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Alleviation of Chromium Stress in *Brassica juncea* L. and Soil Remediation by Plant Growth Promoting (PGP) Bacteria

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Abstract. Several species from the Brassica genus are very important agricultural crops cultivated in different parts of the world and also known to be heavy metals (HMs) accumulators. In our present research work, *Brassica juncea* L. was grown in soil spiked with different concentration levels (0.2 mm, 2 mm and 20 mm) of chromium (Cr) under natural environmental conditions. Plants were treated with Cr resistant bacterial strains (*E.coli, Klebsiella, Staphylococcus* and *Salmonella*). The visible symptoms after the exposure of Cr to *B. juncea* appeared in the form of a reduction in plant growth, leaf length, leaf width, leaf area and lower the plant biomass accumulation. Likewise, the reduction in photosynthetic pigments (chlorophyll and carotenoids) in leaves varied at different concentrations. The accumulation of lipid peroxidation in leaves was determined as 2 thiobarbituric reactive metabolites, chiefly malondialdehyde. The results revealed that the inhibitory responses of both Cr on plant growth which were dependent on concentration and time. It was investigated by applying different HMs resistance bacterial strains to soil. The plant growth increased by decreasing the effect of Cr. In a nutshell, this study indicated that the utilization of bio-fertilizers (bacterial strains) for enhancing the plant's growth and reducing Cr stress in soil which is beneficial in stress reading programs.

Keywords: *Brassica juncea*, chromium accumulation, heavy metal, plant growth promoting bacteria, antioxidants

Introduction

Pollution in the environment and increased in due to heavy metals industrial by mining activities. Currently, the vast production of different HMs like Hg, Cd, Cu and Cr is notable (Pinto *et al*., 2004). An increase in the accumulation of HMs due to various activities of humans is a serious problem (Mishra *et al*., 2017). Chromium (Cr) is the main component which is usually present in all organisms and considered as an essential element for living organisms (Panda and Choudhury, 2005).

Environmental contamination of Cr is considered and managed as a serious threat for human welfare and ecosystem health by (Tseng *et al*., 2019). This perception is driven by the abundance of Cr in the environment owned to its extensive use in a variety of domestic and industrial applications and have extremely high toxicity to all living organisms (Fernández *et al*., 2018).

Chromium has many negative effects on different biological parameters and ultimately effects on vegetation and converts green lands into barren lands (Gbaruko and Friday, 2007; Faisal and Hasnain, 2005). Plants are affected by HM pollution, particularly in the contaminated soils. Bio-accumulation problems and long resistance time in food chain (Al-Hagibi *et al*., 2018).

Heavy metals absorbed by plants both above and underground surfaces. These HMs affect plants' health directly and indirectly. Different toxicity symptoms which can be observed depend on various cellular interactions in the plants (Hall, 2002). Reduction in root growth, early development of seedlings, leaf chlorosis and reduction in biomass are the main symptoms of Cr (Khan *et al*., 2013).

The effects of chromium toxicity on various physiological processes that inhibit plant growth studied by (Kamran *et al*., 2017). Damage to soil texture such as pH, the presence of different elements and the buildup of HMs, reduces plant growth directly or indirectly by interfering with numerous physiological and molecular activities of plants (Hassan *et al*., 2017). These hazardous substances cause morphological and metabolic defects in plants, resulting in lower yields (Amari *et al*., 2017). These abnormalities also cause the formation of reactive oxygen species (ROS) such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical

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(OH^-), which disrupts cell redox equilibrium (Shahid *et al*., 2015; Gill and Tuteja, 2010). *Brassica* species are known metal accumulators and have been evaluated as potential phytoextraction plants and important in oil production (Gall *et al*., 2013). However, the presence of HMs like Cd and Cr has been reported to reduce the amount of oil produced by *Brassica* plants (Ahmad *et al*., 2020).

Microbial bioremediation has emerged as a promising strategy to reduce the concentration of HMs in the environment due to the demonstrated ability of micro-organisms, especially bacteria and sequester/transform these compounds reported by (González-Henao and Ghneim-Herrera, 2021).

One of the most promising techniques for safe crop management strategies is the use of these helpful microbes. Plant soil microbe interactions can play an important role in acclimating plants to metalliferous conditions and investigated to increase microbe-assisted metal tolerance (Tiwari and Lata, 2018).

Heavy metal tolerant-plant growth promoting (HMT-PGP) micro-organisms in the rhizosphere address major issues by modifying plants development and altering soil physico-chemical characteristics which increase in metal bioavailability, resulting in fast HM detoxification or elimination (Mishra *et al*., 2017).

Redox processes mediated by microbes have a significant impact on the transformation of HM into less or nontoxic forms (Amstaetter *et al*., 2010). One such strategy is the use of bacteria which are resistant to HMs. The above findings help us to analyze the effect of Cr on *B. juncea* growth, photosynthetic activity and oxidative stress and alliviation of Cr stress by using four bacterial strains. Thus, the main objectives of this study i.e. to investigate the effect of Cr and heavy metal tolerant-plant growth promoting (HMT-PGP) microbes. antioxidative enzymes (H_2O_2 accumulation and malon-dialdehyde) contents in leaves of *B. juncea* and to assess the effect of Cr on plant growth and photosynthetic activity of *B. juncea*.

Materials and Methods

Experimental design and plant growth. Seeds of *B. juncea* were grown during the winter season in plastic pots (15 ¾ width 15 tall) filled with garden loamy soil under natural conditions of humidity, temperature and light. A completely randomized design (CRD) in a double factorial scheme with three replications was used. After 15 days of growth, the plants were exposed to different concentrations (0.2 mm, 2 mm and 20 mm) of Cr and four heavy metal tolerant bacterial strains (*E. coli*, *Salmonella* sp., *Klebsiella* sp. and *Staphylo-coccus* sp.). Plants were not treated with any bacterial strains and Cr stress to act as a control.

Photosynthetic pigments. *Chlorophyll content (ug/mgFW)*. For the determination of chlorophyll 0.1 g of fresh leaves of all Cr treatment were dissolved in 10 mL of acetone in corning tubes. The tubes were placed in dark for 48 h. All the extract was assayed in UV 1800 spectrophotometer for absorbance at 645 and 663 nm. Chlorophyll contents were determined by using the formula described by (Arnon, 1949) that is chloro-phyll determination = [(0.00802) (D-663) + (0.0202) (D-645) (mL of solvent)/g fresh weight of plant].

Carotenoids content (ug/mgFW). For the carotenoids determination, 0.1 g of fresh leaves of all Cr treatment was dissolved in 10 mL of acetone in corning tubes. The tubes were kept in dark for 48 h. All the leaves extract were examined in UV 1800 spectrophotometer for absorbance at 480 nm. Carotenoids contents were determined by using the formula described by (Wellburn, 1994) which is carotenoids determination = (1000 x 480A-1.29 x chlorophyll a (D-663) – 53.78 x chlorophyll b (D-645) / 220.

H_2O_2 Accumulation. Leaves removed from all treated plants and washed with distilled water to remove any extraneous material which was associated with the tissues for the determination of H_2O_2 accumulation using by method of (Daudi *et al.*, 2012). Leaves placed in test tubes and immersed in (3,3'-diaminobenzidine) DAB solution for detection of H_2O_2. Tubes were wrapped with aluminium foil and kept overnight at room temperature. Leaves were drained off from the DAB staining solution. Chlorophyll removed for proper visualization of the stain. This was done by immersing the leaves in absolute ethanol and heating them in a boiling water bath for 10 min. After that, the leaves were placed on a paper towel saturated with 60% glycerol. H_2O_2 was visualized as a reddish-brown stain formed by the reaction of DAB with the endogenous H_2O_2.

Malondialdehyde (MDA) content determination. The level of lipid peroxidation was measured in terms of MDA content. MDA content was measured using the method of (Dhindsa *et al*., 1981).

The reaction solution contained 20% (w/v) trichloroacetic acid (TCA) and 0.5% (w/v) thiobarbituric acid (TBA). The solution was centrifuged at 12,000 rpm for 10 min. Absorbance of supernatant was measured at 532 nm and 600 nm. MDA content was calculated based on adjusted absorbance and the molar extinction coefficient (155 mm/cm) as described by Heath and Packer (1968).

***Statistical analysis*.** All statistical analysis was done by two-ways analysis of variance (ANOVA). The Duncan's multiple range test was using to determine the statistically significant difference between treatments at $P<0.05$. All data are showed as means ± std. error of mean (SEM) of three independent replicates of every treatment using a completely randomized design.

Results and Discussion

Plant height (cm). Plant growth was measured as plant height. The effect of Cr on plant height of *B. juncea* is shown in Fig. 1. The plant height (cm) was decreased when concentration of chromium was increased. The decrease in plant height (cm) was different at different concentrations of Cr (0.2, 2 and 20 mm) and along with different bacterial strains (*E.coli, Klebsiella, Salmonella,* and *Staphylococcus*).

Maximum increase in plant height with the mean value of 20.1 cm was observed with *Klebsiella* bacterial strains with no addition of any stress than other bacteria and control. Plants were more sensitive to the high treatment level (20 mm) of Cr. At higher concentration plans treated with *Staphylococcus* showed minimum growth at 10 cm. Our study is consistent with the study of Jabeen *et al*. (2016) in which the plant height was decreased under Cr stress. Plants take HMs from soil solution into their roots. After entry into roots, HM ions translocate to shoots primarily through xylem vessels and effect the plant growth.

Photosynthetic pigments. *Chlorophyll content (ug/mgFW)*. The effect of Cr on chlorophyll content of *B. juncea* is shown in Fig. 2. The chlorophyll content was decreased when concentration of Cr was increased.

The inhibition in chlorophyll content was different at different concentrations of Cr (0.2 mm, 2 mm and 20 mm) and along with different bacterial strains (*E. coli, Klebsiella, Salmonella* and *Staphylococcus*).

Maximum increase in chlorophyll content with the mean value of 6.01 (ug/mg FW) was observed with *E. coli* with no addition of any stress than other bacteria and control.

Decrease in the chlorophyll content was recorded at the 20 mm whereas; the photosynthetic pigment was high in the inoculated plants. Kumar *et al*. (2009) had shown that when plants of *B. juncea* were inoculated with two growth promoting bacterial strains (NBRK23 and NBRK24).

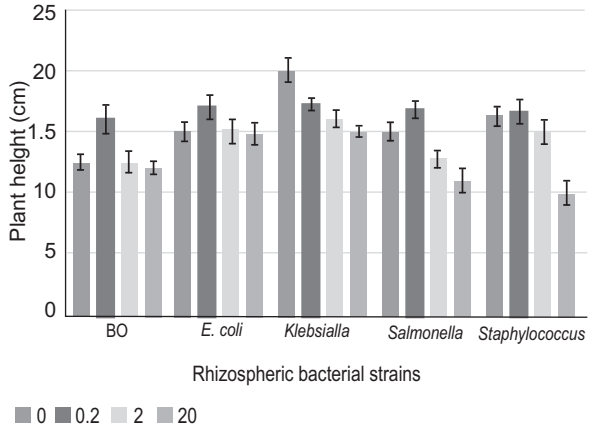

Fig. 1. Effect of Cr on plant height (cm) of *B. juncea* at different concentration levels (0.2, 2 and 20 mM) with the addition of four bacterial strains as compared to control (without addition of Cr). Error bars show the standard error (B0= no bacterial strains).

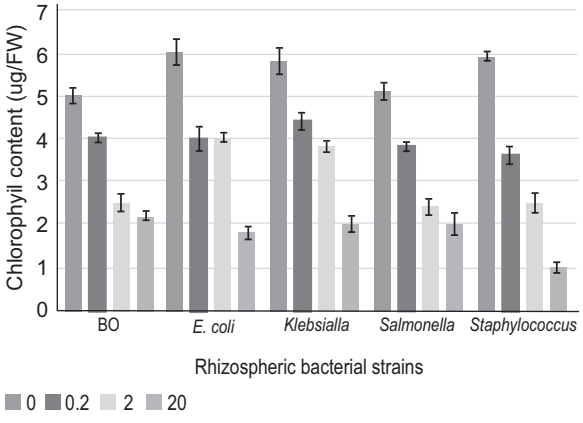

Fig. 2. Effect of Cr on chlorophyll (ug/mgFW) of *B. juncea* at different concentration levels (0.2, 2 and 20 mM) with the addition of four bacterial strains as compared to control (without addition of Cr). Error bars show the standard error (B0= no bacterial strains).

Inoculation of *Klebsiella* bacterial strains showed significantly increase in the chlorophyll content with the average value of 2.5 (ug/mg FW) at 0.2 mm concentration by decreasing the effect of Chromium. Plant growth and chlorophyll content was found more as these strains decreased the toxicity level in plants. MacFarlane and Burchett (2001) investigated that chlorophyll content is reduced in plants due to exposure of HMs in the soil.

Carotenoids content (ug/mgFW). The effect of Cr on carotenoids content of *B. juncea* is shown in Fig. 3. The carotenoids content was decreased when concentration of Cr was increased. The reduction in carotenoids content was different at different concentrations of Cr (0.2, 2 and 20 mm) and along with different bacterial strains. Carotenoids content was noted more when the plants were inoculated with different bacterial strains (*E.coli, Klebsiella, Salmonella* and *Staphylococcus*).

Maximum increase in carotenoids content with the mean value of 195.3(ug/mgFW) was observed with *Staphylococcus* bacterial strains with no addition of any stress than other bacteria and control. The reduction in the carotenoids content of plants was also observed.

Maximum reduction of carotenoids was observed at concentration level of 20 mm. The decline in carotenoids content was high when plants treated with Cr than Cd in soil as described above in the study of (MacFarlane and Burchett, 2001). Carotenoids content was found more in those plants which are inoculated with the bacterial strains. At the higher concentration 20 mm, carotenoids content was higher with the average value of 91 (ug/mgFW) when treated with *Staphylococcus*, while in comparison to *Staphylococcus* control and other bacterial strains showed less increase in contents. Significantly decrease in carotenoids content was observed in plants without inoculation of any bacterial strains at the value of 70 (ug/mgFW).

At the concentration of 2 mm no significant difference was observed between *Salmonella* and *Staphylococcus* both showed maximum content 149 (ug/mgFW) in comparison to other treated and non-treated plants.

Heavy metal concentrations in soil harmed photosynthetic fitness, whereas bacterium inoculation increased photochemical apparatus integrity and functionality, as evidenced by increases in net photosynthetic rate (21%), PSII functionality (Fm and Fv/Fm) and electron transport rate (Mesa-Marín *et al*., 2020).

H_2O_2 Accumulation. Heavy metal accumulation was increased by increasing concentration in lipid accumulation level. Through qualitative analysis of H_2O_2 accumulation in *Brassica* was evaluated that accumulation at the concentration of 0.2 mm, 2 mm and 20 mm. Like lipid accumulation at the concentration of 20 mm, leaves of *B. juncea* showed more accumulation in leaves than other concentrations as indicated in Fig. 4.

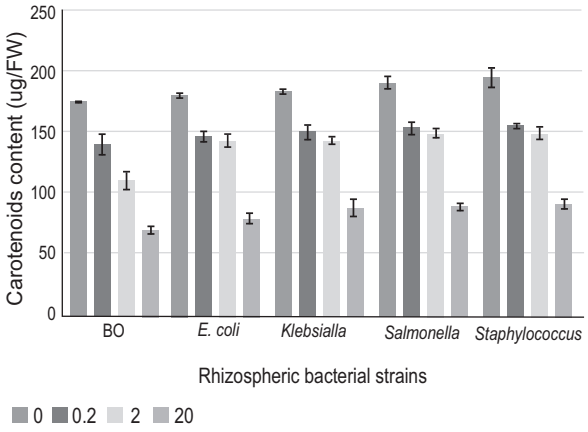

Fig. 3. Effect of Cr on carotenoids (ug/mgFW) of *B. juncea* at different concentration levels (0.2, 2 and 20 mM) with the addition of four bacterial strains as compared to control (without addition of Cr). Error bars show the standard error (B0= no bacterial strains).

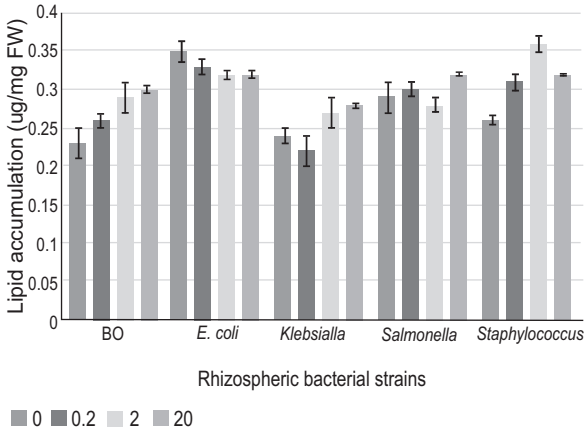

Fig. 4. Effect of Cr on lipid accumulation (ug/mgFW) of *B. juncea* at different concentration levels (0.2, 2 and 20 mM) with the addition of four bacterial strains as compared to control (without addition of Cr). Error bars show the standard error (B0= no bacterial strains).

An increased in concentration of heavy metal produced reactive oxygen in the plants due to which the level of oxidative stress was also increased. The oxidative stress like hydrogen peroxide was increased due to increase in concentration of HMs. It was observed maximum at 20 mm concentration of Cr.

The level of hydrogen peroxide activity was increased greater with chromium. Chaoui *et al*. (1997) observed oxidative reactions in *Phaseolus vulgaris* and peroxidase activity was increased due to increased concentrations of Cr. These results were similar to our results in which peroxidase and MDA activity was increased under enhanced level of HMs.

Malondialdehyde (MDA) content. Lipid accumulation (ug/mgFW) in plants is detected by measuring malonialdehyde (MDA). The effect of Cr on MDA content of *B. juncea* is shown in Fig. 3. The MDA content was increased when concentration of Cr was increased. The increase in MDA content was different at different concentrations of Cr (0.2 mm, 2 mm and 20 mm) and along with different bacterial strain (*E. coli, Klebsiella, Salmonella* and *Staphylococcus*).

Maximum decrease in MDA content with the mean value of 0.23 ug/mgFW was observed with non-inoculated plants (B0) and with no Cr. At 20 mm concentration MDA content was increased with the mean value of 0.39 ug/mgFW with *salmonella* and *staphylococcus* than other inoculated plant. *E. coli, Klebsiella, Salmonella* and *Staphylococcus* all show the same MDA content, no significant difference was observed at these three concentration 0.32 ug/mg FW.

There was increase in MDA content in Cr treated plants. Gill *et al*. (2015) studied that in *Brasicca napus* cultivars accumulation of MDA were high under Cr stress.

Conclusion

Contamination of agricultural soils due to heavy metals is one of serious environmental issues these days. To tackle this problem through ecofriendly and economical ways is the utmost priority. The results of this study indicated the decrease in plant growth, chlorophyll and carotenoids content under stress conditions. As Cr induces reactive oxygen species due to which plant growth and photosynthetic pigments were decreased while MDA and hydrogen peroxide activity were increased. The results also indicated that when plants were inoculated with different bacterial strains they showed better growth than those plants which were not inoculated and clear differences could be observed. So, from our study it is concluded that use of heavy metal resistant bacteria to overcome the toxicity produced by heavy metals is ecofriendly and economical way.

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Conflict of Interest. The authors declare that they have no conflict of interest.

References

Ahmad, R., Ali, S., Abid, M., Rizwan, M., Ali, B., Tanveer, A., Ahmad, I., Azam, M., Ghani, M.A. 2020. Glycinebetaine alleviates the chromium toxicity in *Brassica oleracea* L. by suppressing oxidative stress and modulating the plant morphology and photosynthetic attributes. *Environmental Science and Pollution Research*, **27:** 1101-1111.

Al-Hagibi, H.A., Nagi, H.M., Al-Selwi, K.M., Al-Shwafi, N.A. 2018. Assessment of heavy metals concentration in mangroves leaves of the red sea coast of Yemen. *Journal of Ecology and Natural Resources*, **2:** 120.

Amari, T., Ghnaya, T., Abdelly, C. 2017. Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. *South African Journal of Botany*, **111:** 99-110.

Amstaetter, K., Borch, T., Larese-Casanova, P., Kappler, A. 2010. Redox transformation of arsenic by Fe (II)-activated goethite (α-FeOOH). *Environmental Science and Technology*, **44:** 102-108.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, **24:** 1.

Chaoui, A., Mazhoudi, S., Ghorbal, M.H., El-Ferjani, E. 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Science*, **127:** 139-147.

Daudi, A., Cheng, Z., O'Brien, J.A., Mammarella, N., Khan, S., Ausubel, F.M., Bolwell, G.P. 2012. The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. *Plant Cell*, **24:** 275-287.

Dhindsa, R.S., Dhindsa, P.P., Thorpe. 1981. Leaf senescence, correlation with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, **32:** 93-101.

Faisal, M., Hasnain, S. 2005. Bacterial Cr (VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. *Biotechnology Letters*, **27:** 943-947.

Fernández, P.M., Viñarta, S.C., Bernal, A.R., Cruz, E.L., Figueroa, L.I. 2018. Bioremediation strategies for chromium removal: current research, scale-up approach and future perspectives. *Chemosphere*, **208:** 139-148.

Gall, J.E., Rajakaruna, N. 2013. The physiology, functional genomics, and applied ecology of heavy metal-tolerant Brassicaceae. In: *Brassicaceae: characterization, functional genomics and health benefits*, M. Lang (eds.), pp. 121-148, Nova Science Publishers, Inc., NY. USA.

Gbaruko, B.C., Friday, O.V. 2007. Bioaccumulation of heavy metals in some fauna and flora. *International Journal of Environmental Science and Technology*, **4:** 197-202.

Gill, R.A., Zang, L., Ali, B., Farooq, M.A., Cui, P., Yang, S., Ali, S., Zhou, W. 2015. Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. *Chemosphere*, **120:** 154-164.

Gill, S.S., Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, **48:** 909-930.

González-Henao, S., Ghneim-Herrera, T., 2021. Heavy metals in soils and the remediation potential of bacteria associated with the plant microbiome: evidence points to *Klebsiella* and *Enterobacter* as the best candidates for bioremediation and bacteria-assisted phytoremediation strategies in soils contaminated with arsenic, cadmium and lead. *Frontiers in Environmental Science*, **9:** 15.

Hall, J.Á. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*, **53:** 1-11.

Hassan, T.U., Bano, A., Naz, I. 2017. Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field. *International Journal of Phytoremediation*, **19:** 522-529.

Heath, R.L., Packer, L. 1968. Photoperoxidation in isolated chloroplasts. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125:** 189-198.

Jabeen, N., Abbas, Z., Iqbal, M., Rizwan, M., Jabbar, A., Farid, M., Abbas, F. 2016. Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. *Archives of Agronomy and Soil Science*, **62:** 648-662.

Kamran, M.A., Bibi, S., Xu, R.K., Hussain, S., Mehmood, K., Chaudhary, H.J. 2017. Phyto-extraction of chromium and influence of plant growth promoting bacteria to enhance plant growth. *Journal of Geochemical Exploration*, **182:** 269-274.

Khan, M.Y., Asghar, H.N., Jamshaid, M.U., Akhtar, M.J., Zahir, Z.A. 2013. Effect of microbial inoculation on wheat growth and phytostabilization of chromium contaminated soil. *Pakistan Journal of Botany*, **45:** 27-34.

Kumar, K.V., Srivastava, S., Singh, N., Behl, H.M. 2009. Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *Journal of Hazardous Materials*, **170:** 51-57.

MacFarlane, G.R., Burchett, M.D. 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Marine Pollution Bulletin*, **42:** 233-240.

Mesa-Marín, J., Pérez-Romero, J.A., Redondo-Gómez, S., Pajuelo, E., Rodríguez-Llorente, I.D., Mateos-Naranjo, E. 2020. Impact of plant growth promoting bacteria on *Salicornia ramosissima* ecophysiology and heavy metal phytoremediation capacity in estuarine soils. *Frontiers in Microbiology*, **11:** 2148.

Mishra, J., Singh, R., Arora, N.K. 2017. Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere micro-organisms. *Frontiers in Microbiology*, **8:** 1706.

Panda, S.K., Choudhury, S. 2005. Chromium stress in plants. *Brazilian Journal of Plant Physiology*, **17:** 95-102.

Pinto, A.P., Mota, A.D., De Varennes, A., Pinto, F.C. 2004. Influence of organic matter on the uptake of

cadmium, zinc, copper and iron by sorghum plants. *Science of the Total Environment*, **326:** 239-247.

Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., Aslam, M., Dumat, C. 2015. Heavy metal stress and crop productivity. In: *Crop Production and Global Environmental Issues*, Hakeem, K. (eds.), pp. 1-25, Springer International Publishing, Switzerland.

Tiwari, S., Lata, C. 2018. Heavy metal stress, signaling, and tolerance due to plant-associated microbes: an overview. *Frontiers in Plant Science*, **9:** 452.

Tseng, C.H., Lee, I.H., Chen, Y.C. 2019. Evaluation of hexavalent chromium concentration in water and its health risk with a system dynamics model. *Science of the Total Environment*, **669:** 103-111.

Wellburn, A.R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, **144:** 307-313.