

Physiological exegesis of growth parameters in *Amranthus tricolor* inoculated with Cd resistant bacteria

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ABSTRACT

The aim of this study is to understand the ability of *Bucillus halotolerance* DSM 8802 (as Cd-resistant bacteria isolate) for enhancing cadmium (Cd) phytoextraction in *A. tricolor* and the interaction between them through growth traits (fresh weight FW, dry weight DW, length L for both root and shoot and leaf area LA) and Cd tolerance index (CdTI%) estimated for all measurement. Our results showed that *Bucillus halotolerance* improved plant growth of *A. tricolor* with rates (1.15 and 1.29 times) for root and shoot FW respectively, and (3.26 and 1.32 times) for root DW respectively at 30 mg/l Cd concentration. Shoot FW was significantly increased by (50 mg Cd compared to the same Cd concentrations only. Root and shoot lengths with inoculum were significantly enhanced by (1.49 and 1.14 times) respectively at a little Cd concentration, while LA was significantly uptrend to (1.23 times) at 50 mg/l Cd with inoculum from the same concentration of Cd without inoculum. Also, Accumulation of Cd in root and shoot inoculated was enhanced from (242.5 and 255.5 µg /gDW) to (304.75 and 266 µg /gDW) respectively at 50 mg/l Cd, also BCF was increased from (1.250 and 2.643) under only high Cd concentration to (1.644 and 2.747) for root and shoot of *A. tricolor* respectively. This study demonstrated that *A. tricolor* is a good accumulator model in phytoremediation, and *Bucillus halotolerance* DSM 8802 promoted growth and enhanced water relationships values, and improved physiology parameters of *A. tricolor* against Cd toxicity and increase removal, accumulation and phytoextraction Cd efficiency of *A. tricolor* and succeed in enhancing of phytoremediation combination with PGPB system.

Keywords: Cadmium; phytoremediation; *Bucillus halotolerance*; PGPR and *A. tricolor*.

INTRODUCTION

By directly ingesting contaminated food, coming into physical contact with polluted soil, passing through the food chain (soil-plant-human or soil-plant-animal-human), drinking polluted water, lowering food quality, and decreasing the amount of land suitable for farming, metal contamination damages ecosystems and harms human health, leading to food insecurity (Hussain *et al.*, 2021a). Cd is a heavy metal that is known to be extremely hazardous to people and other living things. It also exhibits biological activity in both terrestrial and aquatic species (Chellaiah, 2018). A higher level of Cd has been found in agricultural soils as a result of advancement in both agriculture and industry (Bojorquez *et al.*, 2016). Due to its mobility and toxicity at relatively low quantities, Cd is the main cause for concern. The main human-caused sources of Cd in the environment are industrial and mining sources as well as those used in agriculture such as chemical fertilisers and pesticides (Manzoor *et al.*, 2019; Rizwan *et al.*, 2018). On the other hand, uncontaminated soils typically have Cd concentrations below 0.5 mg.kg⁻¹, however depending on the parent materials of the soil, this can increase to 3.0 mg/kg (Vahter *et al.*, 1991). Cd is a non-

essential element with no biological function. The World Health Organization (WHO) has set a maximum limit concentration of 0.003 mg.l⁻¹ for Cd in drinking water. Numerous studies have demonstrated that this metal may suppress root elongation, hinder root germination, limit the number of leaves per plant, and inhibit overall plant length, all of which may result in the death of the plant (Bae *et al.*, 2016). The ability to thrive in polluted soils by building morphological and physiological defence mechanisms is one way that plants in contaminated soils adapt to Cd stress (Mei *et al.*, 2018).

The impacts of Cd on plant growth indices included a drop in total leaf number and size, a reduction in shoot biomass, an inhibition of root extension, due to chlorosis and necrosis of leaves (Rai and Tripathi, 2009). After prolonged Cd exposure, the root develops necrotic, disintegrating, and mucilaginous characteristics that inhibit root extension (Abbas *et al.*, 2017). Almost all studies reveal that Cd has an inhibitory influence on plant growth in terms of fresh and dry mass accumulation, height, root length, LA, and other biometric parameters. Cd poisoning resulted in a considerable decrease in the total dry weights of the leaf, stem, and roots of

cabbage (*Brassica oleracea* L.) (Jinadasa *et al.*, 2016). Similar to this, Cd reduced the development and biomass of plant species that produce leaves, roots, and legumes, including lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.), and soybean (*Glycine max* L.) (Monteiro *et al.*, 2008; Wang *et al.*, 2016). The buildup of Cd ions in tissues may affect how well soil retains water, and it tends to reduce the water interactions in plants under Cd stress (Chen *et al.*, 2004). The unintentional absorption of Cd by plant contributes to a necessary unfavourable impact on plant health, including biomass reduction and photosynthetic efficiency (Drava *et al.*, 2012). Reduction of biomass as a result of the Cd toxicity effect can be regarded as phytotoxicity symptoms. The biomass of rice root was drastically reduced when grown in Cd contaminated soil (Yixia *et al.*, 2020). The biomass of canola and Indian mustard were also identified to be affected with more than 70% of reduction (Turan and Esringu, 2007). The same symptom was observed in the hydroponic system plantation of *Pistia stratiotes*. The plant has tolerated up to 20 mg/L of Cd but still resulted in plant biomass reduction with high Cd treatment (Das *et al.*, 2014). Inhibition of plant growth by Cd is the vital reason for plant biomass lessening.

A plant's heavy metal accumulation potential can also be determined via the calculation of the plant's bioconcentration factor (BCF) (Kötschau *et al.*, 2014), which is defined as the ratio of metal concentration in the aerial (shoot biomass) part of the plant (dry weight basis) to the total metal concentration in soil (Li *et al.*, 2017). A plant's BCF is a function of the properties of the metal itself, soil properties, plant genotype (Yang *et al.*, 2009), and plant growth stage (Li *et al.*, 2018). A BCF ≥ 1 indicates a high accumulation of an element in the plant shoot (Kötschau *et al.*, 2014). However, the BCF is not a constant value and may vary for the same element in soils with different chemical properties (Boim *et al.*, 2016). The bio-translocation factor/index (TF, metal concentration in leaves/stems to the content found in the roots), are considered the most important (Krzciuk, 2015).

The *Amaranthus* genus of plants has numerous species that are found around the world (Li *et al.*, 2012). It meets the requirements to be categorised as a Cd hyperaccumulator since it can accumulate more than 100 mg Cd kg⁻¹ in aboveground dry materials (Baker *et al.*, 2000). A few research has focused on the microbe-assisted

phytoextraction by *Amaranthus* species in soils contaminated with heavy metals, despite the fact that it has been demonstrated that heavy metal-tolerant bacteria can enhance the uptake of metal by plants (Chen *et al.*, 2013). However, due to the fact that the plants are often small and grow slowly, making them challenging to harvest mechanically, these hyperaccumulators may not be appropriate for many large-scale phytoextraction initiatives (Ning Yu Li, *et al.*, 2012). So, the objectives of this study are to understand ability of *Bucillus halotolerance* DSM 8802 (as Cd-resistant bacteria isolate) for enhancing Cd phytoextraction in *A. tricolor*, and the interaction between them through FW, DW, L of root and shoot and LA values of as growth traits *A. tricolor*, and calculate Cd tolerance index (CdTI%) for all according to growth parameters.

MATERIAL AND METHODES

Experimental design

The study was carried out in plant physiology lab., Agriculture Faculty, Al azhar University, Cairo, Egypt. *Amaranthus tricolor* seeds were surface-sterilized by soaking them in ethanol for 1 min, rinsed shortly in 0.1 % of HgCl₂ and then thoroughly washed then with distilled water several times according to the method described by El-Abyad *et al.*, (1993). Then seeds have germinated in hydroponic system with half strong Hoagland's solution according to (Hoagland and Arnon, 1953). After 25 days (ds) from germination, uniform in lengths, strong and healthy seedlings were chosen randomly in triplicate and maintained in agriculture plate to treat them with Cd (as CdCl₂ · H₂O) with a Cd solution freshly prepared by 134 dissolving CdCl₂ in deionized water (Turgut *et al.*, 2004). concentrations and bacterial inoculum. Eight different treatments were included in three sets of replications, namely (1) control absence of Cd and bacterial inoculum, (2) present of bacterial inoculum only and absence of Cd, (3) Cd with 30 mg/l concentration, (4) Cd with 40 mg/l concentration, (5) Cd with 50 mg/l concentration, (6) bacterial inoculum within 30 mg/l Cd concentration, (7) bacterial inoculum within 40 mg/l Cd concentration, (8) bacterial inoculum within 50 mg/l Cd concentration. Then, 2 mL of bacterial suspension of *B. halotolerance* was added per nutrient solution (6, 7 and 8 treatments) plate for 27 ds. the experiment of study contains two controls; negative control is a treatment that don't have any adding of bacterial inoculum or Cd,

positive control is containing Cd with any concentration. The nutrient solution is renewed weekly. After 27 ds from being exposed to Cd and bacterial inoculum, the seedlings were harvested and rinsed with distilled water to remove any trace of Cd on root of seedlings, then physiological and biochemical parameters have been measured. Then seedlings divided to root and shoot to determinate Cd metal accumulation in different tissues of plants.

Growth parameter measurements.

The growth parameters of plants seedling were recorded after 50 ds from Cd and bacterial inoculum treatments in all experiments for plats under study as following: fresh weight (FW) was recorded. Dry weight (DW) and leaf area (LA) were measured using petoil application of android software.

The tolerance index (TI) was calculated by the following formula (Wilkins, 1978):

Tolerance index (%) = growth in solution in treatment/ growth in solution in control x 100%

Bioconcentration factor (BCF): The BCF according to Baker *et al.*, (1981) by

$BCF (\text{mg biomass Cd mg}^{-1} \text{ soil Cd}) = \text{Cd in root or shoot} / \text{total Cd in medium}$

Bio translocation factor (BTF) was calculated according to Moradi and Ehsanzadeh, (2015) by equation $BTF = \text{Cd in shoot} / \text{Cd in root}$

Determination of Cd and macro-micro nutrients in seeds and different tissues seedling of plants:

After 50 days of germination under study conditions, 0.2g fresh weight of plant martials were taken and washed in distilled water, then wet digestion was applied on representative portions by using the modified method of (Kaiser *et.al*, 1972). The concentration of Cd in plant root, stem and leaf were measured by the following method used by Shakoor *et al.* (2014).

$\text{Cd concentration (mg/g. DW)} = \text{reading of AAS} \times \text{dilution factor} / \text{DW of plant organ}$. The accumulation of Cd in plant shoot and root was estimated by the following formula: $\text{Cd accumulation } \mu\text{g. plant}^{-1} = \text{conc. of Cd} \times \text{DW of plant organ}$

Statistical analysis

Data were analyzed using ANOVA of statistical package SPSS for Window (v 23.0; SPSS, IBM Corporation, Armonk, NY, USA) (IBM Corp., 2015) software, differences were compared using Duncan's multiple range test at 0.01 level.

RESULTS

Effect of Cd and *B. halotolerance* on growth parameters in *A. tricolor* seedlings: -

Shoot and root fresh weights of *A. tricolor* seedlings.

A. tricolor plant was used to estimate plant growth parameters after 50 days of the experiment. In general, the total biomass includes fresh weight (FW), dry weight (DW), length (L) for root and shoot and leaf area (LA) was significantly decreased progressively with increasing Cd concentrations in treated plant (Figure 1). At the same time, the growth of plants inoculated with *B. halotolerance* significantly increased as compared with control non-inoculated, and alleviated the injurious effects of Cd stress at the same concentrations used. Shoot and root F.W of *A. tricolor* was decrease gradually with increasing of Cd doses as compared with control (not any treatments) in fig. (1-A). *A. tricolor* shoot FW was significantly ($P \leq 0.01$) decreased by (0.74, 0.79 and 0.53 g/plant) and by root F.W by (0.337, 0.307 and 0.297 g/plant) under Cd concentrations (30, 40 and 50 mg/l) respectively as compared with control. The plants inoculated with *B. halotolerance* showed a one-fold-higher development of the plants compared to the un-inoculated control. *B. halotolerance* inoculation increased the growth of *A. tricolor* to a certain extent. In non-stressed and inoculated plants, shoot and root F.W of *A. tricolor* was increased by (3.71, 0.83 g/plant) compared to the un-inoculated control. In addition to the inoculation of *B. halotolerance* to plants stressed with Cd concentrations due to enhancing in shoot and root FW of *A. tricolor* was (0.96, 0.73 and 0.503 g/plant) and (0.38, 0.217 and 0.217 g/plant) for shoot and root F.W of *A. tricolor* respectively as compared with the non-inoculated plant and stressed with concentrations (30, 40 and 50) of Cd respectively.

Shoot and root dry weights of *A. tricolor* seedlings.

Effect of Cd and *B. halotolerance* inoculum on DW of shoot and root of *A. tricolor* after 50 ds from treatment was documented in (Fig. 1-B). In general, increasing of Cd concentrations caused linearly decreased in DW of shoot and

root of plant seedlings used as compared with control. For example, in *A. tricolor*, shoot DW was decreased by (0.204, 0.269, and 0.341 g/plant) and by root D.W by (0.019, 0.17 and 0.031 g/plant) under Cd concentrations (30, 40 and 50 mg/l) respectively as compared with non-inoculated and stressed plants. On another hand, inoculation with *B. halotolerance* w increased was DW of shoot and root of *A. tricolor* compared to control *A. tricolor* seedlings without Cd and inoculum. The plants with *B. halotolerance* displayed less decrease in DW due to the Cd stress and a gradual increase in DW than the plants without inoculum application under concentrations of Cd. Addition the inoculation of *B. halotolerance* to plant stressed with Cd concentrations due to enhancing in shoot and root DW for *A. tricolor* used by (0.271, 0.201 and 0.176 g/plant) and (0.062, 0.034 and 0.032 g/plant) respectively as compared with the non-inoculated plant and stressed with concentrations (30, 40 and 50) of Cd respectively.

Shoot and root lengths (cm²/plant) of *A. tricolor* seedlings.

Shoot and root lengths of *A. tricolor* under Cd concentrations and *B. halotolerance* inoculum after 50 ds showed in Fig. (1-C). Due to Cd stresses to decrease the lengths of root and shoot of plant used. The results showed significant ($p \leq 0.01$) reduction in the lengths of *A. tricolor* shoots by (21.0, 18.0 and 15.0 cm²/plant) in plant subjected to (30, 40 and 50 mg/l) Cd concentrations respectively, where root lengths of *A. tricolor* were decreased by (23.00, 23.0 and 20.33 cm²/plant) under at the same of Cd concentrations as compared with control non-inoculated. Inoculation with bacterial cells resulted in increased lengths of shoot and root of plants used both in untreated and Cd-treated plants. In the absence of Cd stress, inoculation with *B. halotolerance* inoculum significantly ($p \leq 0.01$) increased length of shoot by (45.0 and 40.50 cm²/plant) for *A. tricolor* compared to the control non-inoculum. In the presence of Cd, inoculation with bacteria reduced the negative effect of Cd on lengths of shoot and root. An increase in the length of shoot was observed after inoculation with the bacteria under Cd doses, with data (18.50 cm²/plant) for *A. tricolor* under high Cd concentration, where, increasing value of root lengths of *A. tricolor* under at the same Cd concentrations was (26.0 cm²/plant) in comparison with the un-inoculated plants.

leaf area (cm²/leaf) of *A. tricolor* seedlings.

LA was examined as one of the important parameters in monitoring plant growth and development under the experimental conditions studied. Effect of Cd and *B. halotolerance* inoculum on LA of *A. tricolor* was measured after 50ds in Fig. (1-D). The increasing of Cd treatments resulted in a significant linearly reduction in LA of *A. tricolor*. For example, the decreasing of LA was by (2.80, 2.46 and 1.46 cm²/leaf) at 30, 40 and 50 mg/l Cd respectively as compared with control any addition, while with PGPR inoculum treatments it is shown that LA of *A. tricolor* was increased compared to plants without PGPR both under normal and stressed plant conditions. *A. tricolor* LA was increased by (2.76, 2.17 and 1.81 cm²/leaf) at 30, 40 and 50 mg/l Cd respectively at the same Cd concentrations.

Effect of Cd concentrations and *B. halotolerance* on growth parameters CdTI (%) of *A. tricolor* seedlings.

CdTI according to growth parameters of *A. tricolor* was documented in Fig. (2). The results showed that decreasing of CdTI by Cd concentrations increase. For example, CdTI of FW root was (87.21%, 79.44% and 76.85%) under Cd doses (30, 40 and 50 mg/l) used respectively as compared with control non-inoculated, where CdTI of *A. tricolor* shoot was (31.0%, 33.46% and 22.20%) under the same Cd concentrations. Addition of *B. halotolerance* to plant stressed with Cd concentrations duo to increased CdTI by up (40.22%, 30.65% and 21.08%) for shoot and by up (98.44%, 56.13% and 65.13%) for root FW respectively of *A. tricolor*. as compared with the non-inoculated plant and stressed with concentrations (30, 40 and 50) of Cd respectively. Moreover, the results in Fig. (2) showed that CdTI of shoot DW of *A. tricolor* (26.34%, 34.79% and 44.07%) under 30, 40 and 50 mg/l Cd doses respectively as compared with control non-inoculated. Root DW CdTI was significantly decreased by (10.27%, 9.02% and 7.757%) of *A. tricolor* under the same Cd doses used respectively as compared with control non-inoculated. Addition of *B. halotolerance* to plant stressed with Cd concentrations due to increased CdTI of root DW of *A. tricolor* by up (33.514%, 18.378% and 17.29%) at the gradually Cd concentrations respectively as compared with the non-inoculated and seedlings stressed with concentrations (30, 40 and 50) of Cd respectively, where CdTI of DW shoot was increased at the little Cd concentration by (35.058%) as compared with the same only Cd

concentration. it doesn't show any increase at the moderate and high Cd concentration used as compared with the non-inoculated and seedlings stressed with concentrations (40 and 50) of Cd. CdTI of shoot and root lengths for *A. tricolor* plants was calculated in Fig. 2 to know the percentage of tolerance index for plants used according to shoot and root lengths parameter under all treatments. Inoculation with bacterial cells resulted in increased length of shoot and root of *A. tricolor* in untreated and Cd treated plants. Shoot length CdTI of *A. tricolor* was increased with little rate after inoculum addition and increased from (72.41%, 62.07% and 51.72%) under 30, 40 and 50 mg/l Cd respectively to up (82.76%, 67.24% and 63.79%) at the same Cd concentrations combination with bacterial inoculum used. Inoculation with *B. halotolerance* inoculum significantly ($p \leq 0.01$) increased in root length CdTI of *A. tricolor* from (66.89%, 66.89% and 59.14%) to up (99.85%, 77.56% and 75.62%) respectively as compared to the same Cd concentrations. Our results indicated that LA CdTI was decreased with Cd concentrations used increase (figure 2). For example, at the few Cd concentrations (30 - 50 mg/l) and without inoculation LA TI of *A. tricolor* was (75.56% - 39.26%) respectively. However, bacterial inoculum application was displayed less decrease in LA CdTI at the high Cd concentration by (48.70%) compared without inoculated and stressed.

Effect of Cd and *B. halotolerance* on Cd accumulation ($\mu\text{g/gDW}$) and BCF in root and shoot and BTI of *A. tricolor*

Fig. 3-E shows the Cd uptake in root and shoot under different concentrations of Cd in the presence and absence of inoculum bacterial used. The Cd concentrations in both root and shoot markedly augmented with increasing Cd treatment as comparison with control, also proves the actual uptake of Cd. The Cd concentrations in shoot and root of *A. tricolor* plants enhanced in response to increasing Cd levels, root and shoot of *A. tricolor* attaining to levels as high as 242.5 and 255.5 $\mu\text{g gDW}^{-1}$, respectively at 50mg/l Cd. Evidently, the root Cd concentrations were much lower than those of the shoot in comparison with the control. Effects of *B. halotolerance* inoculum on phytoextraction are shown. Differences were observed in Cd concentrations in inoculated plants compared to the control group. however, total Cd contents in inoculated plants were enhanced, *B. halotolerance* inoculum increased total Cd content by 319 $\mu\text{g gDW}^{-1}$ old in roots and 266 $\mu\text{g gDW}^{-1}$ fold in

shoots, respectively at 50 mg/l Cd concentration. BCF and BTI were calculated and showed in Fig. 3-F. The results revealed that BCF was much more in shoots than in roots. The highest values of BCF in root and shoot were (1.250 and 2.634) respectively at high Cd concentration. After bacterial inoculum application, BCF was increased in all Cd concentrations compared with only Cd doses. BCF was 1.644 and 2.742 for root and shoot respectively under bacteria inoculum and high Cd concentration combination as compared with the same only Cd concentration. Similarity, BTF of *A. tricolor* was increased under Cd doses as comparison with control (Fig. 6-K). The increasing of BTF was at average between 0.942 to 1.054 at 30 and 50 mg Cd/l as compared with control. Similarly, BTF value was increased 0.834 in the presence bacteria at 50 mg Cd/l concentration from absence of bacteria at the same Cd concentration.

DISCUSSION

Effect of Cd and *B. halotolerance* on growth parameters in *A. tricolor* seedlings:

Cd is not thought to be a necessary component for plants and its toxicity causes widespread irregularities in development and suppression in many plant species (Zhang *et al.*, 2019). After prolonged Cd exposure, the root becomes necrotic, decaying, and mucilaginous, which reduces the lengthening of plant roots and shoots and results in leaf rolling and chlorosis (Abbas *et al.*, 2017). Cd toxicity reduces meristematic cells' ability to divide mitotically, which results in shorter roots and more dry biomass while increasing root diameter (Seth *et al.*, 2008; Gratao *et al.*, 2015). Under Cd stress, a rise in root diameter can be caused by an increase in the size of cortical tissues and parenchymal cells, which contribute to increasing the resistance of plants to solute movement and water (Ismael *et al.*, 2018). Due to Cd poisoning, alterations in leaf phenology result in altered chloroplast ultrastructure with reduced chlorophyll levels, which leads to chlorosis and reduces photosynthetic activity (Miyadate *et al.*, 2011). Under severe Cd stress, decreased root length, surface area, and root tip number indicate lower resource storage potential (i.e., nutrition and water in the plant) (Lu *et al.*, 2013). There has also been a discernible decline in LA and DW of many plant components under Cd stress (Jinadasa *et al.*, 2016). Reduced nutrition and water absorption, respiration, photosynthesis, assimilation of nitrogen and

carbon, and antioxidant activity may all contribute to the stunted growth of plants under Cd stress (Rizwan *et al.*, 2017). The growth of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* L.), alfalfa (*Medicago sativa* L.), wheat, maize, spinach, and soybean in high Cd concentrations has been shown to be reduced (Younis *et al.*, 2016; Abbas *et al.*, 2017; Zhang *et al.*, 2019). Additionally, PGPRs boost FW and DW and deal with hazardous metals by reducing their negative effects on plants (Dary *et al.*, 2010). According to earlier studies (Pociniczak *et al.*, 2016; Mitra *et al.*, 2018; Julia *et al.*, 2020), inoculation with metal-resistant PGPR can lessen the toxicity of heavy metals and aid plant growth in contaminated media. Numerous heavy metal-resistant microorganisms have been found to have the ability to promote plant development in heavily Cd-contaminated environments. According to Moreira *et al.* (2016), maize (*Zea mays* L.) grown in mine soils and inoculated with five Cd-resistant PGPR (*Ralstonia eutropha*, *Chryseobacterium humi*, *Pseudomonas fluorescens*, *Rhizobium radiobacter*, and *Pseudomonas reactans*) resulted in an increase in plant biomass and height. These effects grew with Mutualistic associations created by PGPR, particularly free-living rizospheric bacteria, have a negative impact on plant ability to withstand a variety of environmental challenges (Mantelin and Touraine, 2004). Two Cd-resistant bacterial strains, *Pseudomonas sp.* RJ10 and *Bacillus sp.* RJ16 were found to boost *Brassica napus* biomass and stimulate IAA secretion, according to other investigations (Sheng and Xia, 2006). The activity of ACC deaminase in *Bacillus* was initially discovered by Ghosh *et al.*, (2003), ACC deaminase may cleave the ethylene precursor ACC into NH₃ and -ketobutyrate, decreasing ethylene synthesis in plants under abiotic stress (Bal *et al.*, 2013). Decreases in ethylene via PGPR-produced ACC deaminase lower the toxicity of heavy metals and boost plant tolerance since ethylene is hypothesized to contribute to H₂O₂ buildup and heavy metal-induced apoptosis (Etesami and Maheshwari, 2018). In addition, PGPR produces indole acetic acid (IAA), which enhances plant biomass under toxic metal stress circumstances and lengthens the plant's roots and shoots. Since PGPR uses IAA as its sole source of nitrogen, it promotes plant development and the creation of biomass (Amico *et al.*, 2008). According to Dary *et al.*, (2010), *Bradyrhizobium sp.* inoculation significantly improved the growth of *Lupinus luteus* plants under metal stress conditions as compared to uninoculated plants.

Overproduction of ethylene is a common phenomenon in plants under stress. Different plants depend on ethylene for their growth, but if it is present in large enough amounts, it can hinder plant growth (Grichko and Glick, 2001). A number of PGPR, including *Pseudomonas putida*, *Bacillus amyloliquefaciens*, *Azospirillum brasiliense*, and *Bacillus subtilis*, are crucial to plants' ability to withstand drought (Kumar, *et al.*, 2016). By absorbing 1-aminocyclopropane-1-carboxylate, PGPRs boost plant growth and total biomass output while controlling the amount of ethylene produced (Grichko and Glick 2001; Mayak *et al.*, 2004a). According to Baharlouei *et al.* (2011), Under heavy metal stress, PGPRs improved the root and shoot biomass in canola and barley, which boosted plant growth. Similar to this, *B. halotolerance* in *Brassica napus* plants encouraged the formation of root and shoot biomass when stressed (Belimove *et al.*, 2001).

Effect of Cd and *B. halotolerance* on Cd accumulation ($\mu\text{g/gDW}$) and BCF in root and shoot and BTI of *A. tricolor*: -

The most likely initial reaction of plants is metal buildup as a result of phytochelatin's ability to immobilize Cd in cells. With increased Cd treatments, however, the absorption and translocation of Cd by plants may decline and/or be blocked. This is consistent with what Shi *et al.*, (2010) and De Maria *et al.*, (2013) found in safflower and sunflower, respectively. The findings suggested that the rhizosphere bacteria were crucial to the plants' ability to absorb metal in their shoots. Similar findings were made by Gomes *et al.*, (2012) who reported that increasing Cd supply increased *Pfaffia glomerata*'s Cd absorption. Plants use their root tissue as a defensive mechanism to shield their above-ground components from too much Cd and to prevent Cd from moving to their shoots. There have been reports of diverse plant processes for Cd absorption and accumulation in various regions (Zhao *et al.*, 2002). Additionally, according to Moradi and Ehsanzadeh (2015), genotypes of safflower exposed to higher Cd concentrations showed an increase in net Cd accumulation via roots. *Pseudomonas putida* UW4-inoculated *Nicotiana tobacco* plants displayed an increase in growth and metal buildup from polluted soil (Li *et al.*, 2007). Similar to this, Belimov *et al.* (2005) found a link between increased cd accumulation in *Brassica juncea* tissues due to increased root growth and the bacteria's activity as ACC deaminase. The authors

proposed using bacteria with ACC deaminase to perform phytoremediation on soils with metal contamination. It was discovered that inoculating *Sedum alfredii* plants cultivated hydroponically or in soil with rhizobacterial strains from the genus *Burkholderia* increased metal tolerance, biomass production, and mostly Cd uptake and extraction (Li *et al.* 2007; Guo *et al.* 2010). Furthermore, we examined the effects of inoculating *Sedum plumbizincicola* plants with advantageous endophytic bacteria, as the process of phytoextraction by various plants depends on both plant biomass output and metal We also evaluated whether inoculating *Sedum plumbizincicola* plants with advantageous endophytic bacteria altered the absorption of metals (Ma *et al.*, 2011). (Cd, Zn and Pb). In comparison to non-inoculated controls, the ACC considerably boosted the overall absorption of Cd and Zn by *S. plumbizincicola* plants. In comparison to uninoculated controls, the best Cd mobilizer E2S2 inoculation considerably boosted the accumulation of Cd in *S. plumbizincicola* by 43%. Rhizobacteria are a viable alternative for microbial assisted phytoremediation because of their significance in plant heavy metals tolerance and their capacity to encourage growth in metal contaminated soils (Rajkumar *et al.*, 2008). We also evaluated whether bacteria that produce ACC deaminase, siderophores, hydrocyanide (HCN), IAA, and nutrient solubilization (for example, phosphate solubilization) can promote plant growth and shield plants from metal toxicity (Ma *et al.*, 2011; Glick, 2010). Sheng and Xia, (2006) demonstrated how inoculating *Brassica napus* with microorganisms improved Cd uptake.

CONCLUSIONS

This study showed that *A. tricolour* is an effective accumulator model for phytoremediation, and *Bucillus halotolerance* DSM 8802 Optimized has the ability to increase the study's parameters: growth, water relationships, and physiology of *A. tricolour* against Cd toxicity, and increase removal, accumulation, and phytoextraction Cd efficiency of *A. tricolour*. This study also succeeded in enhancing phytoremediation combination with PGPB system. We have demonstrated a about 50% increase in *Busillus halotolerance* DSM 8802 quantity and biomass when co-planting. Coplanting in induced phytoextraction is therefore extremely important for application.

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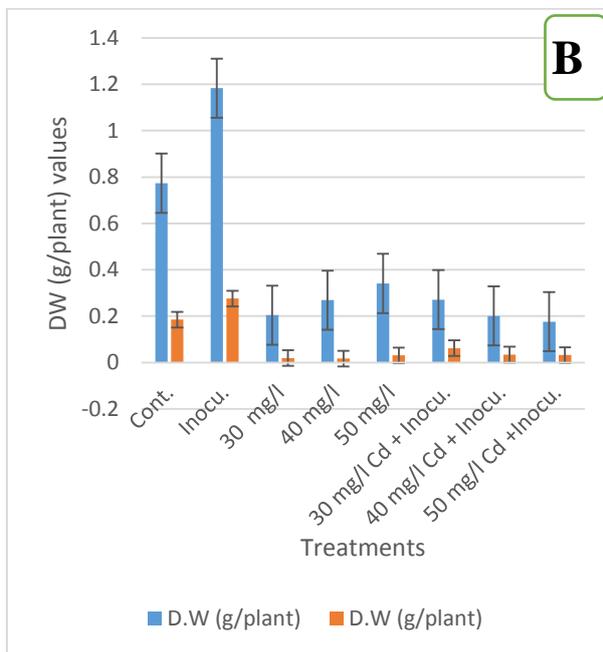
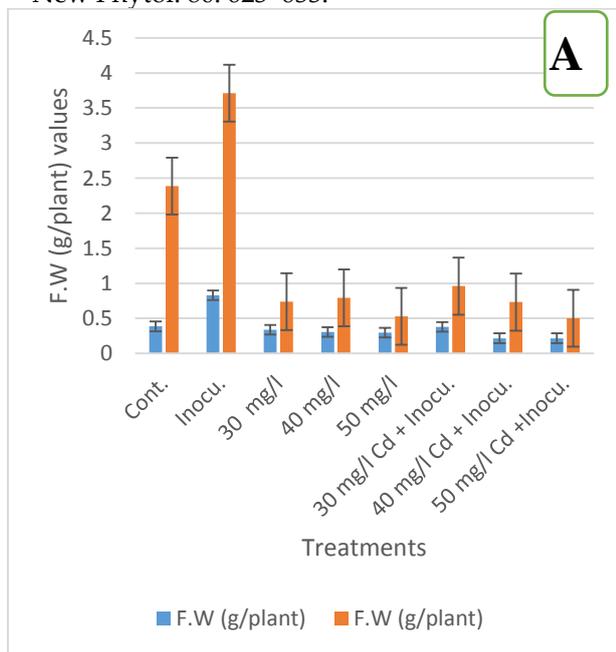
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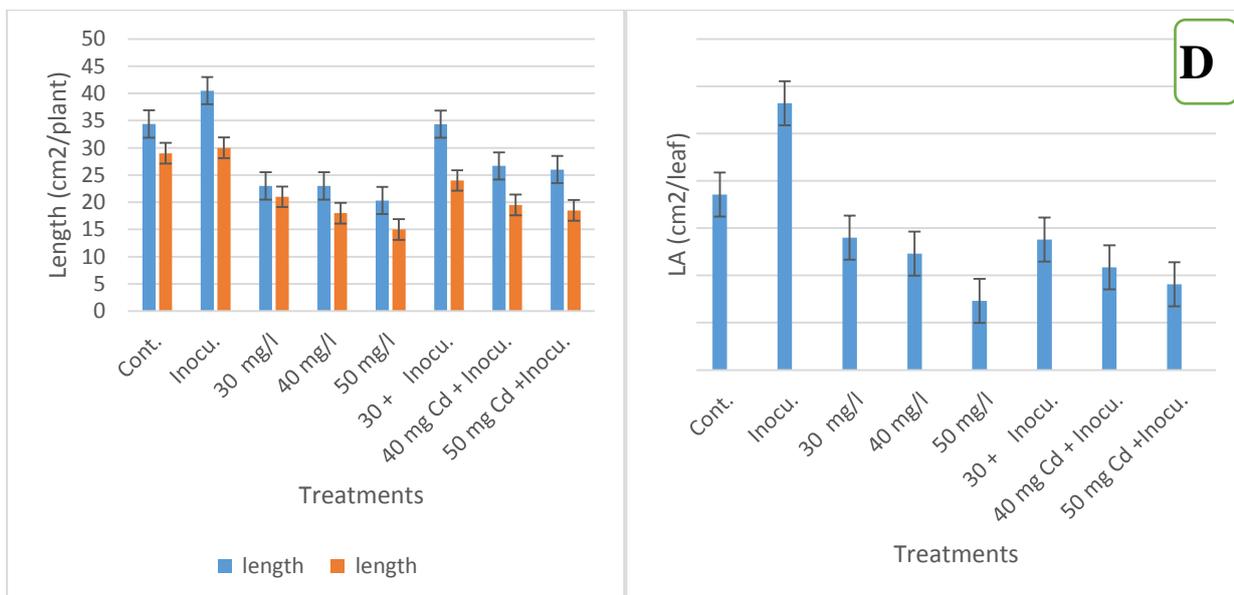


Figure1: Effect of Cd concentrations and *B. halotolerance* on biomass of seedlings of *A. tricolor* seedlings.

A: Effect of Cd concentrations and *B. halotolerance* on shoot and root fresh weights (FW) of *A. tricolor* seedlings.

B: Effect of Cd concentrations and *B. halotolerance* on shoot and root dry weights (DW) of *A. tricolor* seedlings.

C: Effect of Cd concentrations and *B. halotolerance* on shoot and root lengths of *A. tricolor* seedlings.

D: Effect of Cd concentrations and *B. halotolerance* on leaf area (LA) of *A. tricolor* seedlings.

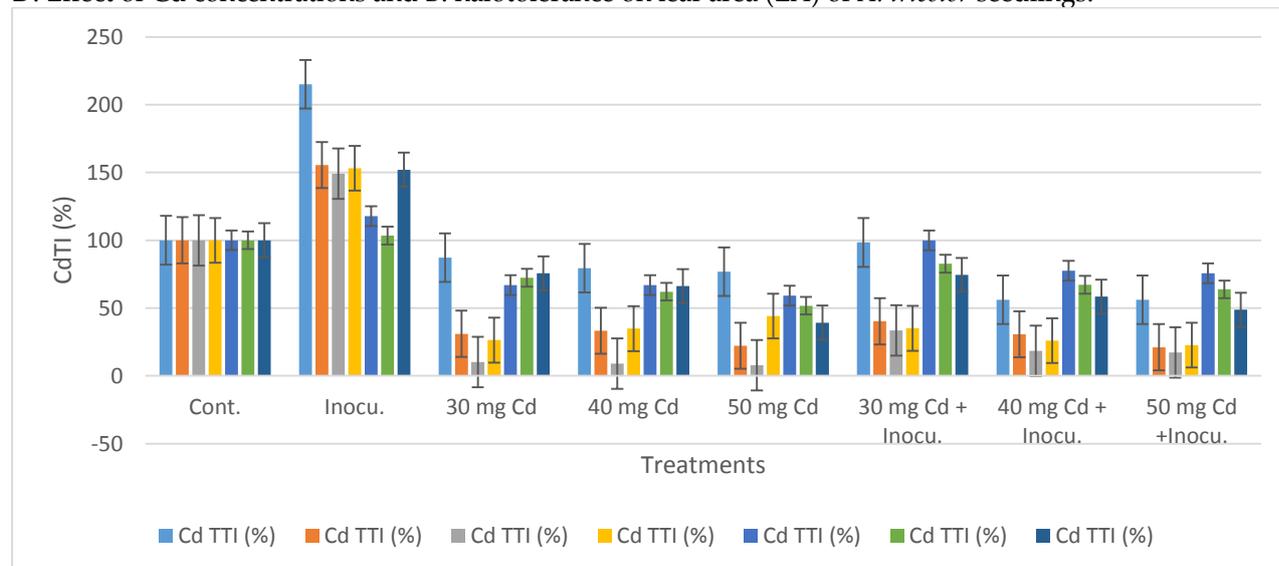


Figure 2: Effect of Cd concentrations and *B. halotolerance* on growth parameters CdTI of *A. tricolor* seedlings.

Where: CdTI: Cd tolerance index, F.W.R: fresh weight root, D.W.R: dry weight root, F.W.Sh: fresh weight shoot, D.W.Sh: fresh weight shoot, LR: root length, LSh: shoot length, LA: leaf area

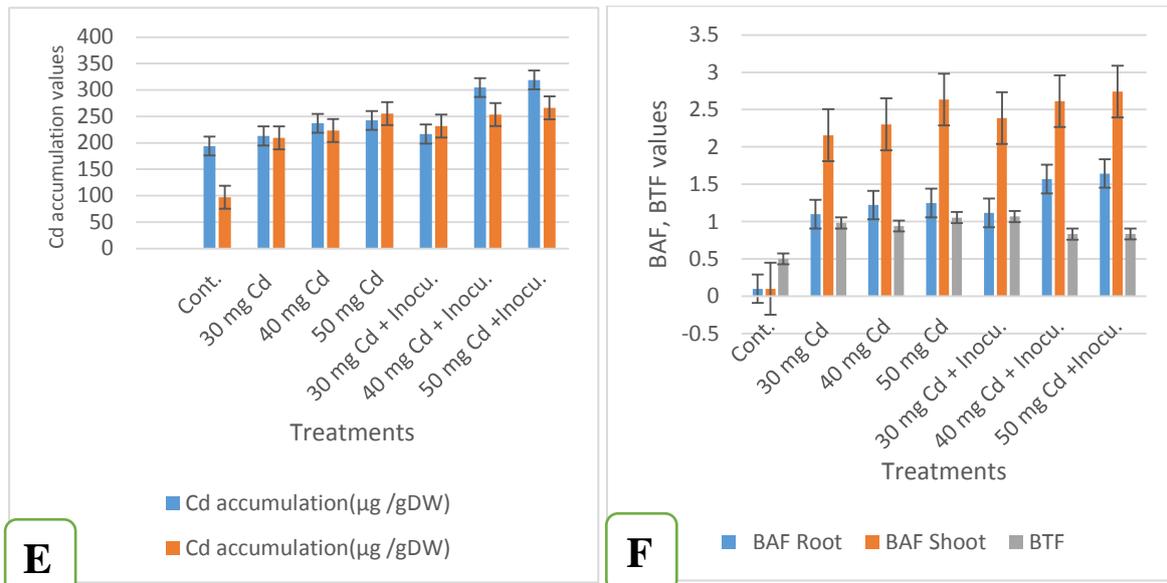


Figure 3: Effect of Cd and *B. halotolerance* on Cd accumulation ($\mu\text{g/gDW}$), BCF in root and shoot and BTF of *A. tricolor*

E: Effect of Cd and *B. halotolerance* on Cd accumulation ($\mu\text{g/gDW}$) of *A. tricolor*

F: Effect of Cd and *B. halotolerance* on BCF in root and shoot and BTF of *A. tricolor*

BCF: bioaccumulation factor, BTF: bioaccumulation factor

التفسيرات الفسيولوجية لمقاييس النمو في الأمرائس ترى كثر والملقح بالبكتريا المقاومة للكاديوم

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الملخص العربى

يعتبر الكاديوم من العناصر غير الأساسية لتغذية النبات وليس له أى وظيفة فسيولوجية داخل الأنظمة الحيوية، وتستخدم المعالجة الحيوية لإزالة هذا العنصر من البيئات الملوثة. يهدف البحث الى دراسة وتفسير الاستجابات الفسيولوجية لمقاييس النمو للنبات المجهد بالكاديوم قبل وبعد المعاملة باللقاح البكتيرى بالسلالة البكتيرية *Busillus halotolerance* DSM 8802 ومحاولة زيادة تراكم الكاديوم داخل أنسجة نبات الأمرائس ترى كثر بعد معاملته بالسلالة البكتيرية محل التجربة. أظهرت النتائج التأثير الضار لإجهاد الكاديوم على النبات محل التجربة على مختلف مقاييس النمو، وفي العموم أدت المعاملة باللقاح إلى تحسن وزيادة معنوية لكل هذه المقاييس والمعايير المختلفة بنسبة قد تصل إلى أكثر من 50% في بعض الصفات والقياسات محل التجربة. وارتفع معدل تراكم الكاديوم داخل أنسجة النبات بنسبة قد تصل الى 75% مما يدل على نجاح نبات الأمرائس ترى كثر في المعالجة النباتية لتراكم الكاديوم داخل أنسجته. ونجاح اللقاح البكتيرى *Busillus halotolerance* DSM 8802 في تحسين نمو النبات المجهد وزيادة معدل تراكم الكاديوم خلال نظام البكتريا المحفزة لنمو النبات.

الكلمات الإسترشادية: الكاديوم، المعالجة النباتية، بباسلس هالوتوليرانس، البكتريا المعززة لنمو النبات و الأمرائس ترى كثر.