitas Jenderal Sudirman of Purwokerto, from February to May 2018.

Preparation of Cd-contaminated soil

The main solution of Cd was made by weighing 0.6846 g of $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ (Merck) into cupker. The solution was then transferred into a 1000 mL baker glass, after which the distilled water was added until the boundary marks were obtained so that the main solution of Cd with a concentration of 1000 ppm. Furthermore, from the main solution of 1000 ppm Cd, diluted to get Cd with a concentration of 5 ppm (Liong et al., 2009). The soil medium used of an Inceptisol was sieved 2 mm and weighed to 5.4 kg/polybag. Measurement of Cd content and soil pH were made before application of heavy metal treatment. A solution of 5 ppm Cd was added into polybag by injection evenly on the planting medium. The pollutant volume used was 100 mL for each planting medium in a polybag (Prayudi et al., 2015). The soil was incubated for one week in a polybag containing 5.4 kg of soil. The soil was incubated in a closed state so that the applied Cd was evenly mixed

Cultivation of *Ipomoea reptans*

Ipomoea reptans seedlings (1 week old) were planted in polybag adjusted with the density of 2

and 4 plants. Each polybag was applied with Bio-T10 (formulation of *Trichoderma* sp. secondary metabolite) (Soesanto, 2015). One week after planting urea was added with a dose of 2 g/polybag (Edi and Julistia, 2010). *Ipomoea reptans* was harvested at the age of 5 weeks after planting. The plant was washed with water until clean. Plant roots, stems and leaves that have been separated and cleaned were then stored in a plastic bag, weighed, oven-dried for 48 hours, and ground for chemical analysis (Liong et al., 2009). A 0.5 g ground 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 sample of the solution was cooled, and distilled water was added, heated and filtered in a hot state into a 50 mL. The sample solution was adjusted to about pH 3 with nitric acid and or sodium hydroxide, mixed with distilled water until the boundary mark, and it was then shaken until homogeneous. Cadmium concentration was measured with AAS (Atomic Absorption Spectrophotometer) using the calibration curve (Liong et al., 2009).

Cultivation of spinach

The planting medium used for the cultivation of spinach was the soil that was formerly used for planting *Ipomoea reptans* combined with the application of *Trichoderma* sp. (as phytobioremediator) that had been allowed to stand for 1 week. Spinach seeds that have been soaked for four hours with distilled water were planted directly on the centre of the polybag with a depth of 1- 2 cm from the soil surface. Water was supplied daily to the polybag. At 10 days after planting, urea fertilizer was applied at a dose of 2 g/polybag (Edi and Julistia, 2010). Spinach was harvested at 5 weeks.

Experimental design

The experimental design used was a completely randomized block design using two factors. The first factor was the density of *Ipomoea reptans* population consisting of K0 (without *Ipomoea reptans*), K1 (2 *Ipomoea reptans* plants/polybag) and K2 (4 *Ipomoea reptans*/polybag). The second factor was the of Bio-T10 (formulation of *Trichoderma* sp. secondary metabolite) consisting of T0 (without application of Bio-T10), T1 (50% dose) and T2 (100% dose). Total treatments were nine combinations with three replications. The variables observed were plant height, leave number, leave area, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and effectiveness of absorption phytobioremediator i.e. the level of success of plants in absorbing heavy metals content with different concentrations This can be seen from the amount of removal

effectiveness of heavy metals on the soil. The effectiveness of the removal of metal content in the phytoremediation process of metals contaminated with heavy metals was calculated using the following formula,

$$RE = \frac{IC - FC}{IC} \times 100\%$$

where, RE = removal effectiveness, IC = initial concentration, FC = final concentration

Data obtained were subjected to analysis of variance followed by Duncan Multiple Range Test at 5% error level when a significant difference was found.

Results and Discussion

Soil pH and cadmium content

Incubation of the soil with 5 ppm Cd increased Cd concentration in the soil from 0.89 to 5.89 ppm, but decreased soil pH from 7.0 to 6.2. According to Hutagalung (1994) and Resmaya (2014), the presence of high concentration Cd in a medium makes the pH down (acid). The higher the concentration of Cd in the medium the lower the pH value of the planting medium; this is because the metal is acidic. Decreased soil pH indicates the greater heavy metal toxicity. A decrease in pH occurs due to the release of heavy metals in the soil so that their mobility increases (Widiyatmoko, 2011).

Growth of *Ipomoea reptans* on Cd-contaminated soil

Based on the results of the analysis (Table 1), the different density of *Ipomoea reptans* plant showed a significant difference in plant shoot dry weight. The K1 treatment (density of 2 plants/polybag) had the highest dry weight of 0.64 g which was significantly different with the K2 treatment with the density of 4 plants/polybag of 0.42 g or which was smaller about 34.38% than the K1 treatment. This was related to other variables of plant growth i.e. plant height, leave number and leave area of spinach plant of the K2 treatment that were smaller than the K1 treatment. *Ipomoea reptans* are assumed to have been able to adapt to the maximum in the gripped environment so that ultimately caused plant growth did not appear to experience significant inhibition, resulted in the increase in biomass even though the environment was gripped by Cd (Yusuf, 2014). The treatment of different population density of *Ipomoea reptans* plants did not show significant differences in the plant height, leave number, leave area, shoot fresh weight, root fresh weight and root dry weight. Healthy plant growth was indicated by no symptoms of morphological damage to plants. Presumably, the presence of Cd only inhibited the growth of plants and did not cause poisoning symptoms in the *Ipomoea reptans* plant. According to Sutrisno and Henny (2015), the effect of Cd pollution on plants is difficult to detect if it is only measured by the rate of growth and yield of plants.

Table 1. Growth and yield of *Ipomoea reptans* in Cd-contaminated soil on different population

Treatment	PH (cm)	LN (sheet)	LA (cm)	WSW (g)	DSW (g)	WRW (g)	DRW (g)
K1	33.42	6.64	1.36	12.96	0.64 b	0.55	0.09
K2	27.03	6.57	1.33	9.17	0.42 a	0.39	0.06
F test	2.72	0.06	0.22	3.9	5.7	3.5	1.48
F table	8.63	0.6	0.12	4.27	0.21	0.18	0.05
CV	12.38	8.78	8.53	15.71	8.94	8.6	3.7

Remarks : PH = plant height, LN=leave number, LA=leave area, WSW= shoot fresh weight, DSW= shoot dry weight, WRW= root fresh weight, DRW = root dry weight. The average number followed by different lower case letters on the column shows a significantly different effect based on the 5% error level of DMRT test. K1= 2 plants/polybag, K2 = 4 plants/polybag

Effectiveness of phytoremediator

The different population density of *Ipomoea reptans* plants significantly affected the effectiveness of phytoremediator (Figure 1). The K1 treatment (2 plants/polybag) had the best effectiveness of phytoremediator (66.31%) that was different with the K2 treatment (4 plants/polybag) of 60.12%, and the K0 treatment (no plant) of 48.34%. Increased level of Cd to

60% in the roots of *Ipomoea reptans* was accompanied by the decreased level of Cd in the soil, indicating that Cd was absorbed by *Ipomoea reptans*. The success of phytoremediation is based on the ability of plants to accumulate contaminants. Plants can absorb contaminants in high concentrations without causing more significant damage to plant growth (Palar, 2008). Furthermore, it is suspected that there was competition between plants in obtaining nutrients,

water and light intensity in the K2 treatment that was greater than in the K1 treatment. This caused the growth of plants in the K2 treatment was slower than that in the K1 treatment so that absorption of Cd was less optimal. According to Yusuf (2014), plant growth causes the working system from absorption of metals by plants to increase, so that the accumulation of metals that can be absorbed by plants is more significant.

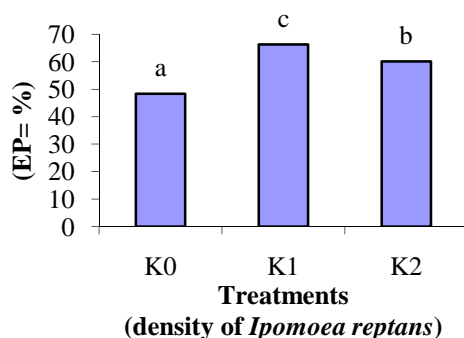


Figure 1. Effectiveness of phytoremediator

Growth of spinach plant

Table 2 shows that the treatment of different population density of *Ipomoea reptans* plants grown on the Cd-contaminated soil significantly affected in plant height, leave number and leave area of spinach plant. This was presumably because of the different content of Cd in the soil due to the previous remediation process that caused the varied growth of spinach. The higher the concentration of Cd in the growing medium, the higher the level of Cd absorbed by the plant roots along with the absorption of nutrients which then inhibited the metabolism and photosynthesis of the plants. Instead, the smaller the concentration of Cd in the planting medium, the smaller Cd content absorbed by the plant roots so that the levels of toxin in plants are low and the growth rate is normal (Wulandari et al., 2014). Different plant density treatment had a significant effect on fresh weight and dry weight of spinach

shoots. Spinach planted on the soil of the former K0 treatment had the smallest shoot fresh and dry weights compared to the K1 and K2 treatments. This condition is in line with the plant height and leave number in the two treatments that were better than that in the K0 treatment. Hardiani et al. (2009) stated that heavy metals affect plant biomass growth, excessive amounts of metals will cause toxic to plants.

Effect of bioremediator

Based on the results of analysis of variance (Table 3), the application of different dosages of Bio-T10 (formulation of *Trichoderma* sp. secondary metabolite) did not result in significant differences in all variables of growth of *Ipomoea reptans*. However, *Ipomoea reptans* plants supplied with Bio-T10 had a higher growth rate than plants without Bio-T10 application although the difference was not significant. It is suspected that Bio-T10 containing secondary metabolite of *Trichoderma* sp. affected the resistance of crops in the environmental conditions gripped. Secondary metabolites of *Trichoderma* sp. according to Prijambada (2014), can be utilized to assist the growth of plants in the environment containing heavy metals. Soil microbes play a significant role in determining the solubility, mobility, and availability of metals for plants by altering the pH in the soil microenvironment, metal speciation and the removal of heavy metal chelating compounds.

Effectiveness of bioremediator

The different dose of Bio-T10 did not give a significant effect on the effectiveness of heavy metal absorption. The results of the analysis showed that the T1 (50% dose Bio-T10) and T2 (100% dose Bio-T10) treatments had the best absorption of Cd by 63.67% and 63.81%, respectively, that were significantly different from the K0 treatment (without Bio-T10 application) of 47.31%.

Table 2. Effects of phytoremediator applied to Cd-contaminated soil on growth of spinach plant

Treatment	PH (cm)	LN (sheet)	LA (cm)	WSW (g)	DSW (g)	WRW (g)	DRW (g)
K0	5.98 a	3.01 a	2.32 a	3.72 a	0.33 a	0.31	0.03
K1	6.95 b	3.51 b	2.74 b	5.58 b	0.47 b	0.48	0.06
K2	7.63 c	3.73 c	2.89 c	5.77 b	0.51 b	0.38	0.07
F test	12.59	28.80	21.2	4.28	10.90	2.62	2.73
F Table	0.69	0.21	0.19	1.63	0.08	0.08	0.04
CV	10.16	6.02	7.23	15.05	19.46	8.23	3.6

Remarks: PH = Plant Height, LN=Leave Number, LA=Leave Area, WSW= shoot fresh Weight, DSW= shoot dry Weight, WRW= Root fresh Weight, DRW= Root dry Weight. The average number followed by different lower case letters on the column shows a significantly different effect based on the 5% error level of DMRT test. K0 = no plant, K1= 2 plants/polybag, K2 = 4 plants/polybag.

Table 3. Effects of bioremediator applied to Cd-contaminated soil on growth of *Ipomoea reptans*

Treatment	PH (cm)	LA (cm)	WSW (g)	DSW (g)	WRW (g)	DRW (g)
T0	32.05	1.38	10.52	0.49	0.46	0.07
T1	28.79	1.29	9.3	0.45	0.46	0.06
T2	30.04	1.39	13.3	0.65	0.49	0.1
F test	0.25	1.47	1.53	1.73	0.05	0.62
F table	10.56	0.14	5.23	0.25	0.11	0.04
CV	12.38	8.53	15.71	8.94	8.6	3.74

Remarks: PH = plant height, LA=leave area, WSW= shoot fresh weight, DSW= shoot dry weight, WRW= root fresh weight, DRW= root dry weight. The average number followed by different lowercase letters on the column shows a significantly different effect based on the 5% error level of DMRT test. T0 (without application of Bio-T10), T1 (50% dose of Bio-T10) and T2 (100% dose of Bio-T10).

The decrease in Cd concentration was influenced by the presence of *Trichoderma* sp. secondary metabolite applied which played a role in reducing the Cd concentration in a large quantity. Remediation of heavy metals using *Trichoderma* sp. secondary metabolite is a bioremediation type of biosorption mechanism because it only uses secondary metabolite results without applying the *Trichoderma* sp. fungus.

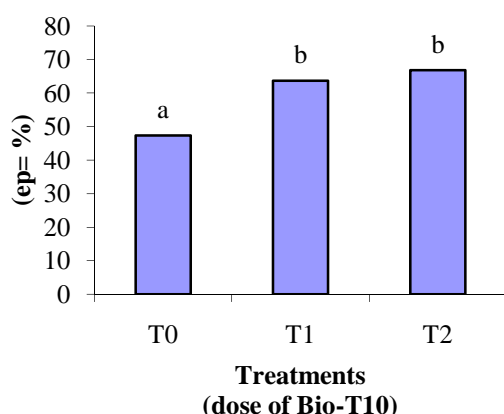


Figure 1. The effectiveness of bioremediator (%)

According to Kurniawan and Ekowati (2016), biosorption can be interpreted as the removal of heavy metals through passive binding to non-living biomass from a solution, and this reduction mechanism is not metabolically controlled.

Growth of spinach plant

Table 4 shows that the application of different doses of Bio-T10 gave a very significant effect on spinach plant height. The application of *Trichoderma* sp. secondary metabolite reduced the Cd content in the soil by biosorption mechanism so that the plant could grow normally. Besides, Bio-T10 contains a growth hormone that can increase the rate of plant growth. Based on the results of the analysis (Table 4), the application of different doses of Bio-T10 significantly affected the shoot dry weight of spinach plant. Spinach plants without bioremediator application had the lowest biomass of 0.33 g. This is because Cd influences plant metabolism activities, such as disturbing photosynthesis and absorption of nutrients. Plants can accumulate large amounts of metal but their growth is, or their plant biomass is low (Hardiani et al., 2009).

Table 4. Effects of bioremediator applied to Cd-contaminated soil on growth of spinach plant

Treatment	PH (cm)	LN (sheet)	LA (cm)	WSW (g)	DSW (g)	WRW (g)	DRW (g)
T0	6.28 a	3.39	2.62	4.88	0.41 a	0.36	0.04
T1	6.75 b	3.41	2.63	4.71	0.39 a	0.36	0.04
T2	7.53 c	3.45	2.70	5.48	0.51 b	0.45	0.08
F test	7.45	0.19	0.48	0.55	4.88	1.06	1.85
F Table	0.69	0.21	0.19	1.63	0.08	0.08	0.04
CV	10.16	6.02	7.23	15.05	19.46	8.23	3.6

Remarks: PH = plant height, LA=leave area, WSW= shoot fresh weight, DSW= shoot dry weight, WRW= root fresh weight, DRW= root dry weight. The average number followed by different lowercase letters on the column shows a significantly different effect based on the 5% error level of DMRT test. T0 (without application of Bio-T10), T1 (50% dose of Bio-T10) and T2 (100% dose of Bio-T10).

Interaction between phytoremediator of *Ipomoea reptans* and bioremediator of *Trichoderma* sp.

Treatment of population density of *Ipomoea reptans* and application of the different dose of Bio-T10 did not show any interaction on all variables of *Ipomoea reptans* growth. The interaction of density treatment of *Ipomoea reptans* population and different application of Bio-T10 dose was shown on the effectiveness variable of phytobioremediator and spinach plant growth (Table 5).

Effectiveness of phytobioremediator

The combination of a different population of a density of *Ipomoea reptans* and different application of dosage of Bio-T10 had a very significant effect on the effectiveness of the absorption of Cd. The best treatment combination of K1T1 (combination of the 2 plants density and 50% dose of Bio-T10) amounted to 71.19% of phytobioremediator effectiveness (Table 5). It is predicted that the secondary metabolite of *Trichoderma* sp. was able to interact with *Ipomoea reptans* plant by affecting the resistance of the plant to Cd stress so that the plant was able

to grow well and was able to absorb higher Cd concentration without showing morphological or physiological damage. Prijambada (2014) stated that regarding microbiology, the effort to increase phytoremediation utilization using plants is to utilize organic acid producing microorganisms or siderophores to increase the ability of plants to absorb heavy metals and transmit heavy metals to higher levels of plants.

Growth of spinach plant

Based on Table 6, the interaction treatment of population density of *Ipomoea reptans* plant and dosage of Bio-T10 gave a significant effect on the height of the spinach plant. The results of statistical analysis showed that the K2T2 treatment yielded the highest plant height of 7.8 cm (Table 6). It is suspected that the lower the concentration of Cd contained in the soil, the less absorbed content and this does not cause toxics to the plant. According to Hamzah et al, (2017), the existence of Cd in the soil at high concentration that exceeds a threshold generally can inhibit plant growth; cadmium will restrict the plant growth and change the plant structure.

Table 5. Interaction of treatments on the effectiveness of phytobioremediator

Density of <i>Ipomoea reptans</i>	Effectiveness of Phytobioremediation (%)		
	Dose of <i>Trichoderma</i> sp.		
	T0	T1	T2
K0	25.12 a (B)	59.36 c (A)	60.61 c (A)
K1	59.37 c (C)	71.19 f (A)	68.36 e (B)
K2	57.44 b (C)	60.44 c (B)	62.48 d (A)

Remarks: The average number followed by different lower case letters in the column shows a significantly different effect based on the 5% error level of DMRT test.

Table 6. Interaction of the treatment of plant spinach height

Density of <i>Ipomoea reptans</i>	Spinach Plant Height (cm)		
	Dose of <i>Trichoderma</i> sp.		
	T0	T1	T2
K0	4.43 a (B)	6.29 b (A)	6.59 c (A)
K1	6.96 c (A)	6.48 b (B)	7.48 d (A)
K2	7.53 de (AB)	7.5 de (B)	7.80 e (A)

Remarks: The average number followed by different lower case letters in the column shows a significantly different effect based on the 5% error level of DMRT test.

The interaction of *Ipomoea reptans* density and dose of Bio-T10 treatments significantly affected leave number and leave area of spinach plant (Table 7). Statistical analysis showed that the K2T2 treatment yielded the highest leave number (3.79 sheets) and leave area (2.94 cm²), that were significantly different from other treatments. The smallest leave number (2.72 sheets) and leave area

(2.04 cm²) were observed on the K0T0 treatment. Significant differences in the variables of the number and width of leaves are thought to be the content of Cd in different soils that affecting the absorption of heavy metals in plants and disrupting the photosynthesis process. The smaller size of the leaves shows plants exposed to heavy metals and the colour of the leaves turns yellow.

This shows the inhibition of chlorophyll formation. According to Haryati et al. (2012), the presence of heavy metals in the disruption of the process of photosynthesis is due to disruption of enzymes that contributes to chlorophyll

biosynthesis. Besides, phytohormone contained in secondary microbial metabolites can spur leaf expansion and nutrient metabolism of plants (Santi et al., 2015).

Table 7. Interaction of the treatment of the number of leaves and leaf width of spinach plant

Density of <i>Ipomoea reptans</i>	Number of Leaves (sheet)			Leaf Width(cm)		
	Dose of <i>Trichoderma</i> sp.					
	T0	T1	T2	T0	T1	T2
K0	2.2 a (C)	3.23 c (A)	3.0 b (B)	2.04 a (B)	2.48 b (A)	2.45 b (A)
K1	3.52 d (A)	3.55 d (A)	3.46 d (A)	2.75 c (A)	2.74 c (A)	2.72 c (A)
K2	3.96 f (A)	3.46 d (B)	3.79 f (B)	3.06 e (A)	2.68 d (C)	2.94 d (C)

Remarks: The average number followed by different lower case letters on the column shows a significantly different effect based on the 5% error level of DMRT test.

Conclusion

Population of 2 *Ipomoea reptans* plants/polybag effectively reduced Cd to 66.31% and increased the growth of spinach by 20% on plant height, leaf number, and leaf area. Also, increased shoot fresh weight and shoot dry weight of spinach by 35%. Application of 100% dose of Bio-T10 effectively reduced Cd by 63.81% and increased spinach growth by 18% on plant height and increased shoot dry weight of spinach by 23%. There were interactions of phytoremediator of *Ipomoea reptans* population of 2 plants/polybag with bioremediator of 50% dose of Bio-T10 that reduced Cd to 71.19% and influenced spinach plant growth by 43% of plant height and leaf number, 31% of leaf area, and 63% of shoot dry weight of spinach plant.

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