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Plant Growth Regulators with a Balanced Supply of Nutrients Enhance the Phytoextraction Efficiency of *Parthenium hysterophorus* for Cadmium in Contaminated Soil

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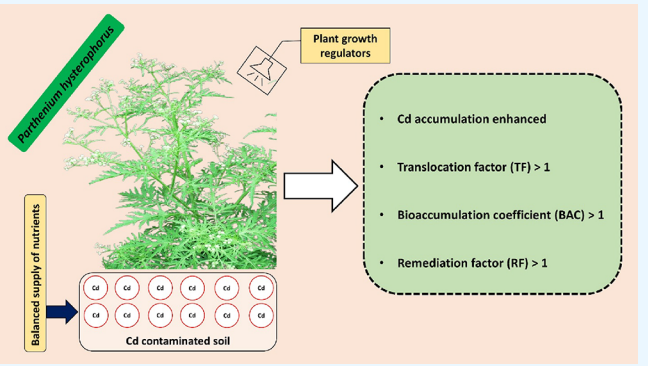
ABSTRACT: Heavy metal contamination in soil, such as cadmium (Cd), poses a serious threat to global food security and human health. It must be managed using environmentally friendly and cost-effective technologies. Plants with high resistance to Cd stress and high biomass production could be potential candidates for the phytoremediation of Cd-contaminated soils to improve Cd phytoextraction. In this regard, the present study was carried out to determine the effect of gibberellic acid (GA_3), indole acetic acid (IAA), and fertilizers (N, P, and K) on *Parthenium hysterophorus* growth and biomass production as well as Cd phytoextraction capabilities. A pot experiment was conducted with various combinations of PGRs and fertilizers, with treatments arranged in five replicates using a completely randomized design. After harvesting, each plant was divided into various parts such as stems, roots, and leaves, and different growth, physiological, and biochemical parameters were recorded. Results showed that under Cd stress, growth, physiological, and biochemical parameters were all significantly decreased. With the combined application of plant growth regulators (GA_3 and IAA) and nutrients, Cd stress was alleviated and all parameters significantly improved. In comparison to the control treatment, the combined application of N + P + K + GA_3 + IAA resulted in the highest fresh and dry biomass production of the root (12.31 and 5.11 g pot^{-1}), shoot (19. 69 and 6.99 g pot^{-1}), leaves (16.56 and 7.09 g pot^{-1}), and entire plant (48.56 and 19.19 g pot^{-1}). Similarly, the same treatment resulted in higher chlorophyll a and b and total chlorophyll contents under Cd stress, which were 2.19, 2.03, and 3.21 times higher than the control, which was Cd stress without any treatment. The combination of N + P + K + GA_3 + IAA also resulted in the highest proline and phenolic contents. In the case of different enzyme activities, the combined application of N + P + K + GA_3 + IAA under Cd stress led to a high increase in catalase (2.5 times), superoxide (3.5 times), and peroxidase (3.7 times) compared to the control. With the combined application of N+ P+ K + GA_3 + IAA, the maximum values of BCF (8.25), BAC (2.6), and RF (5.14%) were measured for phytoextraction potential. On the basis of these findings, it is concluded that *P. hysterophorus* has a high potential to grow, produce the most biomass, and act as a Cd hyperaccumulator in Cd-contaminated soil.



INTRODUCTION

Soil contamination by toxic heavy metals is a global issue that endangers human health and food safety.[1−3] Electroplating, smelting, mining, producing electricity and fuel, industrial effluents, intensive agriculture, solid waste, and air pollution all contribute to the spread of heavy metals in the environment, which can be harmful to human health.[4−6] Cadmium (Cd) is one of the most toxic heavy metals due to its persistent nature, and it enters the soil through a variety of anthropogenic sources such as phosphate fertilizer application, wastewater, sewage sludge, etc., before being assimilated by plants.[7,8] Cd stress affects cell functions and the uptake of essential nutrients such as zinc, calcium, iron, magnesium, and manganese,[9] as well as photosynthesis and respiration processes, reducing plant growth and productivity.[10−12] Cd stress disrupts plant

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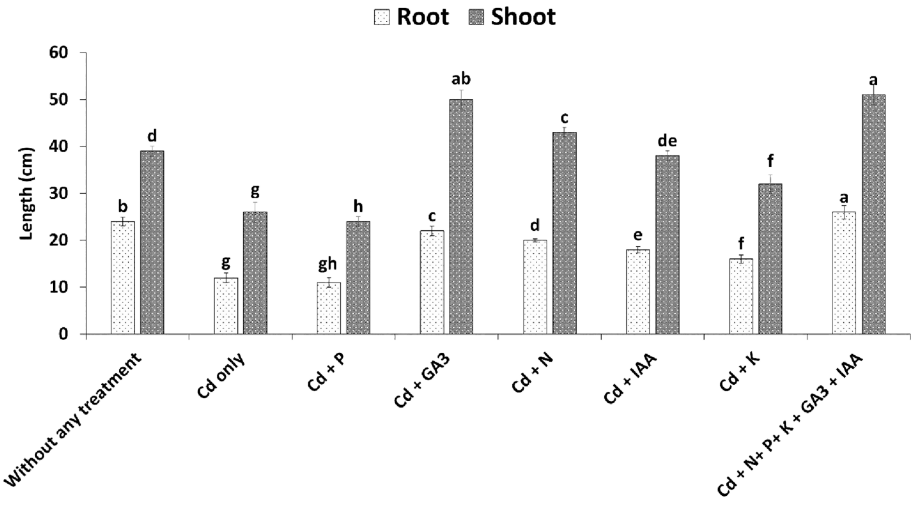

Figure 1. Effect of different growth regulators and fertilizer treatments on root and shoot length (cm) of *P. hysterophorus*. The bars sharing the same letters are statistically nonsignificant with each other at $p \leq 0.05$. Cd was applied at a rate of 50 mg Cd kg^{-1} soil in all Cd treatments. GA_3, IAA, N, P, and K were used at concentrations of 500, 400, 1000, 500, and 700 ppm, respectively.

Table 1. Effect of Different Growth Regulators and Fertilizer Treatments on Fresh and Dry Biomasses of *Parthenium hysterophorus*[a]

	fresh biomass (g pot^{-1})				dry biomass (g pot^{-1})			
treatments	root	shoot	leaves	entire plant	root	shoot	leaves	entire plant
without any treatment	11.69 ± 0.07^{b}	15.00 ± 0.11^{d}	14.52 ± 0.41^{c}	41.21 ± 2.13^{c}	4.10 ± 0.01^{b}	5.76 ± 0.01^{b}	5.32 ± 0.12^{d}	15.18 ± 0.23^{c}
Cd only	6.21 ± 0.03^{f}	11.00 ± 1.14^{e}	10.40 ± 0.11^{f}	27.61 ± 0.14^{f}	2.01 ± 0.02^{ef}	3.43 ± 0.02^{c}	2.24 ± 0.33^{f}	7.68 ± 0.09^{e}
Cd + P	5.41 ± 0.08^{g}	10.23 ± 1.22^{ef}	9.31 ± 0.22^{f}	24.95 ± 1.41^{g}	2.92 ± 0.01^{e}	4.21 ± 0.11^{d}	3.00 ± 0.23^{e}	10.13 ± 0.06^{d}
Cd + GA_3	9.00 ± 0.14^{c}	18.43 ± 0.24^{b}	15.22 ± 0.15^{b}	42.65 ± 1.03^{b}	4.33 ± 0.04^{b}	6.40 ± 0.04^{ab}	6.90 ± 0.01^{b}	16.63 ± 0.12^{b}
Cd + N	8.00 ± 0.05^{d}	15.00 ± 1.00^{d}	13.01 ± 0.55^{d}	36.01 ± 2.09^{d}	3.45 ± 0.09^{c}	6.34 ± 0.08^{ab}	6.06 ± 0.03^{b}	15.85 ± 0.03^{c}
Cd + IAA	8.44 ± 0.71^{d}	14.70 ± 0.21^{cd}	14.02 ± 0.11^{c}	37.16 ± 2.11^{d}	3.21 ± 0.01^{cd}	6.11 ± 0.11^{ab}	5.34 ± 0.66^{bc}	14.66 ± 0.09^{cd}
Cd + K	7.45 ± 0.01^{e}	17.00 ± 0.22^{c}	11.04 ± 0.11^{e}	35.49 ± 2.22^{e}	3.44 ± 0.08^{cd}	6.00 ± 0.12^{ab}	6.05 ± 0.03^{bc}	15.49 ± 0.05^{c}
Cd + N+ P + K + GA_3 + IAA	12.31 ± 0.11^{a}	19.69 ± 1.19^{a}	16.56 ± 0.12^{a}	48.56 ± 2.00^{a}	5.11 ± 0.11^{a}	6.99 ± 0.09^{a}	7.09 ± 0.06^{a}	19.19 ± 0.09^{a}

[a]Mean values ± SD (n = 5) sharing the same letter(s) in a column are statistically nonsignificant with each other at $p \leq 0.05$. In all Cd treatments, Cd was applied @ 50 mg Cd kg^{-1} soil. GA_3, IAA, N, P, and K were applied @ 500, 400, 1000, 500, and 700 ppm, respectively.

metabolic processes, causing ionic imbalance, osmotic stress, and the production of reactive oxygen species (ROS). In plant cells, hydrogen peroxide (H_2O_2), superoxide (O^{-2}), and hydroxyl radicals (HO^{-1}) are important ROS produced by mitochondria, chloroplasts, and peroxisomes.[13,14] To scavenge increased ROS production, an effective antioxidant system is required.[15] The enzymatic antioxidant system includes peroxidase (POX), superoxide dismutase (SOD), ascorbate (ASC), and catalase (CAT).[16] Flavonoids, phenolics, and tocopherols are nonenzymatic antioxidants found in plants.[13,17−20] Both enzymatic and nonenzymatic antioxidant systems protect and stabilize the plant cell.[21,22]

Traditional soil remediation techniques for Cd contamination necessitate a large amount of technological resources as well as the addition of various chemicals.[23,24] Phytoextraction is an approach that holds promise for treating soils contaminated with heavy metals because of its low cost and in situ advantages.[25] It involves the use of plants to extract high concentrations of metals from the soil in various parts of the plant that can be harvested.[26,27] These extracted metals are then processed using a variety of techniques such as drying, ashing, and anaerobic digestion in addition to other microbial and physicochemical methods.[28] The plants must display robust growth and produce a greater amount of biomass to achieve maximum metal extraction. In this regard, the researchers used a variety of amendments to boost plant growth and biomass. Plant growth regulators (PGRs) are biosynthesized by plants and regulate plant growth and development.[2,29−34] The PGR gibberellic acid (GA_3) increases plant yield and dry biomass by stimulating cell elongation.[35] Plants rely heavily on fertilizers and PGRs to control their physiological processes and improve their resistance to stress.[36] Fertilizers are beneficial because they increase a plant's biomass and improve its metal absorption efficiency.[37] The activation of enzymes and the transport of photosynthates from the source to the sink during photosynthesis also rely on a wide variety of nutrients.[38]

There is a possibility that wild native plant species, which are an important part of the ecosystem, could play an important role in preventing the contamination of the environment with heavy metals. A typical example of a wild plant is the *Parthenium hysterophorus* L., which belongs to the family Asteraceae and is found all over the world. Because of its rapid growth, amazing capacity for regeneration, stress resistance,

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Table 2. Effect of Different Growth Regulators and Fertilizer Treatments on Photosynthetic Pigments and Relative Water Content of *Parthenium hysterophorus*[a]

	chlorophyll (μg g^{-1} FW)			relative water contents (g)			
treatments	a	b	total	root	shoot	leaves	entire plant
without any treatment	6.36 ± 1.12^{e}	5.56 ± 0.11^{c}	7.88 ± 0.61^{e}	4.90 ± 0.37^{bc}	8.49 ± 0.94^{c}	6.04 ± 0.46^{c}	22.52 ± 1.39^{c}
Cd only	4.17 ± 0.99^{f}	3.93 ± 0.13^{d}	4.06 ± 0.44^{f}	1.47 ± 0.17^{f}	4.33 ± 0.95^{e}	5.00 ± 0.57^{d}	13.83 ± 1.69^{fg}
Cd + P	3.00 ± 0.45^{f}	3.74 ± 0.98^{d}	3.69 ± 0.13^{g}	0.97 ± 0.30^{g}	3.74 ± 0.95^{ef}	4.01 ± 0.94^{e}	11.76 ± 2.19^{gh}
Cd + GA_3	8.93 ± 0.12^{b}	7.14 ± 0.45^{ab}	12.03 ± 0.66^{b}	5.00 ± 0.23^{ab}	9.33 ± 0.95^{b}	7.07 ± 0.22^{b}	25.21 ± 1.25^{b}
Cd + N	7.58 ± 1.13^{c}	6.54 ± 2.34^{b}	10.07 ± 0.55^{c}	3.00 ± 0.01^{d}	7.03 ± 0.34^{d}	6.06 ± 0.04^{c}	19.09 ± 2.34^{d}
Cd + IAA	7.93 ± 0.09^{c}	7.53 ± 0.56^{a}	11.42 ± 0.12^{bc}	2.54 ± 0.01^{de}	6.30 ± 0.97^{d}	6.12 ± 0.03^{c}	17.99 ± 0.99^{e}
Cd + K	7.14 ± 0.99^{cd}	6.80 ± 1.12^{b}	9.90 ± 0.12^{d}	2.01 ± 0.01^{e}	4.07 ± 0.07^{e}	5.09 ± 0.03^{d}	14.17 ± 0.07^{f}
Cd + N+ P+ K + GA_3 + IAA	9.13 ± 0.01^{a}	7.99 ± 0.51^{a}	13.03 ± 0.44^{a}	5.71 ± 0.08^{a}	11.09 ± 0.95^{a}	8.07 ± 0.70^{a}	27.19 ± 1.88^{a}

[a]Mean values ± SD (n = 5) sharing the same letter(s) in a column are statistically nonsignificant with each other at $p \leq 0.05$. In all Cd treatments, Cd was applied @ 50 mg Cd kg^{-1} soil. GA_3, IAA, N, P, and K were applied @ 500, 400, 1000, 500, and 700 ppm, respectively.

Table 3. Effect of Different Treatments on Proline and Phenolic Contents and Total Soluble Protein in *Parthenium hysterophorus*[a]

	proline contents (μg g^{-1} FW)		phenolic contents (μg g^{-1} FW)		total soluble protein (mg g^{-1} FW)
treatments	roots	leaf	roots	leaf	leaf
without any treatment	24.0 ± 2.10^{h}	16.0 ± 1.12^{h}	19.0 ± 2.32^{h}	26.0 ± 2.06^{h}	0.60 ± 0.02^{d}
Cd only	54.6 ± 1.39^{g}	45.1 ± 1.22 ^{g}	34.3 ± 0.98^{g}	58.1 ± 1.15^{g}	0.20 ± 0.01^{e}
Cd + P	60.0 ± 2.54^{f}	47.0 ± 2.11^{f}	42.0 ± 1.09^{f}	64.0 ± 2.12^{f}	0.08 ± 0.03^{f}
Cd + GA_3	75.5 ± 1.32^{e}	64.1 ± 1.23^{c}	84.0 ± 1.02^{d}	124.0 ± 1.16^{c}	1.02 ± 0.01^{b}
Cd + N	85.1 ± 3.21^{b}	66.0 ± 1.06^{b}	89.0 ± 2.12^{b}	131.0 ± 0.34^{b}	0.90 ± 0.03^{c}
Cd + IAA	64.0 ± 1.15^{d}	55.3 ± 2.13^{e}	66.0 ± 1.51^{e}	116.0 ± 2.01^{d}	0.85 ± 0.01^{c}
Cd + K	68.0 ± 3.02^{c}	57.0 ± 2.02^{d}	77.0 ± 0.44^{c}	91.0 ± 1.33^{e}	0.80 ± 0.01^{c}
Cd + N+ P+ K + GA_3 + IAA	88.0 ± 0.25^{a}	77.3 ± 3.41^{a}	98.0 ± 1.78^{a}	159.0 ± 3.32^{a}	1.40 ± 0.03^{a}

[a]Mean values ± SD (n = 5) sharing the same letter(s) in a column are statistically nonsignificant with each other at $p \leq 0.05$. In all Cd treatments, Cd was applied @ 50 mg Cd kg^{-1} soil. GA_3, IAA, N, P, and K were applied @ 500, 400, 1000, 500, and 700 ppm, respectively.

and unpleasant nature, *P. hysterophorus* was chosen as a trial plant for the current study. The growth and biomass of *P. hysterophorus* may be improved through the balanced use of nutrients and various PGRs, which may also improve the plant's ability to extract Cd under Cd stress. On the basis of this hypothesis, the present study was carried out to elucidate the effects of various nutrients such as nitrogen (N), phosphorus (P), and potassium (K) in different combinations with GA_3 and indole acetic acid (IAA) in enhancing the phytoextraction potential of *P. hysterophorus* under Cd stress. According to our knowledge, no study has examined the capability of *P. hysterophorus* under the influence of balanced nutrients and PGRs applied through foliar application.

RESULTS

Treatment Effects on Growth Parameters under Cd Stress. *Root and Shoot Length.* The effects of a variety of treatments, including cadmium (Cd), indole acetic acid (IAA), gibberellic acid (GA_3), nitrogen (N), phosphorus (P), and potassium (K), on the root and shoot length of *P. hysterophorus* are illustrated in Figure 1. Shoot and root length was significantly reduced under Cd stress when compared to the control (C) and other treatments. Cd stress was significantly reduced by using plant growth regulators (PGRs) such as IAA and GA_3 in various N, P, and K combinations. The combined application of N+ P+ K + GA_3 + IAA (T_6) under Cd stress resulted in the maximum root (26 cm) and shoot lengths (51 cm), which were 2.17 and 1.96 times greater than those of the positive control (C_1) and 1.08 and 1.31 times greater than those of the negative control (C), respectively. In the case of PGRs, GA_3 had a greater impact than IAA, whereas P had a greater impact than N and K applications.

Fresh and Dry Biomass. Table 1 shows the effect of various Cd, IAA, GA_3, and NPK treatment combinations on fresh and dry biomass (root, shoot, leaves, and entire plant) of *P. hysterophorus*. A similar trend to that observed in root and shoot length was observed in fresh and dry biomass of *P. hysterophorus* under different treatments. With the application of P under Cd stress, the minimum fresh weight of the root, shoot, leaves, and entire plant was observed (T_1). Under Cd stress, the minimum dry weight of the root, shoot, leaves, and entire plant was observed without the application of any treatment (C_1). The combined application of N + P + K + GA_3 + IAA resulted in the maximum fresh and dry biomasses of the root (12.31 and 5.11 g pot^{-1}), shoot (19. 69 and 6.99 g pot^{-1}), leaves (16.56 and 7.09 g pot^{-1}), and entire plant (48.56 and 19.19 g pot^{-1}) (T_6). In comparison to the control treatment, C_1, the maximum fresh and dry biomasses of the root, shoot, leaves, and entire plant were 1.98 and 2.54, 1.79 and 2.04, 1.59 and 3.17, and 1.76 and 2.50 times higher, respectively, than the positive control, i.e., Cd stress without any treatment (C_1). In comparison to other PGRs tested, GA_3 had a greater impact on fresh and dry biomasses of the root, shoot, leaves, and entire plant. Similarly, N had a greater impact on fresh and dry biomasses of the root, shoot, leaves, and entire plant than P and K applications.

Treatment Effects on Physiological Parameters under Cd Stress. *Photosynthetic Pigments and Relative Water Content.* The effects of various treatments, such as Cd, IAA, GA_3, N, P, and K, on the photosynthetic pigments (chlorophyll a and b and total) and relative water contents (RWCs) of *P.*

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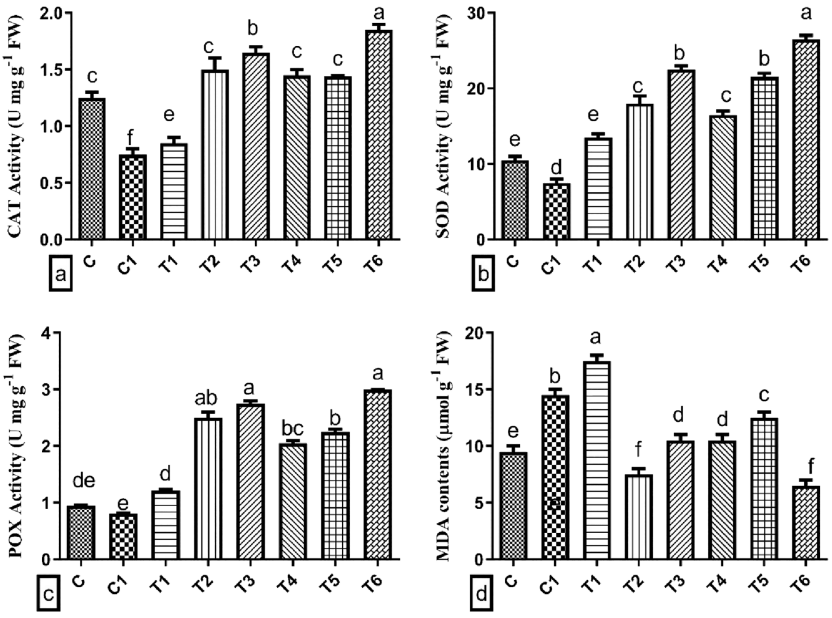

Figure 2. Effects of various treatments on antioxidant enzymatic activities such as (a) CAT activity, (b) SOD activity, (c) POX activity, and (d) MDA levels. The bars sharing the same letters are statistically nonsignificant with each other at $p \leq 0.05$. C = without any treatment; C_1 = Cd only; T_1 = Cd + P; T_2 = Cd + GA_3; T_3 = Cd + N; T_4 = Cd + IAA; T_5 = Cd + K; and T_6 = Cd + N+ P+ K + GA_3 + IAA. Cd was applied at a rate of 50 mg Cd kg^{-1} soil in all Cd treatments. GA_3, IAA, N, P, and K were used at concentrations of 500, 400, 1000, 500, and 700 ppm, respectively.

Table 4. Effect of Different Treatments on Phytoextraction Potential of Cd in *Parthenium hysterophorus*[a]

	Cd concentration (mg kg^{-1} DW)			Cd accumulation (mg Cd pot^{-1} dry biomass)			
treatments	root	stem	leaves	root	stem	leaves	entire plant
Cd only	160.64 ± 0.23^{g}	90.00 ± 0.09^{g}	170.00 ± 1.98^{g}	0.16 ± 0.11^{e}	0.13 ± 0.02^{d}	0.21 ± 0.01^{g}	0.50 ± 0.02^{f}
Cd + P	380.00 ± 2.01^{f}	140.00 ± 1.18^{e}	283.00 ± 2.12^{f}	0.35 ± 0.08^{d}	0.17 ± 0.01^{c}	0.31 ± 0.02^{f}	0.83 ± 0.02^{e}
Cd + GA_3	623.01 ± 4.22^{b}	155.5 ± 2.21^{d}	294.00 ± 2.08^{e}	1.66 ± 0.2^{b}	0.37 ± 0.08^{b}	0.94 ± 0.03^{e}	2.97 ± 0.09^{c}
Cd + N	608.08 ± 3.09^{c}	265.05 ± 3.22^{a}	648.00 ± 0.12^{a}	1.21 ± 0.06^{b}	0.62 ± 0.09^{a}	1.63 ± 0.4^{b}	3.46 ± 0.34^{b}
Cd + IAA	483.00 ± 1.09^{e}	109.00 ± 0.99^{f}	361.00 ± 4.44^{d}	0.85 ± 0.05^{c}	0.23 ± 0.01^{c}	1.05 ± 0.01^{d}	2.13 ± 0.11^{d}
Cd + K	524.00 ± 1.11^{d}	211.00 ± 1.34^{c}	583.00 ± 4.09^{b}	0.83 ± 0.06^{c}	0.42 ± 0.03^{b}	1.47 ± 0.02^{c}	2.73 ± 0.12^{c}
Cd + N+ P+ K + GA_3 + IAA	825.00 ± 4.05^{a}	224.00 ± 0.09^{b}	463.00 ± 2.13^{c}	2.44 ± 0.11^{a}	0.67 ± 0.02^{a}	1.95 ± 0.04^{a}	5.06 ± 0.21^{a}

[a]Mean values ± SD (n = 5) sharing the same letter(s) in a column are statistically nonsignificant with each other at $p \leq 0.05$. In all Cd treatments, Cd was applied @ 50 mg Cd kg^{-1} soil. GA_3, IAA, N, P, and K were applied @ 500, 400, 1000, 500, and 700 ppm, respectively.

hysterophorus are shown in Table 2. Chlorophyll a and b and total pigments as well as relative water contents (RWCs) under Cd stress were significantly lower than they were under control (C) and other treatments. Using plant growth regulators (PGRs), such as IAA and GA_3, along with various N, P, and K combinations, this stress was significantly lessened. The maximum chlorophyll a and b and total chlorophyll contents were 9.13, 7.99, and 13.03 $\mu g\ g^{-1}$ FW with the combined application of N+ P+ K + GA_3 + IAA (T_6) under Cd stress, and these were 2.19, 2.03, and 3.21 times more compared to the positive control, i.e., Cd stress without any treatment (C_1), and 1.43, 1.44 and 1.65 times more compared to the negative control, i.e., without Cd stress and any treatment (C), respectively. In the case of PGRs, the effect of GA_3 was greater than that of IAA, but it was statistically nonsignificant in the case of chlorophyll b and total chlorophyll contents, whereas the effect of N was greater than that of P and K application. A similar trend could be seen in the relative water content of the leaves.

Proline and Phenolic Contents and Total Soluble Protein. Under Cd stress, the proline, phenolic, and total soluble protein contents in root and leaf samples, as well as their levels in comparison to the control (C) and other treatments, were all significantly reduced (Table 3). This stress was significantly reduced by using plant growth regulators (PGRs), such as IAA and GA_3, along with various N, P, and K combinations. With the combined application of N+ P+ K + GA_3 + IAA (T_6) under Cd stress, the maximum proline and phenolic contents in the root (88.0 and 98.0 $\mu g\ g^{-1}$ FW) and leaf (77.3 and 159.0 $\mu g\ g^{-1}$ FW) samples, respectively, were noted. These were 1.61 and 2.86 times more in roots and 1.72 and 2.74 times more in leaf samples, respectively, compared to the positive control, which is Cd stress without any treatment (C_1), and 3.66 and 5.16 times more in roots and 4.83 and 6.12 times more in leaf samples compared to the negative control, which is without Cd stress and any treatment (C). In the case of PGRs, GA_3 had a greater impact than IAA, whereas N had a greater impact than P and K applications. A similar pattern was observed in the case of soluble protein contents in leaves. The same treatment, N+ P+ K + GA_3 + IAA (T_6), led to the highest protein content (1.40 mg g^{-1} FW) in leaf samples, whereas the lowest protein content (0.08 g g^{-1} FW) was observed with the application of P alone under Cd stress.

Antioxidant Enzymatic Activities. The use of various treatments, including Cd, IAA, GA_3, N, P, and K, had a significant effect on the antioxidant enzyme activities of *P.*

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Table 5. Effect of Different Treatments on the Translocation Factor (TF), Bioconcentration Factor (BCF), Bioaccumulation Coefficient (BAC), and Remediation Factor (RF) of Cd in Different Parts of *Parthenium hysterophorus*[a]

	translocation factor (TF)					
	root to stem	root to leaves	stem to leaves	BCF	BAC	RF (%)
Cd only	0.56 ± 0.02[a]	1.06 ± 0.01[a]	1.89 ± 0.01[c]	1.61 ± 0.01[e]	0.90 ± 0.02[d]	0.68 ± 0.01[e]
Cd + P	0.37 ± 0.06[b]	0.74 ± 0.02[b]	2.02 ± 0.02[b]	3.80 ± 0.02[d]	1.40 ± 0.03[c]	0.96 ± 0.03[e]
Cd + GA_3	0.25 ± 0.01[d]	0.47 ± 0.03[b]	1.89 ± 0.07[c]	6.23 ± 0.04[b]	1.56 ± 0.02[c]	2.62 ± 0.01[d]
Cd + N	0.44 ± 0.04[c]	1.07 ± 0.09[a]	2.44 ± 0.06[b]	6.08 ± 0.02[b]	2.24 ± 0.02[b]	4.60 ± 0.01[b]
Cd + IAA	0.23 ± 0.06[d]	0.75 ± 0.01[b]	3.31 ± 0.01[a]	4.83 ± 0.03[c]	1.09 ± 0.02[d]	2.56 ± 0.04[d]
Cd + K	0.40 ± 0.09[c]	1.11 ± 0.03[a]	2.76 ± 0.03[b]	5.24 ± 0.01[c]	2.11 ± 0.01[b]	3.78 ± 0.03[c]
Cd + N+ P+ K + GA_3 + IAA	0.27 ± 0.02[d]	0.56 ± 0.02[b]	2.07 ± 0.02[b]	8.25 ± 0.02[a]	2.65 ± 0.01[a]	5.14 ± 0.02[a]

[a]Mean values ± SD (n = 5) sharing the same letter(s) in a column are statistically nonsignificant with each other at $p \leq 0.05$. In all Cd treatments, Cd was applied @ 50 mg Cd kg^{-1} soil. GA_3, IAA, N, P, and K were applied @ 500, 400, 1000, 500, and 700 ppm, respectively.

hysterophorus (Figure 2). In contrast to the control (C) and other treatments, malondialdehyde (MDA) contents increased under Cd stress, whereas catalase (CAT), superoxide (SOD), and peroxidase (POX) enzyme activities were significantly reduced. The application of IAA and GA_3 in various N, P, and K combinations significantly reduced Cd stress. Under Cd stress, the combined application of N+ P+ K + GA_3 + IAA (T_6) resulted in the maximum CAT (1.85 U mg g^{-1} FW), SOD (26.5 U mg g-1 FW), and POX (2.99 U mg g^{-1} FW) activities that were 2.5, 3.5, and 3.7 times higher than the positive control, which is Cd stress without any treatment (C_1). The same treatment resulted in the greatest reduction in MDA contents. The impact of GA_3 was greater in the case of PGRs compared to IAA, and the impact of N was greater compared to the P and K applications.

Treatment Effects on Phytoremediation Potential. *Cd Accumulation in Root, Stem, and Leaves.* The application of various treatments, including IAA, GA_3, N, P, and K, significantly increased the accumulation of Cd in the root, stem, and leaves of *P. hysterophorus* (Table 4). The application of N+ P+ K + GA_3 + IAA (T_6), followed by the application of GA_3 (T_2), N (T_3), K (T_5), IAA (T_4), and P (T_1) under Cd stress, resulted in the highest concentration of Cd in the root (825.0 mg kg^{-1} DW). With the application of N under Cd stress, the highest Cd concentrations (265.0 and 648.0 mg kg^{-1} DW) in the stem and leaves were noted (T_3). The combined application of N+ P+ K + GA_3 + IAA (T_6) caused the greatest accumulation of Cd (5.06 mg kg^{-1} DW) in the entire body of *P. hysterophorus* followed by N (T_3), GA_3 (T_2), K (T_5), IAA (T_4), and P (T_1). On the basis of the dry biomass, the root and leaves bioaccumulated the most Cd at 55.8 and 54.0%, respectively, whereas the stem bioaccumulated the most Cd at 26.0%.

Phytoextraction Potential. Using the translocation factor (TF), bioconcentration factor (BCF), bioaccumulation coefficient (BAC), and remediation factor (RF), the phytoextraction potential of different treatments, such as IAA, GA_3, N, P, and K, was determined (Table 5).

The phytoextraction potential of *P. hysterophorus* for Cd was significantly improved by the application of various amendments. Without any amendments, the maximum translocation of Cd from root to stem and root to leaves was observed in the control. Under Cd stress, K (T_5) application showed the greatest translocation from stem to leaves. Meanwhile, the maximum translocation from stem to leaves was observed with the application of K (T_5) under Cd stress. With the application of N+ P+ K + GA_3 + IAA, the highest values of BCF (8.25), BAC (2.6), and RF (5.14%) were observed (T_6). These findings allow us to classify *P. hysterophorus* as a Cd hyperaccumulator. These findings suggest that *P. hysterophorus* is a hyperaccumulator of the metal Cd.

DISCUSSION

The accumulation of cadmium (Cd) has a detrimental effect on the growth of plants. The reduction in plant growth may be attributable to the fact that cadmium has a deleterious effect on the uptake and distribution of nutrients within plant cells, in addition to physiological processes such as respiration and photosynthesis.[19] There is a correlation between the toxicity of Cd and the loss of biomass in many plants, including *Cucumis sativus* and *Lemna polyrrhiza*.[19] According to the findings of this study, Cd caused a significant reduction in the growth and biomass of *P. hysterophorus*. It was discovered that growth regulators (GA_3) and fertilizers (NPK) had a beneficial effect on plant growth and biomass when the plants were subjected to Cd stress. The total amount of metal that plants can extract is primarily determined by two factors: the plant biomass and the metal concentration in the biomass. The current study found that Cd accumulation and biomass production in various parts of *P. hysterophorus* were significantly influenced by a balanced nutrient supply. Prior research has shown that heavy metal stress inhibits plant growth and development, which eventually lowers yield and biomass.[19,37] As a result of the potential negative effects of heavy metals on photosynthetic pigments, the production of reactive oxygen species (ROS), and the uptake of Cd rather than micronutrients, plants that are subjected to metal stress exhibit reduced growth and biomass accumulation.[10,39,40] *P. hysterophorus* had significantly shorter root and shoot lengths, as well as lower biomasses, because of Cd exposure in this study. The plant also had significantly lower relative water content. The findings of our study are in line with those of numerous researchers from different countries all over the world.[41,42] When the cabbage was subjected to Cd stress, Kamran et al.[43] discovered that its growth parameters experienced a significant decline. Stressful situations could cause cell division to stop and cell cycle to lengthen, which would slow down growth and metabolism. There may have been a reduction in the number of roots and lateral roots, as well as inhibition of enzymatic activity, which affected the growth.[44] It has been demonstrated that Cd exposure causes a decrease in biomass in *Zea mays* L., *Raphanus sativus* L., and *Lycopersicum esculentum* L.[29,35] Growth inhibition caused by Cd stress may be the cause of low water intake and nutrient absorption.[19] In the current study, foliar treatment of PGRs increased development and biomass under Cd stress. The increased growth and biomass

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ACS Omega 2023, 8, 18940−18950

could be due to more plant nutrient availability.[19] Plant growth regulators (PGRs) increase resistance to metal stress, hasten cell division, and promote the development and growth of plant tissues.[45] These also strengthen the plant's roots, which can result in an increased intake of both nutrients and water for the plant.[29]

The findings of this investigation demonstrated that PGRs significantly increased the concentration and accumulation of Cd in a variety of plant parts. These findings are consistent with the results obtained by Chen et al.[46] The effect of phytohormones on protein, RNA, and DNA synthesis[47] as well as polyribosome multiplication may be responsible for increased biomass production.[48] When plant growth regulators were applied as a foliar spray, there was a significant increase in the amount of Cd that was accumulated in *P. hysterophorus*.[29,49] Hyperaccumulator plants exhibited halted growth and biomass when grown in soil contaminated with metals, which eventually affected their ability to extract various metals from the soil.[50] Previous research has shown that when plants are subjected to Pb stress, both *Vigna unguiculata* and *Raphanus sativus* experience a reduction in their overall growth and biomass, which leads to a lower rate of nutrient uptake.[51,52] PGRs improve plant growth, biomass, and abiotic stress forbearance, which lead to increased metal accumulation in plants. This is accomplished by promoting cell division within plants.[53] The use of a plant growth regulator, which also improves the rate of transpiration and nutrient absorption, ultimately increases metal uptake.[53] Increased cellular development may result in increased Cd accumulation in plants.[54] The current study found that the order of Cd concentration and accumulation in various plant parts was as follows: roots > leaves > stems. Other researchers have discovered similar results.[55,56]

In the present study, Cd stress resulted in a significant reduction of the amount of photosynthetic pigments in *P. hysterophorus* when compared to the control. It is possible that a decrease in enzyme activity disrupted the synthesis of photosynthetic pigments, which ultimately led to a reduction in the accumulation of those pigments in leaf samples.[57,58] Under Cd stress, the chloroplast structure is altered because of the replacement of Mg with Cd, resulting in a decrease in mesophyll cells, photosynthetic pigments, and guard cells.[12,39,57,59] The production of stress-related metabolites such as proline and phenolic contents plays a key role in protecting the cell from being damaged.[60,61] Plants produce proline in addition to other metabolites when they are subjected to stressful conditions.[14] In the present study, the production of stress-related metabolites such as proline and phenolic contents was significantly increased under Cd stress. In plant cells, proline is necessary for Cd detoxification. Proline, which functions as a buffer, protects and stabilizes the macromolecules of the cell. Proline also prevents the accumulation of free radicals.[62] Inside the cell, the amino acid proline and the heavy metal Cd combine to form a nontoxic compound.[63] Numerous plant species, including wheat, sunflower, tomato, and *Solanum nigrum*, have been observed to exhibit increased levels of proline production when subjected to metal stress.[29,31,55,64,65]

Phenolic compounds act as a defense mechanism against biotic and abiotic stresses.[66] As a direct result of exposure to a wide range of environmental conditions, numerous plant species significantly increased the biosynthesis of phenolic compounds.[67,68] The phenolic compounds have antioxidant properties, as demonstrated by their ability to scavenge reactive oxygen species (ROS) produced in response to metal stress. Because of the effects of Cd stress, the total phenolic contents of *P. hysterophorus* were found to be significantly higher in the present investigation. The findings are consistent with those that were found by Chen et al.,[69] who reported a substantial increase in phenolic contents as a response to Cd stress. On the other hand, when a plant is subjected to metal stress, phenolic compounds act as antioxidants.[68] The chelation of metal ions is the source of their antioxidative capabilities.[70] In the current study, the production of total phenolic contents of *P. hysterophorus* was significantly increased by plant growth regulators. There were also reports of comparable results for maize that had been subjected to lead (Pb) stress.[29] Oxidative stress occurs when the accumulation of ROS reaches a certain threshold.[71,72] ROS buildup contributes to oxidative stress by promoting the oxidation of a wide range of biomolecules, including lipids and proteins, in a wide range of organelles.[57,73] To protect itself from oxidative damage, the plant triggers the activities of its antioxidant enzymatic system.[39,74] In this investigation, increased levels of the antioxidant enzymes SOD, CAT, and POX were discovered in this study.

In terms of phytoremediation, *P. hysterophorus* demonstrated a substantial potential to extract Cd from the contaminated medium. The root portion of the plant was where an accumulation of approximately 55.8% of the Cd was found. Moreover, the combined application of Cd + N+ P+ K + GA_3 + IAA resulted in the maximum values of BAC, BCF, and TF, i.e., >1. From these values, it is clear that *P. hysterophorus* could be regarded as a potential hyperaccumulator of Cd with the potential to phytoextract Cd from the contaminated soil. The optimal distribution of nutrients, in conjunction with the presence of plant growth regulators, led to an increase in the availability of cadmium in the soil[47,40,45] and, as a consequence, led to the highest possible level of Cd accumulation in *P. hysterophorus*. Earlier, Jan et al.[2] found that the combined application of plant growth regulators and EDTA significantly enhanced the phytoextraction of Cd from the Cd-spiked soil with *Dysphania ambrosioides*. In comparison to Jan et al.,[2] the values of BAC, BCF, and TF observed in the present study were less. However, the values of BAC, BCF, and TF were >1 in the present study, which are in line with other studies of Cabello-Conejo et al.,[30] Maric et al.,[50] and Sun et al.[55] The authors in these studies noted that the values of BAC, BCF, and TF were >1 and regarded the tested plants as hyperaccumulators. In the present study, *P. hysterophorus* could be regarded as a potential hyperaccumulator of Cd-contaminated soils based on the values of BAC, BCF, and TF.

CONCLUSIONS

The growth and biomass production of *P. hysterophorus* under Cd stress were significantly improved by the foliar application of GA_3 and IAA as well as other nutrients. The application of PGRs (GA_3, and IAA) in conjunction with a balanced supply of nutrients (N, P, and K) led to a notable improvement in the development, physiological, and biochemical characteristics of *P. hysterophorus*, which in turn led to its increased biomass production under Cd stress. The same treatment led to the greatest amount of Cd being extracted by *P. hysterophorus* from the Cd-contaminated soil. On the basis of the bioconcentration and remediation factor values, *P. hysterophorus* showed promising results in the phytoextraction of Cd from Cd-polluted soil. On the basis of these findings, *P. hysterophorus*

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may be a hyperaccumulator for Cd-contaminated soils, and it may be possible to use *P. hysterophorus* for Cd phytoextraction. However, the use of *P. hysterophorus* as a hyperaccumulator plant would require additional research to be conducted under field conditions in the future.

MATERIALS AND METHODS

Plant Materials. Plantlets of *P. hysterophorus* with a 2 cm root and 3 cm shoot were collected from the village of Akhgarm (34°56′2.4102″N, 72°2′6.7632″E) in District Dir Upper, Khyber Pakhtunkhwa. Plantlets were grown in pots under controlled glasshouse conditions (temperature = 30 ± 1 °C, relative humidity = 70−80%, and light/dark duration of 14/10 h) to assess the potential of selected plants for Cd phytoextraction.

Soil Analysis. The soil was taken from the University Research farm area and air-dried before being ground to a final particle size of 2 mm. After sifting, 1 kg of soil was placed in each plastic pot (18 × 15 cm). Various physicochemical properties (water holding capacity, electrical conductivity, pH, etc.) of the soil were investigated using established techniques.[75] In terms of volume, the soil had a water-holding capacity of 21.8% (v/w). The pH (6.75) and EC (1.43 dS m^{-1}) were calculated from a 1:2 (w/v) ratio of soil to water suspension using electrical conductivity (WTW 330i) and a pH meter. The soil was loamy sand in texture and contained 1.24% organic matter, 30% silt, 54% sand, 2.5% lime, and 16% clay. The soil was normal with Cd contents 0.026 ± 0.002 mg kg^{-1}.

Pot Experiment. The soil was amended with 50 mg Cd kg^{-1} of cadmium acetate dihydrate. After the addition of Cd, the pots were stored for a few days in a glass house. There were seven different combinations of fertilizer nutrients and PGRs. The following are the details of the treatments: C = untreated with Cd; C_1 = Cd only; T_1 = Cd + P; T_2 = Cd + GA_3; T_3 = Cd + N; T_4 = Cd + IAA; T_5 = Cd + K; and T_6 = Cd + N+ P+ K + GA_3 + IAA. The application rates of gibberellic acid (GA_3), indole acetic acid (IAA), nitrogen (N), phosphorus (P), and potassium (K) were 500 mg L^{-1}, 400 mg L^{-1}, 1000 mg kg^{-1}, 500 mg kg^{-1}, and 700 mg kg^{-1}, respectively. The N, P, and K treatments were thoroughly combined using a steel spoon. Every week, foliar application of seven doses of two different treatments (GA_3 and IAA) was used. Before seedling implantation, the pots received sufficient irrigation. After 24 h, two identical seedlings were placed in each pot and arranged in a randomized design (CRD) with five replicates. Pots were watered with tap water twice each week. Throughout the study, weeding, hoeing, and other recommended agronomic practices were employed. Harvesting of the aboveground and underground portions was done during the 50 day trial. After harvesting, the following growth and physiological parameters were recorded using standard protocols.

Growth, Yield, Physiological, and Biochemical Parameters. *Plant Growth and Biomass.* Various growth parameters, such as root and shoot length, as well as dry and fresh biomass, were measured. The harvested plants were labeled and separated into different positions such as root, stem, and leaf samples. A measuring scale was used to determine root and shoot length. For fresh biomass of root and shoot samples, a digital balance was used. The dry weight of each sample was determined using an analytical balance. With a grinder, each dry portion was ground into a fine powder and stored for further analysis.

For relative water contents (RWCs), fresh weight (FW) from the flag leaves was recorded. Turgid weight (TW) was obtained after soaking the leaves for 24 h. For dry weight (DW), the samples were dried for 72 h in an oven at 60−62 °C. The RWC of the samples was calculated using the following formula:

$$\text{RWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Analysis of Free Proline, Total Phenolics, and Protein Contents. Plants produce certain proteins such as proline in addition to other metabolites, i.e., phenolic compounds, when they are subjected to stressful conditions.[60] In plant cells, proline is necessary for Cd detoxification as it functions as a buffer and protects and stabilizes the macromolecules of the cell from the damage caused by free radicals.[60,61] Phenolic compounds act as a defense mechanism against biotic and abiotic stresses as these have antioxidant properties, as demonstrated by their ability to scavenge reactive oxygen species (ROS) produced in response to metal stress.[66] Fresh plant tissues (roots and leaves) were taken and crushed, and free proline was extracted using a slight modification of the method of Bates et al.[76] The method of Singleton and Rossi[77] was used to extract total phenols from the shade-dried root and leaf samples. The absorbance of total phenolic content and proline content in the samples was measured using a spectrophotometer at 760 and 520 nm, respectively. The concentrations of free proline and total phenols in each sample were determined by comparing spectrometric absorbance with standard curves. The absorbance from various concentrations of proline standard solutions was used to create the standard curve for proline quantification. The standard curve was used to calculate the concentration of proline in various samples. On the other hand, the standard curve for quantifying total phenolics was made using gallic acid at 10, 30, 50, 100, and 150 mg/L in 80% methanol, and it was expressed as mg gallic acid equivalent/g dry weight of the plant material (mg GAE/g DW). A bovine serum albumin (BSA) protein assay kit (Jiancheng, Nanjing, China) with BSA as the standard was used to estimate leaf protein content.[78]

Chlorophyll Content. Using Arnon's method, the total chlorophyll was calculated.[79] From each treatment, 200 mg of fresh leaves was taken and ground in 2 mL of acetone (80%). The ground material was put into a 2 mL Eppendorf tube and centrifuged at 10,000 rpm for 5 min. The supernatant was collected and put into a fresh test tube. Six milliliters of acetone was added to the test tube. The sample's absorbance was calculated using a spectrophotometer at 645 and 663 nm. Using the following formula, the amounts of chlorophyll a and b and total chlorophyll in leaves were determined:

$$\text{Chlorophyll a}\left(\frac{\mu g}{g}\right) = 12.7(\text{A663}) - 2.69(\text{A645})$$

$$\text{Chlorophyll b}\left(\frac{\mu g}{g}\right) = 22.9(\text{A645}) - 4.68(\text{A663})$$

$$\text{Total chlorophyll}\left(\frac{\mu g}{g}\right) = 20.2(\text{A645}) + 8.02(\text{A663})$$

Antioxidant Compounds. The fresh leaves were mixed with sodium phosphate buffer (50 mM, pH 7.8) and centrifuged.

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ACS Omega 2023, 8, 18940−18950

The antioxidant activities of superoxide dismutase and catalase from the supernatant were determined using standard procedures.[80,81] For peroxidase (POX) activity, the Nakano and Asada[82] method was used, with sodium phosphate (50 mM pH 5.5) containing EDTA-Na_2 (0.2 mM).

Measurement of Membrane Damage. The Hodges et al.[83] method was used to estimate MDA (malondialdehyde) levels. Absorbance was measured using a spectrophotometer at 440, 532, and 600 nm.

$$A = [(\text{Abs } 532_{+\text{TBA}}) - (\text{Abs } 600_{+\text{TBA}}) - (\text{Abs } 532_{-\text{TBA}} - \text{Abs } 600_{-\text{TBA}})]$$

$$B = [(\text{Abs } 440_{+\text{TBA}} - \text{Abs } 600_{+\text{TBA}}) \times 0.0571]$$

$$\text{MDA equivalents } (\mu\text{mol mL}^{-1}) = (A - B/157{,}000) \times 10^3$$

Acid Digestion and Cd Determination. The soil that had adhered to the plant roots was carefully removed, and the roots were rinsed with an EDTA solution. The plant samples were dried, ground, and digested using the acid digestion method.[84] The powdered plant sample (250 mg) was weighed and mixed with 6.5 mL of an acid solution containing nitric and sulfuric acid in a 5:1 ratio. After adding an acidic solution, the flasks were placed in a furnace overnight. The flasks were carefully placed on a hot plate the following day and heated until a clear supernatant was obtained. Using distilled water, the supernatant volume was made up to 50. The samples were cooled, filtered through Whatman no. 42, and analyzed for Cd concentration in the root, stem, and leaf samples using an atomic absorption spectrophotometer (Perkin-Elmer, AAnalyst 800).

Phytoremediation Potential. The phytoremediation potential of the plant was determined through the bioconcentration factor, bioaccumulation coefficient, translocation factor, and phytoextraction efficiency. The bioconcentration factor (BCF) is the ratio of metal concentration in plants to metal supplied in the growth medium (BCF). The BCF for each sample was calculated to determine how much metal the plant tissue (shoot + root) absorbed from the growing medium. In general, BCF calculates a plant's hyperaccumulation potential. The following formulae were used to calculate the translocation factor (TF) and bioaccumulation coefficient (BAC):

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Cd conc. in plant tissue } (\mu\text{g g}^{-1})}{\text{Cd conc. in soil } (\mu\text{g g}^{-1})}$$

$$\text{Bioaccumulation coefficient (BAC)} = \frac{\text{conc. of Cd in shoot } (\mu\text{g g}^{-1})}{\text{Cd conc. in soil } (\mu\text{g g}^{-1})}$$

$$\text{Translocation factor (TF)} = \frac{\text{conc. of Cd in shoot } (\mu\text{g g}^{-1})}{\text{Cd conc. in root } (\mu\text{g g}^{-1})}$$

The remediation factor (RF) was used to determine the percentage of Cd bioaccumulated in the plant's dry above-ground biomass from the total elemental contents in the soil during one cropping season to determine the phytoextraction efficiency.[85] During the current study, RF was calculated using the following equation:

$$\text{RF} = \frac{\text{Cd}_{\text{plant}} \times B_{\text{plant}}}{\text{Cd}_{\text{soil}} \times W_{\text{soil}}} \times 100\ (\%)$$

where B_{plant} is the plant dry above-ground biomass (g), Cd_{plant} is the Cd content in the plant dry above-ground biomass (mg kg^{-1}), Cd_{soil} is the total Cd content in soil (mg kg^{-1}), and W_{soil} is the amount of soil taken in one pot (g).

Statistical Analysis. One-way ANOVA was used to analyze the collected data statistically using the Statistix version 8.1 computer software (Tallahassee, Florida, USA). Tukey's HSD (honestly significant difference) test was used to determine whether there was a significant difference between the treatment means at $\alpha = 0.05$. The figure and tables were created with Microsoft Office Excel 2016 and GraphPad Prism version 8.00 for Windows (GraphPad Software, San Diego, California).

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