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PHYTOREMEDIATION OF CADMIUM CONTAMINATED SOIL BY AUXIN ASSISTED BACTERIAL INOCULATION

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ABSTRACT

Toxic level of cadmium (Cd) has been built up in soil profile due to over fertilization, industrial activities and irrigation with city wastewater. One of the remediation strategies of such contaminated soil is phytoextraction by using hyper-accumulator plants. Metal stress hinders plant growth in contaminated soils even hyper-accumulator plant cannot attain sufficient biomass for effective phytoremediation. Plant growth promoting bacteria and auxin help plants to cope with metal stress. A pot experiment was conducted where the efficacy of four different Cd tolerant bacterial isolates alone and in combination with auxin (10^{-3} M) was evaluated to promote spinach growth in Cd contaminated soil. Results revealed that Cd contamination severely decreased the spinach growth. However, bacterial inoculation and auxin application improved the spinach growth and Cd uptake compared to control. But combined application of bacterial isolates and auxin was more pronounced for enhancing fresh and dry mass, and phytoextraction of Cd. Bacterial inoculation along with auxin increased the fresh and dry weight up to 261 and 45%, respectively, over control. Moreover, combined use of bacteria and auxin also enhanced the uptake of Cd by spinach up to 5.36 folds compared to control. Thus, synergistic use of bacteria and auxin could be a novel approach for improving plant growth under metals stress as well as for meaningful phytoremediation of metals contaminated soils.

Keywords: Spinach, Phytoextraction, IAA

INTRODUCTION

Metals contamination in soil has been recognized as major threat to environment and human health because of its toxicity. Metals concentration in soil beyond the permissible limits not only reduce the crop growth but also have damaging effect on human health when enter in the food chain (Jiwan and Singh, 2011). The sources of metals in soil might be the parent material or anthropogenic activities that are associated with industrialization such as paint industries, glass manufacturing industries and manufacturer of batteries through industrial process of mining and smelting, disposal of wastes and use of fertilizers and pesticides (Bilos et al., 2001). Cadmium pollution causes deleterious effects on public health and wildlife due to its greater solubility in water which has led to increase interests for development of such techniques that can reduce its toxic effects in soil (Valls and Lorenzo, 2002). Phytoremediation is a promising tool that provides more benefits than conventional physico-chemical approaches to remove toxic metals from the soil because of its lower cost and safety to the environment (Kramer, 2005). It is relatively better approach in which plants are used to reduce toxicity of the contaminants *in-situ*. Some plant species have been identified as hyper-accumulators that have ability to accumulate remarkably high concentrations of heavy metals without hampering their growth and development (Sheng et al., 2008). However, application of hyper-accumulators for phytoremediation in field is not suitable because of their smaller biomass and slower growth due to venomous nature of pollutants (Sheng et al., 2008). Moreover, the success of phytoremediation could be enhanced through exogenous application of plant growth regulators i.e. auxin, which increases metals uptake and translocation capability of plants (Khan et al., 2000). Auxins are natural plant hormones produced in the apical regions of plants and stimulate the cell division, cell enlargement, apical dominance, root elongation and subsequently increase the uptake of metal ions (Parker et al., 1992). Inoculation with plant growth promoting bacteria significantly improves plant biomass and resultantly improves phytoremediation (Glick, 2010; Asghar et al., 2013; Khan et al., 2013). Bacteria

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resistant to toxic level of metals have strong influence on metal transport and speciation in the rhizosphere (Lors et al., 2004). Studies revealed that bacterial inoculation significantly improves the accumulation of lead and Cd in tomato (He et al., 2009), Cd in rape (Sheng and Xia, 2006; Sheng et al., 2008) and Indian mustard (Belimov et al., 2005), and nickel in *Alyssum murale* (Abou-Shanab et al., 2006). The major aim of reported research work was to isolate Cd tolerant bacteria and monitor their effect alone and in combination with exogenously applied auxin on growth of spinach and its ability to hyper-accumulate Cd.

MATERIALS AND METHODS

Isolation of Cd-tolerant bacteria

Soil samples for isolation of Cd tolerant bacteria were collected around the industrial area of Faisalabad. Glucose peptone agar media enriched with different concentrations of Cd (0, 50, 100, 150 and 200 mg L^{-1} by using $CdCl_2$) was used for bacterial isolation by dilution plate technique. The composition of glucose peptone agar medium (L^{-1}) was as follow: peptone, 20 g; dextrose, 10 g; sodium chloride, 5 g; agar, 15 g and pH was maintained 7.2. These isolates were purified by repeated streaking on glucose peptone agar medium.

Pot trial

Pot trial was conducted to evaluate the synergistic effect of bacteria and auxin on the growth of Spinach (*Spinacia oslercea* L.) in Cd contaminated soil. Pots, with 12 kg soil in each, were spiked with 40 mg kg^{-1} of Cd by using $CdCl_2$. The inoculum for pot trial was prepared by growing the selected isolates in glucose peptone broth medium. Flasks containing glucose peptone broth were inoculated with selected isolates and incubated at 28±1°C for 3 days. Bacterial cells were harvested by centrifugation at 4500 rpm for 20 minutes. Then cells were washed and suspended in sterilized phosphate buffer saline and uniform cell density (10^7-10^8 CFU mL^{-1}) was achieved by maintaining optical density of (OD=0.45) at 535 nm. The composition of phosphate buffer saline was as follow: NaCl, 8.01 g; KCl, 0.20 g; $Na_2HPO_4.2H_2O$, 1.78 g; KH_2PO_4, O.27 g and pH was maintained 7.4. The inoculum of each isolate was injected into sterile peat (100 mL kg^{-1}) and was incubated for 24 h at 28±1°C before using it for seed coating. For seed inoculation, seed dressing was carried out with inoculated peat mixed with clay and 10% sugar solution. In case of the un-inoculated control, the seeds were coated with the same but autoclaved inoculum suspension. Seeds of spinach (*Spinacia olercea* L.) inoculated with four different bacterial isolates were sown in pots. Bacterial isolates and auxin @ 10^{-3} M were used alone as well as in combination, along with a control where neither inoculation nor auxin was applied. Indole acetic acid (IAA) as auxin was sprayed after each cut of spinach and total three cuttings were taken. Pots were arranged according to completely randomized design (CRD) in wire house under ambient light and temperature. Fertilizers NP @ 56-72 kg ha^{-1}, respectively, were applied along with other recommended agronomic practices. Data regarding shoot fresh and dry biomass were recorded at three different cuttings of spinach. The Cd contents were determined from the shoot samples by taking one composite sample by mixing grounded shoot tissues of three cuttings of each treatment. The oven-dried plant samples were ground and digested in mixture of concentrated HNO_3 and $HClO_4$ (4:1, v/v) (Chen et al., 2004). After digestion, the volume of each sample was adjusted and Cd content was determined by atomic absorption spectrometer (Aanalyst 100 Spectrometer, Perkin Elmer). The data recorded were subjected to statistical analysis by using Statistix-9 computer software (Copyright 2005, Analytical software, USA).

RESULTS

In this experiment, bacterial isolates and exogenous auxin application alone and in combination were evaluated for plant growth promotion and remediation of Cd contaminated soil. Four bacterial isolates were found Cd tolerant up to 200 mg L^{-1} and these were selected for further experiment. Results (Table 1) indicated a significant reduction in fresh and dry biomass production of spinach under Cd contamination. However, inoculation with Cd tolerant bacterial isolates considerably alleviated the deleterious effects of Cd in spinach and improved the growth of spinach compared with control. Bacterial isolate-4 improved the fresh weight up to 196 and 43% at first and second cutting, respectively, over control under Cd stress. Dry weight was improved up to 16% at both second and third cutting in response to isolate-3 over control. Similarly, exogenous application of auxin also improved the fresh and dry mass production of spinach under Cd contamination. Fresh biomass of spinach was significantly improved by auxin up to 116 and 26% at first and third cutting, respectively, compared to control. However, the effect of auxin was non-significant in improving the fresh biomass at second cutting over control. Maximum improvement up to 25% recorded in dry mass of spinach over control at second cutting under Cd stress. But application of auxin and bacterial isolates in combination dramatically increased the biomass production of spinach compared to control and sole application of both bacterial isolates and auxin under Cd contamination. At first cutting bacterial isolates-4 and isolate-1 along with auxin at 10^{-3} M caused maximum improvement in fresh and dry weight up to 261 and 45%, respectively, over control under Cd contamination. Similarly, at second cutting, isolate-2 along with auxin caused maximum increase up to 75 and 61% in fresh and dry weight, respectively, compared to control. Isolates-1, 2 and 4 were statistically similar in improving dry biomass but significantly higher than un-inoculated control at third cutting. Results also revealed that bacterial inoculation and auxin application facilitated the phytoextraction of Cd in spinach alone as well as in combination, compared to control (Figure 1). However, effect of combined application was more pronounced in improving the Cd uptake by spinach compared to sole application of bacterial isolates and auxin. Bacterial isolates-1, 2, 3 and 4 along with auxin improved the Cd uptake by spinach up to 3.90, 3.36, 5.36 and 2.72 folds, respectively, over control.

Table 1: Synergistic effect of auxin and bacteria on fresh and dry weight of different cuttings of spinach in cadmium contaminated soil

Treatments	**Fresh weight (g) at first cutting**	**Dry weight (g) at first cutting**	**Fresh weight (g) at 2nd cutting**	**Dry weight (g) at 2nd cutting**	**Fresh weight (g) at 3rd cutting**	**Dry weight (g) at 3rd cutting**
Control	39.25 g	9.59 g	80.78 g	9.04 e	92.32 g	13.35 f
Auxin	85.10 f	9.77 fg	90.84 fg	11.34 c	116.27 e	14.50 e
Isolate-1	83.63 f	10.81 de	96.17 ef	11.22 cd	99.79 f	16.55 d
Isolate-2	98.51 e	11.13 cd	100.48 d-f	9.55 e	129.38 d	16.05 d
Isolate-3	105.71 d	11.19 cd	102.61 c-e	10.47 d	132.51 c	15.10 e
Isolate-4	116.48 c	10.27 ef	107.56 cd	11.48 c	114.95 e	16.63 d
Isolate-1 + Auxin	137.06 b	13.94 a	133.64 a	11.31 cd	136.61 ab	18.73 c
Isolate-2 + Auxin	115.57 c	11.55 c	142.10 a	12.72 b	130.02 d	19.77 b
Isolate-3 + Auxin	133.37 b	12.61 b	118.91 b	12.43 b	137.19 a	16.03 d
Isolate-4 + Auxin	141.90 a	11.66 c	112.07 bc	14.60 a	135.57 b	21.39 a

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test *($p<0.05$)*

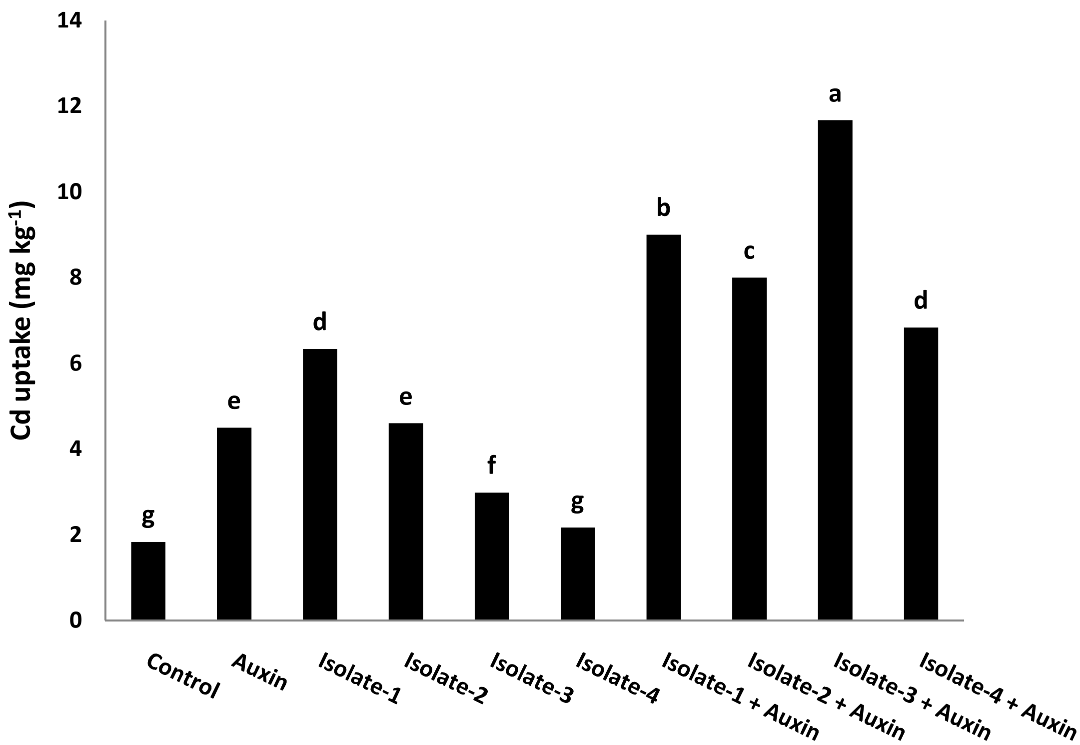

Fig. 1: Synergistic effect of auxin and bacteria on phytoextraction of cadmium by spinach. Bars sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test *($p<0.05$)*

DISCUSSION

Contamination of soils with toxic metals is the most important environmental concern and risk to human health. Higher levels of metals not only lower soil microbial activity and crop production but also threaten human health through accumulation in the food chain (Jiwan and Singh, 2011). In this study, Cd tolerant bacteria were tested alone and in combination with auxin to monitor plant growth and uptake of Cd from contaminated soils. Our results revealed that combined application of bacterial isolates and auxin significantly improved the fresh and dry biomass of spinach over control under Cd stress. This improvement in plant growth might be due to multiple mechanisms, which are adapted by bacteria in response to metal contamination, such as siderophore production, IAA production and phosphate solubilization (Rajkumar et al., 2006). Our results are supported by the work of Hoflich (2000) where he used *Rhizobium leguminosarumbv. trifolii* (known to produce auxin and cytokinin) as an inoculant in wheat and maize under greenhouse condition and found that inoculated plants had significantly more root and shoot growth over un-inoculated control. Moreover, auxin has been reported as a best marker for bacterial efficiency particularly under stress conditions (Boiero et al., 2006) and cytokinin as signal compounds which are involved in amelioration of environmental stresses from root to shoots (Jackson, 1993). Arif et al. (2001) reported that application of 1AA at 10^{-3} M significantly increased the biological yield of spinach. Likewise, Zahir et al. (2000) reported 50% increase in fresh biomass of soyabean by the application of IAA over control. The results of pot experiment were also supported by the findings of Sheng et al. (2008) where they recorded significant increase in biomass of inoculated plant over un-inoculated control under Cd contamination. Similarly, Belimov et al. (2005) also reported remarkable increase in shoot growth of Indian mustard in response to microbial inoculation compared with un-inoculated control in Cd contaminated soil. Several studies showed that accumulation of metals in plant tissue can be enhanced through bacterial inoculation (Chen et al., 2005; Sheng and Xia, 2006). In our experiment, an increase in Cd contents of above ground parts of spinach was observed in response to alone as well as combined application of bacterial isolates and auxin over control under Cd contamination. This might be due to increased bioavailability of Cd in rhizosphere because of organic acid production by the bacterial isolates (Cieslinski et al., 1998). Our results were also resembled to the findings of He et al. (2009) where they recorded 58–104% increase in Cd uptake in the inoculated tomato plants compared to control. Results regarding pot experiment revealed that the combined application of plant growth promoting bacteria and auxin efficiently enhanced the Cd concentration in above ground tissues of spinach besides improving plant growth in Cd contamination. Different researchers have reported the positive role of auxin in metals uptake by plants (Liu et al., 2007; Tassi et al., 2008) which strongly support our finding.

CONCLUSION

From the study it was concluded that synergistic use of plant growth promoting bacteria and plant growth regulators i.e. auxin could be an effective approach to handle metal contaminated soils. However, the exact mechanism of plant growth promotion and phytoextraction of metal need to be further explored.

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