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To cite this article: Ambrose Okem, Manoj G. Kulkarni & Johannes Van Staden (2015) Enhancing Phytoremediation Potential of *Pennisetum clandestinum* Hochst in Cadmium-Contaminated Soil Using Smoke-Water and Smoke-Isolated Karrikinolide, *International Journal of Phytoremediation*, 17:11, 1046-1052, DOI: [10.1080/15226514.2014.981245](https://doi.org/10.1080/15226514.2014.981245)

To link to this article: <http://dx.doi.org/10.1080/15226514.2014.981245>



Accepted online: 12 Jan 2015.



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Enhancing Phytoremediation Potential of *Pennisetum clandestinum* Hochst in Cadmium-Contaminated Soil Using Smoke-Water and Smoke-Isolated Karrikinolide

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The use of plant growth regulators (PGRs) and biostimulants to enhance phytoextraction is gaining popularity in phytoremediation technology. This study investigated the stimulatory effects of smoke-water (SW), a smoke-derived compound karrikinolide (KAR₁) and other known plant growth regulators (PGRs) [gibberellic acid (GA₃), kinetin (Kin) and indole-3-butyric acid (IBA)] to enhance the phytoextraction potential of *Pennisetum clandestinum*. *Pennisetum clandestinum* seedlings were grown for 10 weeks in vermiculite using Hoagland's nutrient solution and were treated with cadmium (Cd) (2, 5, and 10 mg L⁻¹) and SW, KAR₁ and PGRs. KAR₁ exhibited positive effects on shoot and root dry weight (140 and 137 mg respectively) at the highest concentration of Cd (10 mg L⁻¹) compared to all the other treatments. KAR₁ and SW treatments used in the present study significantly improved the phytoextraction potential of *P. clandestinum* (602 and 575 mg kg⁻¹ respectively) compared to the other tested PGRs. This is the first report on the use of SW and KAR₁ to enhance phytoremediation potential in *P. clandestinum*. Further studies are needed to elucidate the exact mechanisms of smoke constituents involved in phytoextraction potential of plant species.

Keywords: heavy metal, plant growth regulator, phytoremediation, phytoextraction, *Pennisetum clandestinum*, smoke solution

Introduction

Phytoremediation is an emerging cost-effective green technology that uses plants to ameliorate lands contaminated with toxic heavy metals. The conventional remediation methods have several limitations such as cost and technical complexities, irreversible changes in soil properties and disturbance of native soil microflora as well as secondary pollution problems (Ali, Khan, and Sajad 2013). Phytoremediation on the other hand is an eco-friendly, efficient and a novel technique for decontamination of heavy metal-polluted soils (Ali *et al.* 2013). Hyperaccumulator plants have tolerance to heavy metals through various detoxification mechanisms, which may include selective metal uptake and translocation of metals to the shoots via the xylem loading. Plants can also detoxify and sequester heavy metals in tissues by excretion process, complexing specific ligands and compartmentation of metal-ligand complexes (Barazani *et al.* 2004; Bulak, Walkiewicz, and Brzezinska 2014). In order for easy uptake by plants, heavy metals need to be disassociated from soil compartments and become bioavailable in a soil solution. This process of soil-

bound metals can be achieved by a number of approaches such as application of soil acidifiers, commercial nutrients or the use of some chelating agent like EDTA (Chen, Shen, and Li 2004).

Plant growth regulator (PGR) are chemicals which enhance plant growth when applied in a very minute quantity. For instance, gibberellic acid (GA₃) was found to accelerate bud development and stem elongation in a number of plant species (Kabar 1990; Hernandez 1997; Naeem *et al.* 2004). Indole-3-butyric acid (IBA) is well known for its beneficial effects in enhancing adventitious root formation in many plant species (Ludwig-Müller 2000). Kinetin regulates cell division and differentiation in plants thereby improving germination and emergence of crop seeds as well as enhancing early flowering (Pan *et al.* 1999).

The use of PGR-assisted phytoextraction is a novel strategy that has been used to improve the efficiency of metal uptake by plants. PGR enhance both shoot and root growth and biomass yield as well as the effectiveness of antioxidation systems in plants, thereby facilitating the greater acquisition and accumulation of heavy metals (Liphadzi, Kirkham, and Paulsen 2006; Israr *et al.* 2011). The use of conventional PGRs to enhance the efficiency of phytoextraction has been documented in a number of plant species such as *Zea mays* (Meers 2005), *Medicago sativa* (Lopez *et al.* 2005) and *Brassica juncea* (Fuentes *et al.* 2000). The use of plant growth hormones for restoration

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of growth rate and biomass affected by heavy metal stress has been encouraged (Falkowska *et al.* 2011).

Unlike the conventional use of PGRs, SW is simple and cost-effective. It can be easily prepared by bubbling plant-derived smoke through water. SW is now widely used as a germination cue for fire-dependent as well as non-fire-dependent plant species (Jäger, Light, and Van Staden 1996; Light and Van Staden 2004). Smoke treatments are used for propagation of economically important plants, as germination percentage can be increased significantly (Brown and Van Staden 1998). These treatments also improve seedling vigor and enhance plant growth under normal and stress conditions (Kulkarni, Light, and Van Staden 2011). Karrikinolide (KAR₁) isolated from smoke and which is commercially available is now recognized as a naturally occurring plant growth regulator (Chiwocha *et al.* 2009). Thus smoke and smoke-derived compounds have good potential in horticulture, agriculture and weed management (Roche, Koch, and Dixon 1997; Light and Van Staden 2004). Smoke technology has recently been used in post-mining habitat restoration and conservation practices (Boucher and Meets 2004; Daws *et al.* 2014). However, the use of SW and KAR₁ to enhance phytoremediation has not been investigated to date.

Recently, phytoremediation studies have focused more on the use of high-biomass plants. *Pennisetum clandestinum* (Kikuyu) is a prostrate perennial yellowish-green grass, stoloniferous and rhizomatous often turf forming which grows up to 18 inches high when ungrazed, but under grazing or mowing it forms a dense turf (Mears 1970; Muscolo, Panuccio, and Eshel 2013). Kikuyu grass spreads vigorously by numerous large, fleshy, creeping stolons and rhizomes that sometimes reach 2 m or more in length and depth. This plant species is considered as an excellent colonizer and soil stabilizer due to its ability to recover rapidly from wear and divot injury, and competes well with weed invasion. It can tolerate a wide range of water supply and soil pH (between 5.5 and 8.0) conditions. Kikuyu grass has varying adaptive features due to its high growth rate and well-developed root system, which enables the plant to tolerate drought, heat and salinity stress. The potential of *P. clandestinum* to accumulate Cu (copper) in roots has been explored for phytostabilization of copper-polluted sites (Sögüt *et al.* 2005; Ortiz-Calderon, Alcaide, and Likao 2008). *Pennisetum clandestinum* also has the ability to accumulate high amounts of cadmium, copper, lead and zinc in its roots (Beavington 1976; Bramley and Barrow 1994). The aim of this study was to investigate the effects of SW and KAR₁ in enhancing the phytoremediation potential of *P. clandestinum* in cadmium-contaminated soil and compare the responses to known PGRs.

Material and Methods

Effects of PGRs, SW and KAR₁ on Seedling Vigor

Seeds of Kikuyu, *P. clandestinum* (Hochst) were obtained from McDonald Seed Company, Pietermaritzburg, South

Africa. The seeds were germinated in disposable Petri dishes (90 mm) on two layers of Whatman No. 1 filter papers. Petri dishes (6 replicates per treatment) containing 20 seeds in each were placed in a plant growth chamber at 25°C with 16:8 h light:dark conditions. The photosynthetic photon flux density of lamps in the growth chamber was $80.4 \pm 3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. Seeds were moistened with 4.5 mL of different concentrations of gibberellic acid (GA₃) (10^{-5} , 10^{-6} and 10^{-7} M), kinetin (KIN) (10^{-5} , 10^{-6} and 10^{-7} M), indole-3-butyric acid (IBA) (10^{-5} , 10^{-6} and 10^{-7} M), karrikinolide (KAR₁) (10^{-7} , 10^{-8} and 10^{-9} M) and smoke-water (SW) (1:500, 1:1000 and 1:2000 v/v). Distilled water was used to moisten control seeds. Smoke-water was prepared according to the method of Baxter and Van Staden (1994) and karrikinolide was isolated from plant-derived smoke-water as described in Van Staden *et al.* (2004). Concentrations of PGRs, SW and KAR₁ that exhibited the best seedling vigor index [SVI = seedling length (mm) X germination (%)] after 15 days were selected for the growth experiments.

Effects of PGRs, SW and KAR₁ on Cd Uptake

The experiment was conducted in a greenhouse ($60 \pm 5\%$ relative humidity; $22 \pm 2^\circ\text{C}$; average photosynthetic photon flux density of $450 \mu\text{mol m}^{-2} \text{s}^{-1}$) in the Botanical Garden, University of KwaZulu-Natal Pietermaritzburg, South Africa. Three seeds of *P. clandestinum* were sown in each pot (15 cm) with 10 replicates containing vermiculite as a substrate. The seedlings were irrigated with 100 mL half-strength (50%) Hoagland's nutrient solution on alternate days for 10 weeks, after which the plants were well established. Thereafter, plants were treated every alternate day with different concentrations of cadmium (Cd) (2, 5 and 10 mg L⁻¹) (Street *et al.* 2009) which was applied in the form of cadmium nitrate [Cd(NO₃)₂ 4H₂O]. Each concentration of Cd was supplemented with either IBA 10^{-7} M, KIN 10^{-7} M, GA₃ 10^{-5} M, SW 1:2000 v/v or KAR₁ 10^{-8} M. The test solutions were prepared using half-strength Hoagland's nutrient solution. Two controls were included in this experiment i.e. one treated with Hoagland's nutrient solution only and the second with Cd but without PGRs. The experiment was terminated after 5 weeks from the initiation of treatments and different growth parameters were recorded and subsequently samples were oven dried at 70°C for 7 days.

Acid Digestion of Plant Sample

Acid digestion of plant samples was conducted according to the method of Okem *et al.* (2012). Plant samples (root and shoot) (0.5 g) were placed in borosilicate glass digestion tubes. To each of the tubes, 10 mL HNO₃-HCl-H₂O₂ (8:1:1, v/v/v) was added and the tubes placed on a heating block with the temperature set to increase to 150°C for 3 h or until the solutions were completely digested. After the digestion was completed, the clear solutions were transferred to 50 mL volumetric flasks and made to volume with ultrapure (UP) water. The diluted samples were stored in high density polyethylene bottles until analysis. The samples were analyzed in triplicate.

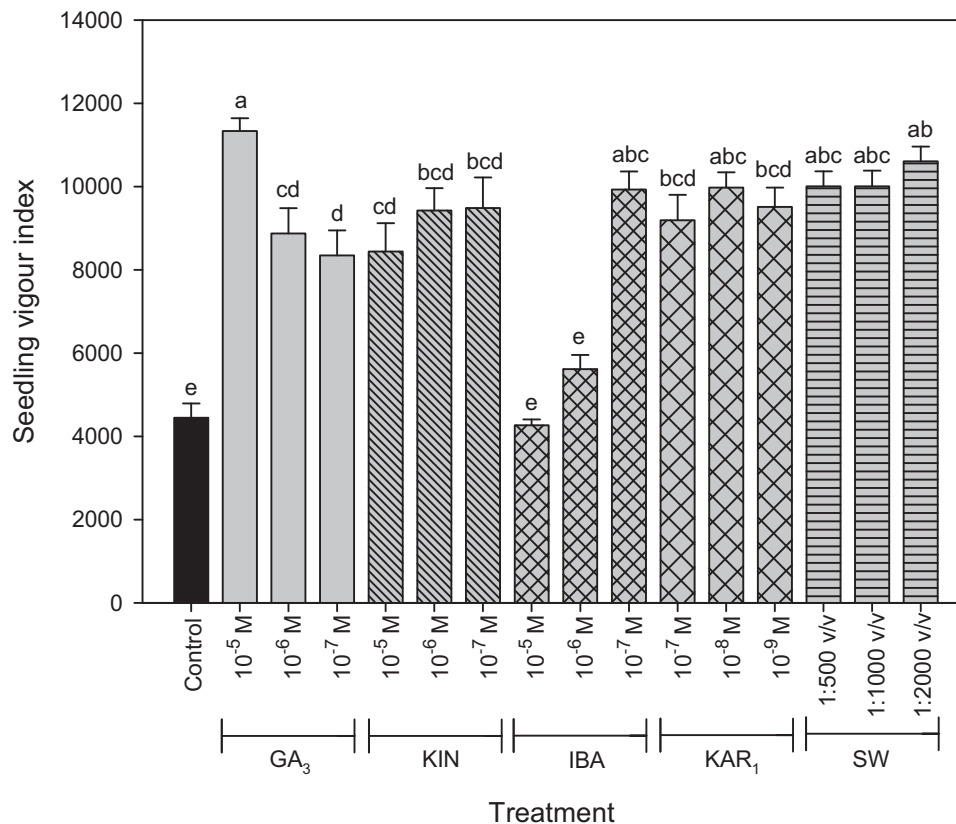


Fig. 1. Effect of different plant growth regulators (GA₃, gibberellic acid; KIN, Kinetin; IBA, indole-3-butyric acid), smoke-water (SW) and karrikinolide (KAR₁) on seedling vigor of *Pennisetum clandestinum*. Seeds were germinated at 25°C under 16:8 h light:dark conditions for 15 days. Bars (\pm SE) with different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

A blank solution containing 10 mL HNO₃-HCl-H₂O₂ (8:1:1, v/v/v) was included.

using Duncan's multiple range test at 5% level of significance ($P < 0.05$).

Analysis of Cd using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

The digested samples were analyzed for Cd composition using ICP-OES (Varian 720-ES, Varian Inc, Palo Alto, CA, USA) (Okem et al. 2012). ICP-OES is a powerful tool that provides multi-elemental analysis and supports a broad linear calibration range and can measure metal concentrations in a variety of different sample matrices. The operating conditions for the ICP-OES instrument were: RF power 1.0 kW; viewing geometry axial; Argon gas was used as plasma gas at flow rate of 15.0 L min⁻¹; auxiliary gas flow rate 1.50 L min⁻¹; nebulizer gas at flow rate 0.75 L min⁻¹; replicate reading time 9.0 s. All analyses were performed in triplicate.

Statistical Analysis

The data of the experiment were analyzed with GenStat (release 14.1) statistical package. One-way analysis of variance was conducted and the means of the treatments were separated

Results

Effects of PGRs, SW and KAR₁ on Seedling Vigor of *P. clandestinum*

The results of the seedling vigor index showed that GA₃ 10⁻⁵ M was significantly higher (11335) compared to the control (4445) (Fig. 1). All the other treatments also exhibited a higher increase in seedling vigor index compared to the control. The only exceptions were IBA 10⁻⁵ and 10⁻⁶ M concentrations, which were not significantly different from the seedling vigor index of the control. The best results were recorded for SW 1:2000 v/v, KAR₁ 10⁻⁸ M, GA₃ 10⁻⁵ M, IBA 10⁻⁷ M and KIN 10⁻⁷ M concentrations. These concentrations were therefore chosen for the growth studies discussed below.

Effects of PGRs, SW and KAR₁ on Growth of *P. clandestinum* under Cd Stress

The control plants treated with Hoagland's nutrient solution alone exhibited significantly higher growth compared to all

Table 1. Effect of different plant growth regulators (GA₃, gibberellic acid; KIN, Kinetin; IBA, indole-3-butyric acid), smoke-water (SW) and karrikinolide (KAR₁) on growth of *Pennisetum clandestinum*. Plants were grown in the greenhouse and treatments were initiated after 10 weeks. Fifteen-week-old plants were harvested and growth parameters were measured.

Treatment	Shoot length (cm)	Root length (cm)	Leaf (no)	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot dry weight (mg)	Root dry weight (mg)
Control	37.9 ± 1.4	17.1 ± 1.2	11.5 ± 1.2	613 ± 10	781 ± 185	255 ± 22	182 ± 39
Cd 2 mg L⁻¹							
Control	28.9 ± 1.82 ab	12.3 ± 0.37 bc	11.5 ± 0.6 a	624 ± 60 a	865 ± 205 a	55 ± 12 c	158 ± 35 a
GA ₃ (10 ⁻⁵ M)	29.4 ± 1.56 ab	11.2 ± 0.61 c	10.7 ± 0.6 a	700 ± 70 a	451 ± 73 b	145 ± 18 ab	105 ± 14 ab
KIN (10 ⁻⁷ M)	31.3 ± 1.19 a	14.8 ± 0.82 a	7.9 ± 0.4 b	616 ± 50 a	425 ± 72 b	108 ± 8 b	80 ± 10 b
IBA (10 ⁻⁷ M)	28.5 ± 1.4 ab	14.1 ± 0.64 ab	9.1 ± 0.5 b	409 ± 40 b	518 ± 70 b	165 ± 32 a	119 ± 21 ab
KAR ₁ (10 ⁻⁸ M)	28.1 ± 1.1 ab	12.7 ± 0.69 bc	8.0 ± 0.5 b	584 ± 50 a	403 ± 80 b	125 ± 11 ab	78 ± 13 b
SW (1:2000)	25.6 ± 1.3 b	8.4 ± 0.41 d	8.9 ± 0.6 b	639 ± 40 a	423 ± 90 b	117 ± 11 ab	86 ± 14 b
Cd 5 mg L⁻¹							
Control	23.8 ± 1.8 c	11.7 ± 0.60 c	10.5 ± 1.2 ab	741 ± 120 ab	618 ± 150 ab	164 ± 24 a	117 ± 28 a
GA ₃ (10 ⁻⁵ M)	28.9 ± 1.5 b	15.8 ± 0.80 b	9.5 ± 0.5 b	547 ± 60 bc	339 ± 54 b	144 ± 10 a	92 ± 10 a
KIN (10 ⁻⁷ M)	33.6 ± 1.6 a	18.7 ± 1.39 a	12.1 ± 0.6 a	928 ± 90 a	743 ± 144 a	163 ± 19 a	110 ± 20 a
IBA (10 ⁻⁷ M)	28.6 ± 1.0 b	15.0 ± 0.79 b	11.0 ± 0.6 ab	619 ± 50 bc	503 ± 80 ab	138 ± 10 ab	91 ± 14 a
KAR ₁ (10 ⁻⁸ M)	24.2 ± 1.2 c	18.8 ± 0.94 a	8.8 ± 0.5 b	434 ± 40 c	551 ± 70 ab	98 ± 9 bc	90 ± 13 a
SW (1:2000)	24.9 ± 1.0 bc	10.2 ± 0.60 c	8.7 ± 0.6 b	566 ± 50 bc	405 ± 80 b	81 ± 8 c	74 ± 12 a
Cd 10 mg L⁻¹							
Control	18.8 ± 0.87 c	16.0 ± 1.34 ab	9.1 ± 0.4 bc	407 ± 30 b	412 ± 56 b	93 ± 8 b	64 ± 11 b
GA ₃ (10 ⁻⁵ M)	28.7 ± 2.8 a	13.8 ± 0.89 bc	8.4 ± 0.6 c	466 ± 70 b	442 ± 47 b	111 ± 12 ab	59 ± 7 b
KIN (10 ⁻⁷ M)	22.9 ± 1.33 bc	14.0 ± 0.89 bc	8.2 ± 0.6 c	431 ± 50 b	328 ± 72 b	92 ± 11 b	107 ± 30 ab
IBA (10 ⁻⁷ M)	23.1 ± 0.9 bc	14.7 ± 0.90 b	10.6 ± 0.6 ab	486 ± 50 b	408 ± 60 b	111 ± 10 ab	74 ± 10 b
KAR ₁ (10 ⁻⁸ M)	27.1 ± 1.3 ab	18.4 ± 0.75 a	11.0 ± 0.5 a	753 ± 50 a	857 ± 70 a	140 ± 12 a	137 ± 11 a
SW (1:2000)	26.1 ± 1.4 ab	11.3 ± 0.53 c	9.2 ± 0.5 bc	541 ± 80 b	474 ± 70 b	128 ± 12 a	74 ± 7 b

Mean values (±SE) in a column of each Cd treatment with different letter(s) is significantly different according to Duncan's multiple range test at 5% level of significance ($p < 0.05$). * Control treatment without Cd was not included in the analysis.

other treatments (Table 1). At Cd 2 mg L⁻¹, KIN- and IBA-treated plants exhibited a significant increase in root length (14.8 and 14.1 cm respectively) compared to all other treatments (Table 1). The shoot dry weights of all the treatments at Cd 2 mg L⁻¹ were significantly higher than the Cd 2 mg L⁻¹ control plants with IBA having the highest values (Table 1). There was no significant difference for root dry weight in all the PGRs- and smoke solution-treated plants at Cd 5 mg L⁻¹. However, KIN and KAR₁ significantly increased root length compared to the other treatments (Table 1). KAR₁ at Cd 10 mg L⁻¹ showed significantly greater values than the control and other treatments in most of the recorded growth parameters (Table 1).

Effects of PGRs, SW and KAR₁ on Cd uptake in *P. clandestinum*

A significant increase in Cd uptake was recorded in the shoots of KAR₁- and SW-treated plants grown with Cd 2 mg L⁻¹ (441.73 and 304.81 mg L⁻¹ respectively) (Fig. 2A). IBA significantly increased the accumulation of Cd in the root at Cd 2 mg L⁻¹ (215.36 mg L⁻¹) compared to all other treatments. At Cd 5 mg L⁻¹, KIN, IBA, SW and KAR₁ significantly increased uptake of Cd in shoots of *P. clandestinum* compared to the

control with the exception of GA₃ treatment (Fig. 2B) which had very low accumulation of Cd. KAR₁ and SW treatments showed significant increase in the uptake of Cd compared to all other treatments when supplied with Cd 10 mg L⁻¹ (602.34 and 575.28 mg L⁻¹ respectively) (Fig. 2C). In the case of roots, KIN, IBA and KAR₁ recorded significantly higher Cd concentrations compared to the control (Fig. 2C).

Discussion

Effect of PGRs, SW and KAR₁ on Seedling Vigor of *P. clandestinum*

This is the first report on the use of SW and smoke-derived KAR₁ compound to enhance phytoremediation potential in *P. clandestinum* species. The stimulatory effects of SW and KAR₁ on seedling vigor could have played a vital role in enhancing accumulation of Cd in the shoot of *P. clandestinum*. Unlike the other conventional PGRs used in this study, KAR₁ and SW showed better phytoextraction efficiency at all the examined Cd concentrations.

Smoke treatments do not only have the ability to release seed dormancy and improve germination but it can also

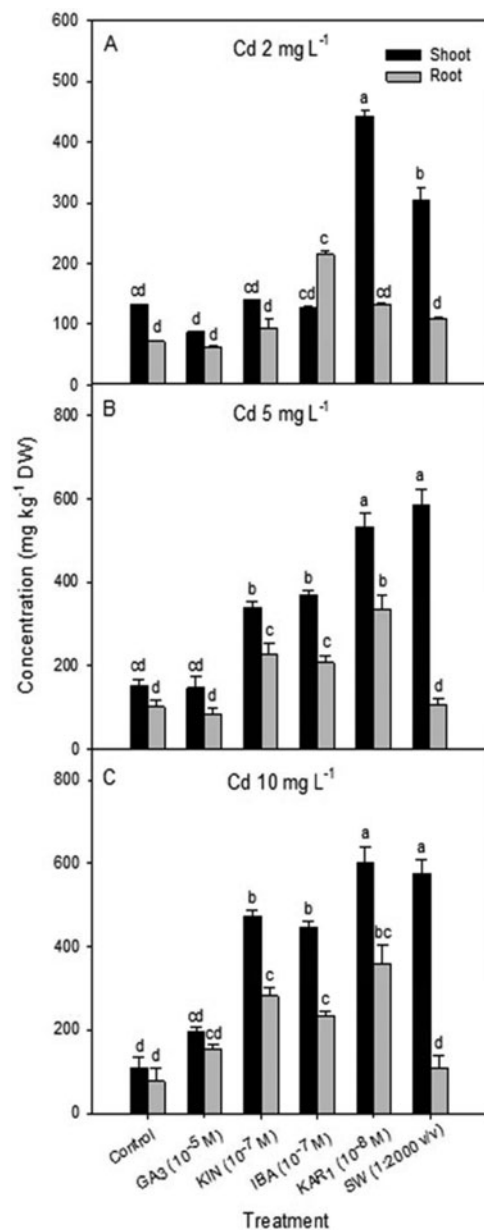


Fig. 2. Cadmium uptake in *Pennisetum clandestinum* under the influence of different plant growth regulators (GA₃, gibberellic acid; KIN, Kinetin; IBA, indole-3-butyric acid), smoke-water (SW) and karrikinolide (KAR₁). Plants were grown in the greenhouse and treatments were initiated after 10 weeks. Fifteen-week-old plants were harvested and dried for 7 days at 70°C before analysis. Bars (±SE) of each Cd concentration with different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

enhance seedling vigor (Baxter and Van Staden 1994; Sparg *et al.* 2005). This suggests that the active compounds in SW influence enzyme systems which control germination and seedling vigor (Blank and Young 1998). Physiological disorders caused by Cd toxicity such as chlorosis, growth inhibition, browning of root tips, and death (Kahle 1993) were observed in plants treated with Cd without growth promoting substances.

Effect of PGRs, SW and KAR₁ on Growth of *P. clandestinum* under Cd Stress

The negative effects of heavy metals on germination, growth rate and biomass can be attenuated by using exogenous PGRs. Lopez *et al.* (2005) reported that combined treatment of PGRs at the concentration of 100 mM indole-3-acetic acid (IAA) and 0.2 mM ethylenediaminetetraacetic acid (EDTA) increased the Pb accumulation in leaves of *Medicago sativa* by about 2800% as well as alleviated the effects of heavy metal stress. Application of brassinolide on *Chlorella vulgaris* exposed to heavy metal stress exhibited significant anti-stress effects. (Bajguz 2010). KIN applied to *Carthamus tinctorius* plants grown on a lead spiked soil significantly alleviated the toxic effects of pollution and increased the dry matter content (Sayed 1999). KIN stimulates cell division and enhances photosynthetic activity (Tassi *et al.* 2008). These effects could have been responsible for the increase in root length as well as accumulation of dry matter of KIN-treated *P. clandestinum* plants. IBA is largely used to promote rooting in varieties of plant species because of its greater stability against degradation in solution and metabolism in plant tissues (Epstein and Ludwig-Müller 1993; Ludwig-Müller 2000). IBA non-significantly improved the root system (fresh and dry weight) of *P. clandestinum* compared to some other tested regulators and accumulated a significantly higher amount of Cd both in the shoot and root at Cd 10 mg L⁻¹. GA₃ was not as effective as other tested plant growth promoting substances which may be due to the mode of application. In this study, *P. clandestinum* plants were drenched with GA₃ solution which might not have promoted the root biosynthesis as did the other growth regulators. However, there is a possibility that foliar GA₃ application may stimulate growth and uptake of Cd in *P. clandestinum*. Foliar treatment with GA₃ has significantly enhanced the plant biomass and phytoremediation potential in *Parthenium hysterophorus* (Hadi, Ali, and Ahmad 2014).

Effect of PGRs, SW and KAR₁ on Uptake of Cd in *P. clandestinum*

In general, a greater quantity of Cd was accumulated in the shoots of *P. clandestinum* than the roots. This indicates that root to shoot translocation for Cd in this plant species was higher when treated with plant growth promoting substances. PGRs alleviate the negative effects by improving plant tolerance to heavy metal stress and enhance certain biosynthetic pathways (Fuentes *et al.* 2000; Liphadzi, Kirkham, and Paulsen 2006; Tassi *et al.* 2008; Piotrowska-Niczypuk *et al.* 2012). *Pennisetum clandestinum* is known for its unique morphological and ecological characteristics such as massive deep root system and its tolerance to a wide range of adverse environmental conditions including salinity. These could have been responsible for the greater acquisition and tolerance to Cd in the present study. In the present study, at the highest concentration of Cd (10 mg L⁻¹), there was a positive effect in the phytoextraction efficiency when KIN, IBA, SW and KAR₁ were used. These results clearly suggest that phytoextraction can be improved by increasing both the dry mass and metal accumulation in the upper parts of plants using SW (1:2000 v/v),

KAR₁ (10⁻⁸ M), KIN (10⁻⁵ M) and IBA (10⁻⁷ M) respectively. It is well known that soil contaminated with elevated levels of heavy metal such as Cd is an inhospitable substrate for plant establishment and growth. Hence, the use of plant growth promoting substances to increase plant biomass as well as efficient root colonization are essential steps to enhance phytostabilisation and phytoextraction of metals from polluted soils (Tassi *et al.* 2008; Bulak, Walkiewicz, and Brzezinska 2014). The cost of using conventional chelating agents to enhance the efficiency of phytoremediation will increase with the severity of metal contamination. Smoke stimulating products on the other hand are cost-effective and very low concentrations are needed to achieve positive effects. Hence, further studies are needed to elucidate the exact mechanism of SW and KAR₁ to enhance the phytoremediation potential in plant species.

Conclusions

The result of the present study showed that all the PGRs as well as KAR₁ and SW treatments significantly enhanced seedling vigor compared to the control plants. The exceptions were IBA 10⁻⁵ and 10⁻⁶ M concentrations, which were not significantly different from the control. At the highest concentrations of Cd (10 mg L⁻¹), GA₃, KAR₁ and SW exhibited positive effects on shoot length as well as shoot dry weight. Application of KAR₁ and SW significantly improved the phytoextraction potential of *P. clandestinum* compared to the other tested PGRs. This plant species have the ability to tolerate adverse environmental conditions and these could have been the reason for the high levels of Cd accumulation noted in this study. However, the phytoremediation potential of *P. clandestinum* has not been established owing to lack of detailed investigations of its capacity to absorb heavy metal contamination in soil. This is the first report on the use of SW and smoke-derived KAR₁ compound to enhance phytoremediation potential in plant species. In the present study, smoke-technology appears to be a good strategy to boost the phytoextraction process of Cd in *P. clandestinum*. However, further studies are needed to elucidate the exact mechanism of smoke treatments (solutions) involved in enhancing uptake of heavy metals in plant species.

Funding

The authors thank the University of KwaZulu-Natal for providing financial support.

References

- Ali H, Khan E, Sajad MA. 2013. Phytoremediation of heavy metals- Concepts and applications. *Chemosphere* 91:869–881.
- Bajguz A. 2010. An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in *Chlorella vulgaris* cultures under heavy metals stress. *Environ Exp Bot* 68:175–179.
- Barazani O, Sathiyamoorthy P, Manandhar U, Vulkan R, Golan-Goldhirsh A. 2004. Heavy metal accumulation by *Nicotiana glauca* Graham in a solid waste disposal site. *Chemosphere* 54:867–872.
- Baxter BJM, Van Staden J. 1994. Plant-derived smoke: an effective seed pre-treatment. *Plant Growth Regul* 14:279–282.
- Beavington F. 1976. Seasonal enrichment by trace elements of kikuyu grass around heavy metals industries. *Plant Soil* 45:283–286.
- Blank RR, Young JA. 1998. Heat substrate and smoke: influence on seed emergence and plant growth. *J Range Manage* 51:577–583.
- Boucher C, Meets M. 2004. Determination of the relative activity of aqueous plant-derived smoke solution used in seed germination. *S Afr J Bot* 70:31–318.
- Bramley RGV, Barrow NJ. 1994. Differences in the cadmium content of some common Western Australian pasture plants grown in a soil amended with cadmium - describing the effects of levels of cadmium supply. *Fertilizer Res* 39:113–122.
- Brown NAC, Van Staden J. 1998. Plant-derived smoke: an effective seed pre-soaking treatment for wildflower species and with potential for horticultural and vegetable crops. *Seed Sci Technol* 26:669–673.
- Bulak P, Walkiewicz A, Brzezinska M. 2014. Plant growth regulators-assisted phytoextraction. *Biol Plantarum* 58:1–8.
- Chen Y, Shen Z, Li X. 2004. The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. *Applied Geochem* 19:1553–1565.
- Chiwocha SDS, Dixon KW, Flematti GR, Ghisalberti EL, Merritt DJ, Nelson DC, Riseborough JAM, Smith SM, Stevens JC. 2009. Karrikins: a new family of plant growth regulators in smoke. *Plant Sci* 177:252–256.
- Daws MI, Downes KS, Koch JM, Willyam D. 2014. Is broad-scale smoke-water application always a useful tool for improving seedling emergence in post-mining restoration? Evidence from jarrah forest restoration in Western Australia. *S Afr J Bot* 90:109–113.
- Epstein E, Ludwig-Müller J. 1993. Indole-3-butyric acid in plants: occurrence, synthesis, metabolism and transport. *Physiol Plant* 88:382–389.
- Falkowska M, Pietryczuk A, Piotrowska A, Bajguz A, Grygoruk A, Czerpak R. 2011. The effect of gibberellic acid (GA₃) on growth, metal biosorption and metabolism of the green algae *Chlorella vulgaris* (Chlorophyceae) Beijerinck exposed to cadmium and lead stress. *Pol J Environ Stud* 20:53–59.
- Fuentes CS, Khoo T, Muir SP, Khan AG. 2000. Phytoremediation of a contaminated mine site using plant growth regulators to increase heavy metal uptake. In: *Proceedings of the 5th International Conference on Clean Technologies for the Mining Industry*. Santiago, Chile. pp 427–435.
- Hadi F, Ali N, Ahmad A. 2014. Enhanced phytoremediation of cadmium-contaminated soil by *Parthenium hysterophorus* plant: Effect of gibberellic acid (GA₃) and synthetic chelator, alone and in combinations. *Bioremed J* 18:46–55.
- Hernandez P. 1997. Morphogenesis in sunflower as affected by exogenous application of plant growth regulators. *Agriscientia* 13:3–11.
- Israr M, Jewell A, Kumar D, Sahi SV. 2011. Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of *S. drummondii*. *J Hazard Mater* 186:1520–1526.
- Jäger AK, Light ME, Van Staden J. 1996. Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environ Exp Bot* 36:421–429.
- Kabar K. 1990. Comparison of kinetin and gibberellic acid effects on seed germination under saline conditions. *Phyton (Horn. Austria)* 30:291–298.
- Kahle H. 1993. Response of roots of trees to heavy metals. *Environ Exp Bot* 33:99–119.
- Kulkarni MG, Light ME, Van Staden J. 2011. Plant-derived smoke: Old technology with possibilities for economic applications in agriculture and horticulture. *S Afr J Bot* 77:972–979.
- Light ME, Van Staden J. 2004. The potential of smoke in seed technology. *S Afr J Bot* 70:97–101.
- Liphadzi MS, Kirkham MB, Paulsen GM. 2006. Auxin-enhanced root growth for phytoremediation of sewage-sludge amended soil. *Environ Technol* 27:695–704.

- Lopez ML, Peralta-Videa JR, Benitez T, Gardea-Torresdey JL. 2005. Enhancement of lead uptake by alfalfa (*Medicago sativa*) using EDTA and plant growth promote. *Chemosphere* 61:525–598.
- Ludwig-Müller J. 2000. Indole-3-butyric acid in plant growth and development. *Plant Growth Regul* 32:219–230.
- Mears PT. 1970. Kikuyu- (*Pennisetum clandestinum*) as a pasture grass-A Review. *Trop Grasslands* 4:139–152.
- Meers E, Ruttens A, Hopgood M, Lesage E, Tack FMG. 2005. Potential of *Brassica rapa*, *Helianthus annuus* and *Zeamays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. *Chemosphere* 61:561–572.
- Muscolo A, Panuccio MR, Eshel A. 2013. Ecophysiology of *Pennisetum clandestinum*: a valuable salt tolerant grass. *Environ Exp Bot* 92:55–63.
- Naeem M, Bhatti I, Ahmad RH, Ashraf MY. 2004. Effect of some growth hormones (GA₃, IAA and Kinetin) on the morphology and early or delayed initiation of bud of Lentil (*Lens culinaris* Medik). *Pak J Bot* 36:801–809.
- Okem A, Southway C, Ndhala AR, Van Staden J. 2012. Determination of total and bioavailable heavy and trace metals in South African commercial herbal concoctions using ICP-OES. *S Afr J Bot* 82:75–82.
- Ortiz-Calderon C, Alcaide O, Likao J. 2008. Copper distribution in leaves and roots of plants growing on a copper mine-tailing storage facility in northern Chile. *Rev Chil Hist Nat* 81:489–499.
- Pan B, Bai YM, Leibovitch S, Smith DL. 1999. Plant-growth-promoting rhizobacteria and kinetin as ways to promote corn growth and yield in a short-growing-season area. *Europe J Agron* 11:179–186.
- Piotrowska-Niczypouk A, Bajquz A, Zambryzycka E, Godlewska-Żylkiewicz B. 2012. Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris*. *Plant Physiol Biochem* 52:52–65.
- Roche S, Koch JM, Dixon KW. 1997. Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restor Ecol* 5:191–203.
- Sayed SA. 1999. Effects of lead and kinetin on the growth, and some physiological components of safflower. *Plant Growth Regul* 29:167–174.
- Söğüt Z, Zaimoğlu BZ, Erdoğan R, Sucu MY. 2005. Phytoremediation of landfill leachate using *Pennisetum clandestinum*. *J Environ Biol* 26:13–20.
- Sparg SG, Kulkarni MG, Light ME, Van Staden J. 2005. Improving seedling vigour of indigenous medicinal plants with smoke. *Biore-source Technol* 96:1323–1330.
- Street RA, Kulkarni MG, Stirk WA, Southway C, Abdillahi HS, Chinsamy M, Van Staden J. 2009. Effect of cadmium uptake and accumulation on growth and antibacterial activity of *Merwillia plumbea* – An extensively used medicinal plant in South Africa. *S Afr J Bot* 75:611–616.
- Tassi E, Pouget J, Petruzzelli G, Barbaferi M. 2008. The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. *Chemosphere* 71:66–73.
- Van Staden J, Jäger AK, Light ME, Burger BV. 2004. Isolation of the major germination cue from plant derived smoke. *S Afr J Bot* 70:654–659.