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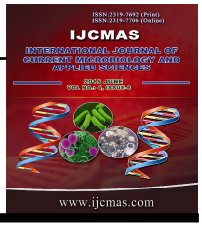

Original Research Article

Efficiency of an Cd-Tolerant Actinomycete Isolate Obtained From Wastewater in Removal of Heavy Metals and Enhancing Plant growth of *Zea mays*, L. Plant

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Keywords

Actinomycetes, Wastewater, Biological treatment, Heavy metals removal

A B S T R A C T

Cadmium, a potent environmental contaminant, is toxic to plants and the microbial community and disrupts many ecological and environmental processes. The aim of the current study was to use actinomycetes, isolated from contaminated places to enhance plant growth and remove the harmful effect of cadmium. For this purpose, the actinomycete isolate HM1 isolated from the influent of Wastewater Treatment Plant, Jeddah was the most resistant isolate for Cd, and thus it was selected, characterized by morphological and physiological characteristics and identified as *Streptomyces* sp HM1. The impact of Cd (10, 20, 40 and 60 ppm) and *Streptomyces* sp HM1 on some physiological traits of the salt sensitive cultivar Giza 122 of *Zea mays*, L. plants grown for 10 weeks in the greenhouse were determined. Irrigating plants with heavy metal containing water decreased root and shoot growth (length, fresh and dry masses), no of leaves, N, P, K and Mg concentrations, but increased Cd concentration in both shoots and roots. Raising HM concentrations decreased chlorophyll (Chl) content of leaves and soluble proteins of shoot but increased total-soluble sugars and proline concentrations. Applying *Streptomyces* sp HM1 to the experimental soil, influenced the most test characters by increasing the heavy metal tolerance of the plant. However, the number of these microorganisms was reduced under the increasing of heavy metal concentrations. In most cases, the interactive effects of heavy metal heavy metal and inoculum seemed to be insignificant. It was concluded that applying *Streptomyces spp*. HM1 to the soil slightly improved the heavy metal tolerance of the tested plant. Therefore, the test cultivar of maize is a promising to be cultivated in heavy metal contaminated soils even in the presence of actinomycetes.

Introduction

Agricultural soils or water contamination with heavy metal by disposal of sewage sludge or atmospheric deposition cause excessive accumulation in the top soil and a risk of leaching metals into the groundwater (Kabata-Pendias and Pendias, 1992). Metal concentrations in soil range from less than 1 mg/kg to high as 100,000 mg/kg, whether due to the geological origin of the soil or as a result of human activity (Blaylock and Huang, 2000). Excess concentrations of some heavy metals in soils such as Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2} and Zn^{+2} have caused the disruption of natural aquatic and terrestrial ecosystems (Gardea-Torresdey*et al*., 1996; Meagher, 2000). Although some metals are immobile and persistent, other metals are mobile and therefore, the potential of transfer either through the soil to groundwater or via plant-root uptake (bioavailability). Cadmium has no known beneficial effects and may become toxic to plants and animals if their concentrations exceed certain values (Adriano, 1986) while Ni, Cu, and Zn are three essential micronutrients for plant nutrition. The physiology and biochemistry of the toxic effects of Cd in plants are likely to be similar to those reported for other heavy metals. Nowadays, cleanup processes of heavy metal pollution are expensive and environmentally destructive (Meagher, 2000). Recently, scientists and engineers have started to generate cost-effective technologies that include the use of microorganisms, biomass, and live plants in the cleaning process of polluted areas (Wasay *et al*., 1998). Moreover, some plants species can tolerate greater than usual amounts of heavy metals or other toxic compounds (Raskin and Ensley, 2000). In a few studies, the seeds have been exposed to the contaminants (Claire *et al*., 1991; Xiong, 1998). The ability of *Zea mays* to germinate and grow in presence of Cd^{+2} was studied in this research. Removal of the adverse effects of Cd by soil inculcation with Cd resistant *Streptomyces* was also studied.

Materials and Methods

Bacterial isolation: The present study was carried out to isolate and identify HM resistant bacteria from wastewater sample collected from Wastewater Treatment Plant (WWTP), at BaniMalek region, western region, Jeddah, Saudi Arabia. Two waste water samples of l00 ml each were collected randomly from the main effluent in sterile glass bottles. The samples were diluted and used for actinomycete isolation on starch nitrate medium (Shirling and Gottlieb, 1966), contained 20 ppm of Cd $Cd(NO_3)_2 4H_2O$. All plates were incubated for 7 days at 30°C and all the obtained actinomycete isolates were purified, grown in different conc. of Cd (0-40 ppm) and the grown and mycelia color were determined. The most resistant isolate HM1 was selected for more detail studies. The isolate HM1 was grown in liquid production broth medium containing 2 mg/ml L-tryptophan with different concentration of Cd (0-40 ppm) and growth (optical density at 550 nm) and IAA was determined. The effect culture supernatant on the *Zea mays*, L.grains germination % was determined as described by Mahmoud *et al*. (2004).

Extraction and detection of IAA: The isolate HM1 was grown production medium (Aly, 1997) supplemented with 2 mg/ml L-tryptophan at a pH of 7.0. The supernatants were filtered using Milipore filter (0.45 mm) and the IAA in the cell free filtrate was extracted from the supernatants with ethyl acetate according to the method described by Ahmad *et al*. (2005). Ethyl acetate extract was applied to TLC plates (Silica gel, thickness 0.25 mm, Merck, Germany)

and developed in butanone/ethyl acetate/ethanol/ water (3:5:1:1v/v/v/v). Spots with Rf. values identical to authentic IAA were identified under UV light (254 nm) by spraying the plates with Ehmann's reagent (Ehmann 1977). Quantification of IAA was determined according to the method of Bano and Musarrat (2003).

Identification of the bacterial isolate: The isolate HM1 was characterized through a number of microbiological, physiological and biochemical tests (spore morphology, the color of aerial and spore masses, diffusible pigment production and carbon sources utilization) as described in Aly *et al.* (2011).

The Effect of HMOn Grain Germination of *Zea mays*, L.: The supernatant of HM1 was filtered (Milipore filter, 0.45 mm) and the cell free filtrate was used to determine IAA concentration. *Zea mays* L. cv. Giza 122 seeds were surface sterilized by soaking in a 10% sodium hypochlorite (NaOCl) for 5 min, followed by rinsing in sterile distilled-water. The surface-sterilized seeds were separately soaked in the culture filtrate or sterile distilled water incubated in the dark until the seedlings emerged (10 days) and germination percentage (%) and index were determined as described by Dhamangaonkar and Pragati (2009).

Germination Index = Sum of germinated grains for a certain period/Total days × Total grains.

Preparation of inoculums: *Streptomyces spp*. HM1 was grown on starch nitrate agar and the bacterial cells were scraped from seven-day-old culture into sterile saline solution to give a suspension containing $2xl0^6$ cells/ml.

Plant growth studies and analysis: A pot experiments were conducted under greenhouse conditions for a 10-week period with *Zea mays*, L. grown in sandy soil. This experiment was carried out during summer 2014. The sterile seeds were germinated in the dark and one week-old seedlings were transferred to each pot containing 2kg of steam sterilized sandy soil. The pots were kept in a glasshouse with a temperature range of 20-22°C. Two groups of pots were established: the first one remained without any inoculation (control), the second was inoculated with *Streptomyces* sp HM1. When plants were grown for certain length, 15 ml of the bacterial suspension ($2x10^6$ CFU/ml) was used to inoculate each pot. For control, only water was added. The plants were irrigated by Hoagland nutrient solution (Hoagland and Arnon, 1950), with different conc. of Cd. The heavy metals of Cd^{+2}, (as Cd $(NO_3)_2 4H_2O$ was used at the concentrations of 0.0, 10, 20 and 40 ppm in this research.

The nutrient solution had the following composition, in mM: KH2PO4, 1.0; KNO3, 5; Ca(NO3)2, 5; MgSO4, 2; Fe- EDTA, 0.1; H3BO3, 0.005; $MnCl_2$, 0.010; $ZnSO_4$, 0.008; CuSO4,0.004; $(NH_4)Mo_7O_{24}$, 0.0002. After an initial growth period of 15 days, the most vigorous seedlings were selected and Cd was added as $Cd(NO_3)_2 4H_2O$ to the nutrient solution at different concentrations and each pot received only 200 ml two times/week and the plants were irrigated with distilled water when needed. 200 ml/week of sterile dist. water was used to wash each pot. After 10 weeks, the plants were harvested and analyzed. The root depth, shoot length and no of leaves were determined. The shoot and root systems were separated and oven-dried for 10 d at 65°C and dry weights for each sample were obtained.

Total accumulation rate of Cd (TAR) in mg $plant^{-1}$ d^{-1} was determined following Zhu *et al*. (1999), and Cd uptake (mg $plant^{-1}$ d^{-1})

following Sharma and Agrawal, (2006). TAR and total Cd uptake were calculated using the following formulas:

$$TAR = \frac{\text{root Cd content} + \text{shoot Cd content}}{(\text{total dry matter} \times T)} \quad (1)$$

$$Uptake = \frac{\text{total Cd content}}{T} \quad (2)$$

T: time of the experimental period

Photosynthetic Pigments: Chlorophyll a, chlorophyll b and carotenoids of *Zea mays*, L. leaves were determined spectrophotometrically as the method described by Arnon (1949) and Metzner et al. (1965). An 85% aqueous acetone extract of a known F.W. of leaf was assayed Spectrometrically (*LKB NOVASPEC*) at 664, 645, 420 nm. The following equations were used to determine the concentration of the pigment fractions as γ/ml.

Chlorophyll a = 10.3 E664 – 0.918 E645……..…...µg / ml. (3)
Chlorophyll b = 19.7 E645 – 3.870 E664……...……..µg / ml(4)
Carotenoids = 403 E452- (0.0264 Chl. a + 0.426 Chl.b)…….µg / ml(5)

The pigment fractions were calculated as µg Chl./mg D.W.

Soluble Sugars Contents: Soluble sugars were determined using the antherone-sulphoric acid method described by (Fales, 1951; Schlegel, 1956 and adapted by Badour, 1959).

Proteins Contents: Dry samples collected during the growth study were analyzed for protein content, after precipitating the protein with 15% TCA at 4^{o}C according to Lowry et al. (1951).

Proline contents: proline contents of the control and treated plants were determined. This was estimated using the acid ninhydrin method described by Bates et al. (1973). Two ml of water extract were mixed 10 ml of 3% aqueous sulfosalicylic acid. Two ml of this mixture was allowed to react with 2 ml acid ninhydrin-reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100oC; the reaction was terminated by cooling the mixture in an ice bath. The reaction mixture was extracted with 4 ml toluene, and mixed vigorously for 15-20s. The chromatophore - containing toluene was aspirated from the aqueous phase, warmed to room temperature, and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve.

Phosphorus, N, K and Mg Concentration were estimated after acid digestion according to methods described by Allen *et al*. (1974) using Shimadzu Atomic Absorption Flame Spectrophotometer (Model *AA-640-12*). Cd content in the plant tissues was determined and any symptoms of metal or salt toxicity exhibited by the plants were visually noted during the whole experimental period. Cd concentrations in the roots and shoots were determined by atomic absorption spectrophotometry (Perkin-Elmer Analyst 400) after a 0.1 g wet digestion of dried material in 5 mL of a strong acid solution of $HNO_3/HClO_4$, 3:1, v/v (Van Assche and Clijsters, 1990).

Statistical analysis: Data of the shoot and root recorded was statistically analyzed by *F* test *ANOVA* and the means were compared using Duncan's multiple range ($P<0.05$). Where relevant, the experimental data was subjected to analysis of variance. Percentage values were transformed into arcsines according to Bliss (1973) and

analysis of variance was carried out according to Snedecor and Cochran (1967).t-Test to determine whether the differences between control and treated samples were significant or not at $P< 0.05$.

Results and Discussion

From TWWP, five actinomyceteisolates grow in starch nitrate agar and were resistant to Cd (20 ppm). Only one isolates HM1was resistant to Cd up 40 ppm. It has gray color which changed to dark gray or black in the presence of Cd (Table 1). In production medium with 2 mg/ml L-tryptophan and in the presence of Cd (0-40 ppm), growth and IAA production decreased by increasing concentration of Heavy metal. The decrease was significantly found at 20 and 40 ppm of Cd in both growth and IAA production. The isolate HM1 was selected for more details studies. It was Gram positive, has substrate mycelia and aerial hyphae, on starch nitrate agar it has gray color and produced chain of conidia with smooth surface. Some cultural, morphological, physiological and biochemical characters were represented in Table (2&3). It was identified as a species belonging to genus *Streptomyces* and identified as *Streptomyces* sp HM1.*Zea mays* seeds were soaked in *Streptomyces* culture filtrate, obtained in the presence of different concentration of Cd and percentage of spore germination was determined (Table 1). Germination percentage of control seeds was 92% and increased to 98 in the presence of bacterial filtrate. It is clear that increasing Cd concentration decreased the germination percentage up to 35% at 40 ppm while presence of *Streptomyces* filtrate increased it to 65%. Germination Index was decreased by increasing the heavy metal concentrations.

Zea mays, L. grainswere grown for 10 weeks in sandy soil and irrigated with different conc. of Cd. Plants were treated with Cd alone or Cd^{+}*Streptomyces* (ST) and the experimental data suggest that increasing Cd uptake by ***plants decreased growth*** and biomass in addition to numbers of leaves while in the case of soil inoculation with *Streptomyces* there was not a clear effect on Cd accumulation in plant tissues whichdeveloped no visible signs of metal toxicity (Table 4).

Table (4): Effect of different concentrations of Cd on root depth (Cm), shoot length (Cm) and root and shoot dry weight (g/plant) of *Zea mays* plants grown in sterile soil and inoculation or un-inoculated with *Streptomyces spp*. HM1

The K and Mg levels of the shoots decreased significantly as Cd exposure increased while the effect of Cd of concentration on phosphorous and nitrogen content of the plants was not significant (Table 5). Inoculation of the soil with *Streptomyces* may remove some of the negative effects of Cd on the K, Mg, N and P contents of the tested pant especially at 40 ppm of Cd.

Cadmium concentration affects plant growth and Cd levels of both roots and shoots were increased as Cd exposure of the plant increased (Table 5). Significant increases in Cd concentrations were seen in shoots and roots at the highest Cd exposures in relation to the control. The Cd contents were always higher in roots than in shoots, regardless of the used Cd solution concentration, and roots showed marked increases as Cd exposure increased. Similarly, total accumulation rates of Cd (TAR) was calculated as (mg/plant/ day) increased as Cd exposure was superior (Table 5). As expected, Cd uptake (mg/plant/ day) was higher in the presence of Cd than in controls, and increased as Cd concentrations in the nutrient solution increased.

Chlorophyll (a+b) content of leaves, protein content of shoot were negatively affected by the Cd metal while there was even an increase in the amount of carotenoids pigments in plants treated with heavy metal Cd (Table 6).

Shoot proteins seems to have a general tendency for decrease in plants treated with the metal in comparison with the respective controls and a statistically significant difference exists in plants treated with the metal specially at 40 ppm and control plants. The difference in proteins content was no significant in *Streptomyces* inoculated plants compared to control (Table 6). Soluble sugars and proline contents in shoot system were significantly increased by heavy metal or *Streptomyces* inoculation compared to control (Table 6).

The data revealed that different resistant actinobacteria were obtained from wastewater samples on agar medium containing heavy metal. Addition of heavy metal to growth medium inhibits growth of unwanted microbes and allows resistant actinobacteria to dominate (Aly and El-Sabagh, 2004, Velho-Pereira and Kamat, 2011). According to the tested characters, the isolate HM1 was belonging to genus *Streptomyces* and identified as *Streptomyces* sp. HM1 (Aly*et al*., 2011, 2013).

Similarly, Ghorbani-Nasrabadi *et al*. (2012) used modified agar to isolate uncommon and resistant actinomycetes. Our results showed that presence of heavy metal decreased bacterial growth and affect bacterial pigment and IAA production. Belimov *et al*. (2005) isolated eleven bacterial strains capable of producing indole acetic acid and/or siderophores from sewage sludge, mining waste highly contaminated with Cd and Cd-supplemented soils from the rhizosphere region of Indian mustard (*Brassica juncea* L. Czern.) seedlings. Production of IAA by bacteria was confirmed by Edi Husen (2003). Similarly, the previous cadmium-tolerant bacterial strains showed increased tolerance to other metals including Zn, Cu, Ni and Co (Belimov *et al*., 2005). Out of the isolated bacteria, *Azotobacter vinelandii* MM1 and *Streptomyces* sp. MM1017 showed positive results for IAA production and soaking wheat seeds in both culture filtrates increased significantly wheat germination (Aly *et al*., 2012).

Cadmium is considered as important potent environmental contaminant and the potential effect of Cd on maize growth was determined in presence and absence of *Streptomyces*. Various plant criteria are affected because of Cd toxicity to the plant and microbial community. Increasing concentrations of Cd as heavy metal (HM), increased the inhibitory effect on seed germination and almost of the measured parameters were reduced by the increased concentrations. Such growth retardation may due to metals toxicity that resulted in damages to various physiological and biochemical processes. Reduced seed germination is observed in corn treated with 20, 50, 100 and 200 µg/ml lead acetate (Kalimuthu and Siva, 1990, Hussain *et al*., 2013)

Cadmium accumulation in plant tissues was kept generally at low levels compared to that found by Gomes *et al*, (2012) who reported that the Cd-tolerance *Pfaffiaglomerata* were exposed to nutrient solutions with increasing Cd concentrations (0, 15, 45, and 90 µmol Cd/l) and found that this species is effectively a Cd hyper accumulator. At the highest Cd concentrations, bad effects of Cd on *Zea mays* growth were observed and roots contained higher Cd contents than shoots as they came into direct contact with Cd in nutrition solution, therefore roots were

subject to the toxic effects of that heavy metal. Mazhoudi *et al.* (1997) reported that roots have a defensive role in protecting above ground plant organs from heavy metal exposure. Similarly, the effects of Lead Nitrate ($Pb(NO_3)_2$) as heavy metal on germination, early growth seedling, root-shoot length, root-shoot fresh and dry weights, total protein content and the uptake of lead by roots and shoots of *Zea mays* were investigated by Hussein *et al*. (2013). They reported that all of the parameters were reduced by the increased lead concentrations. Such growth retardation was due to metals toxicity that resulted in damages to various physiological and biochemical processes. On contrast, although Cd and Pd usually decrease the chlorophyll content and biomass and change water relations in *Atriplexhalimus, which* is halophyte promising plant for remediation of heavy metal contaminated sites under saline condition and can be potentially viewed as an alternative method for soil desalination (Manousaki and Kalogerakis, 2009).

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Visual symptoms like growth and biomass reductions and morphological alterations in roots are commonly seen in plants growing under toxic heavy metal conditions, and occur even in tolerant or accumulator species (Gomes *et al*., 2012). Cd induced decreases in water potential, chlorophyll a biosynthesis and size and number of xylem vessels (Wójcik*et al*., 2005) and caused alterations in plant hormonal balances (Skrebsky *et al*., 2008). Higher Cd levels (200 mg/g of dry shoots) were found in the shoots of *Pfaffiaglomerata* which make this species as a Cd-hyperaccumulator (Carneiro *et al*., 2002).

According to our results, it was noticeable that as Cd exposure increased Cd-uptake increased which led to an increase in TAR of plants which reduced the biomass production. Borah and Devi (2012) reported that higher heavy metals concentrations are toxic to development, growth and productivity of *Pisum sativum* and chlorophyll and proline content also showed a gradual change. Differential Cd tolerance levels observed among several plant species have been attributed to genetic or physiological features, such as the presence of blockers in roots that resulted in Cd allocation to apoplastic spaces (Marchial *et al*., 1996). Plants are able to increase their antioxidant enzyme activities in order to reduce oxidative stress, thus increasing their Cd tolerance (Singh *et al*., 2006).

Some heavy metal tolerant bacteria offer promise as inoculants to improve growth of the metal accumulating plant *Brassica juncea* in the presence of toxic Cd concentrations and for the development of plant inoculant systems useful for phytoremediation of polluted soils (Belimov *et al*., 2005). Our results indicated that inoculation of soil with *Streptomyces* enhanced plant growth even in the presence of heavy metals and decreased Cd accumulation by plants. Similarly, Aly *et al*. (2001, 2003) isolated *Streptomyces* which enhanced plant growth under saline conditions.

The results of Belimov *et al*. (2005) suggested that *Variovorax paradoxus*, particularly strain 5C-2, offers promise as a bacterial inoculant for improvement of root growth of *Brassica juncea* plants in the presence of toxic metal concentrations. Moreover, soil inoculations with the bacterial cells of *Azotobacter vinelandii* MM1 and/or *Streptomyces* sp. MM1017 increased significantly seed germination, growth and development of wheat in normal and saline conditions (Aly *et al*., 2012).

Table.1 Color, growth and IAA production by the Streptomyces isolate HM1 and *Zea mays*, L. seed germination under different concentration of cadmium

Cd content (ppm)	Solid starch nitrate medium		Production medium		% Seeds germination of *Zea mays* in the presence of Cd	
	Color	Growth	Growth (A_{550nm})	IAA	without culture filtrate	with culture filtrate
0 (Control)	Pale gray	+++	1.44	4.9±0.55	93.4	98.0
10	Dark gray	++	1. 35	7.9±0.25*	87.3	90.1
20	Dark gray	++	0.611*	1.9±0.11*	49.3	73.1
40	Black	+	0.32*	0.59±0.15*	33.4	65.2

*: significant results at $p<0.05$

Table.2 Morphological character of the selected isolate HM1

Tested character	Results
Gram stain	Gram positive
Source of isolation	wastewater
Motility of spore	Absent
Shape of spore	Cylindrical (5-7 and, 6-9 µm)
Spore chain	Spiral chain
Spore Surface	Hairy
Number of spore/ chain	5-20
Aerial hyphae	Well developed
Substrate mycelium	Well developed
Zoospore, Sporangium, Sclerichia, Fragmented mycelium	Absent

Table.3 Physiological characteristics of the isolate HM1

Character	Reaction	Character	Reaction
Melanin pigment on Tyrosine agar	-ve	Tolerance to 10% NaCl	+
Enzyme activities:		pH range	6-9
Proteolysis	+ve	Growth temperature:	10 - 50ºC
Lecithinase	-ve	Resistance to antibiotic	
Lipolysis	+ve	Penicillin	-
Hydrolysis activities:		Cephalosporine	-
Chitin	+ve	Kanamycin	-
Gelatin	+ve	Rifampin	+
Pectin	+ve	Streptomtcin	+
H_2S Production	-ve	Resistance to 0.01 $CuSO_4$	+

-ve: negative results, +ve: positive results, : - Sensitive, +: Resistance

Table.4 Effect of different concentrations of Cd on root depth (Cm), shoot length (Cm) and root and shoot dry weight (g/plant) of *Zea mays* plants grown in sterile soil and inoculation or un-inoculated with *Streptomyces spp*. HM1

Treatments	ppm	CFU/g $X10^8$	Root			Shoot			
			Depth cm	Fresh weight g/plant	Dry weight g/plant	Length cm	Fresh weight g/plant	Dry weight g/plant	No. leaves No./plant
Cd	0	ND	14.0	4.4	2.3	78.3	7.2	4.2	14
	10	ND	12.8*	3.3*	2.0*	70.0	6.4	3.4*	14
	20	ND	8.1*	2.8*	1.0*	61.0*	5.6*	1.5*	10*
	40	ND	6.6*	1.1*	0.9*	24.4*	4.9*	1.0*	10*
Cd+ST	0	4.11	17.3*	5.2*	3.3*	84.2*	10.8*	6.6*	14
	10	3.88	14.4	3.9*	2.9	79.3	11.8*	7.2*	14
	20	1.48	11.9*	3.5*	2.0	50.3*	11.6*	3.1	12
	40	0. 90	9.57*	2.3*	1.7*	36.6*	8.9	2.3*	12

ND: Not detected, Cd: Plants treated with Cd, Cd+ST: Plants treated with Cd in soil previously inoculated with Streptomyces, * significant results at $p < 0.05$

Table.5 Effect of different concentrations of Cd on shoot content of minerals, shoot and root contained of Cd and Cd uptake by *Zea mays* plants grown in sterile soil and inoculation or un-inoculated with *Streptomyces spp*. HM1

Treatments	**ppm**	**Shoot content of metal ions (mg/g DW)**					**Root Cd mg/g DW**	**TAR (mg/plant/ day)**	**Cd uptake (mg/plant/ day)**
		K^+	**Mg^{++}**	**N^+ mg/g**	**P^{++} mg/g**	**Cd**			
Cd	0	14.9	5.11	15.62	10.2	0.01	0.13	0.33	1.80
	10	17.4*	4.6*	18.2*	14	8.8*	40.0*	0.64	4.01
	20	11.1*	3.66*	20.2*	10	20*	63.4*	1.25	5.32
	40	11.8	3.2*	20.6*	10.4	33*	80.4*	2.43	5.82
Cd+ ST	0	14.4	6.61	16.9	14.3	0.03	0.13	0.39	1.27
	10	14.8	5.8	18	12.1	4.4*	7.90*	0.32	2.22
	20	11.9*	4.5*	16.9	11.6	8.0*	22.0*	0.64	3.51
	40	11.9*	4.4*	16.4	10.8	14*	30.1*	1.42	4.56

TAR: Total accumulation rate, Cd: Plants treated with Cd, Cd + ST: Plants treated with Cd in soil previously inoculated with Streptomyces, * significant results at $p < 0.05$

Table.6 Effect of different concentrations of Cd on chlorophyll continent of leaves and shoot protein, sugars and proline of *Zea mays* plants grown in sterile soil and inoculated or un-inoculated with *Streptomyces spp*. HM1

Treatments	level of Cd (ppm)	Shoot analysis (mg/g)				
		Chlorophyll (a+b)	Carotenoids	Proline	Soluble sugar	Protein
Cd	0.0 (Control)	9.18	0.77	0.390	63.0	22.19
	10	7.78*	0.80	0.50*	73.0*	21.91*
	20	4.99*	0.90	0.79*	70.3*	20.11*
	40	3.11*	0.99*	0.70*	80.9*	20.08*
Cd + ST	0	9.90	0.77	0.39	78.0*	24.01
	10	8.11	0.79	0.65*	104 *	23.15
	20	3.42*	0.88	0.66*	118*	23.48
	40	3.21*	0.90	0.90 *	130*	23.58

Cd: Plants treated with Cd, Cd+ST: Plants treated with Cd in soil previously inoculated with Streptomyces, * significant results at $p<0.05$

Also, the dark septateendophytes (DSE) *E. pisciphila* H93 successfully colonized and promoted the growth of maize roots and shoots under HM stress conditions by restricting the translocation of HM ions from roots to shoots and the mutual symbiosis between *E. pisciphila* and its host (maize) may be an efficient strategy to survive in the stressful environments (Li *et al*., 2011). Borah and Devi (2012) added that toxicity of heavy metals are on almost all the plants and everywhere but toxicity intensity may vary from plant to plant and consumption of contaminated plants as a food or a feed may cause negative impact on human health. In conclusion, our results demonstrated that *Zea mays* has great potential to grow under Cd stress and inoculation with Cd resistant Streptomyces increase the Cd-tolerance of this species which may be closely related to IAA production, anatomical and physiological features. Due to high Cd-uptake and accumulation in maize roots, roots of plants grown in Cd-contaminated areas must be strictly avoided from animal feed.

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