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RESEARCH ARTICLE

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON PLANT GROWTH AND FLOURIDE (F) UPTAKE BY F HYPERACCUMULATOR PLANT *PROSOPIS JULIFLORA*

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ABSTRACT

Fluoride (F) pollution is a worldwide problem, as there is no cure of fluorosis available yet. The strategies to remediate F contaminates in soils, phytoremediation approach using F accumulating plants is much convincing in terms of F removal efficiency. Plant Growth Promoting Rhizobacteria (PGPR) enhances the efficiency of phytoremediation. Two rhizobacterial strains *Pseudomonas fluorescence* (P.F) and *Pseudomonas aeuroginosa* (P.A) were used to determine the effects of inoculation on growth, antioxidant activity and the tolerance potential of *Prosopis juliflora* plants to accumulate F. These two strains, increases bioaccumulation factor (BF) 2.51-27.06 and translocation factor (TF) 0.66-1.10 of plant accumulated high amount of F in root. The organ wise accumulation showed an accumulation (15.9 $mgkg^{-1}$ dw and 23.5 $mgkg^{-1}$ dw) in shoot and root respectively. Further, increase of superoxide dismutase, catalase and peroxidase activity was also recorded. *Pseudomonas fluorescence* significantly increases the biomass and high bioaccumulation, translocation factor efficiency in comparison to *Pseudomonas aeuroginosa*. Present study suggests that the two PGPR strains could be used to improve the soil quality of F contaminated soil. Further enhances the efficiency of F hyperaccumulator plant *P. juliflora* for phytoremediation purpose.

INTRODUCTION

Soil pollution by F non-metal is one of the main worldwide problem. Several reports suggest that, F is an essential element for the normal growth of plants and in higher concentration it is toxic for plants (Weinstein and Davison, 2004). Seed germination and early seedling growth are important phases for the successful growth and survival of plants and these physiological parameters of plants are affected by F stress (Sabal *et al*., 2006). Several physiological and biochemical processes are known to be affected by F such as chlorosis and necrosis of leaf, low nutrient uptake, reduction of plant biomass, and enzymatic activities (Gupta *et al*., 2009; Chakrabarti and Patra, 2013). Several states are F endemic (Choubisa, 2012) including Rajasthan where ground water contains a high amount F in all the 33 districts (Choubisa *et al*. 2001). In Rajasthan, due to irregular and low rainfall and drought, ground water is the main water source for irrigation for agricultural purposes (Chaudhary *et al*., 2009). Higher soil salinity increases the catalase, peroxidase and superoxidase activity among tolerant and sensitive varieties of plant (Vaidyanathan *et al*., 2003). The relationship between antioxidants and salinity indicate that O^{-}_{2} radical and H_2O_2 could play an important role in the mechanisms system (Chookhampaeng, 2011).

Plant growth-promoting rhizobacteria (PGPR) effect on plants by improving growth and enhancing root development, or increasing plant tolerance to various environmental stresses (Ahemad and Khan, 2011; Bhattacharyya and Jha, 2012). Plant growth promoting bacteria can enhance plant biomass by a wide variety of mechanisms system including phosphate solubilization, siderophore production, phytohormone production, 1-aminocyclopropane-1-carboxylate deaminase for root elongation (Ahemad and Khan, 2011; Bhattacharyya and Jha, 2012). Metal phytoremediation, is often facilitated by soil microorganisms living in close association with plant roots (Shilev *et al*., 2001).

The contribution of the rhizomicrobial population to phytoremediation of contaminate site is usually referred to as rhizoremediation (Kuiper *et al*., 2004). In hyperaccumulators, PGPR can increase the absorption of heavy metals through increasing plant biomass. *Pseudomonas fluorescens* and *Pseudomonas aeuroginosa*, that can tolerate high concentrations of heavy metals in polluted waters (Wasi *et al*., 2010). *Pseudomonas spp.* may therefore result in strains with enhanced biodegradative activities and a potential for bioremediation applications in metal-polluted sites (Valls *et al*., 2000). The present investigation employs *Pseudomonas fluorescence* enhancing the availability of heavy metals in the rhizosphere (Petriccione *et al*., 2013).

The bacteria that have been used for these studies are often first selected for resistance to the (F) and tested for the presence of one or several plant-growth promoting (PGP) properties. These studies are considered for the F-resistant bacteria, which will be able to proliferate and promote plant growth in the presence of high level of toxic F. In the present studies leguminous species (*P. juliflora*) hyperaccumulator plant promoted us to assess the potential use of this plant in the reclamation of F contaminated soils. This is also naturally grown in F widespread areas of Rajasthan (India) without showing any morphological distortion. It is tolerant to very high temperature (like 48°C). The tree can grow in different areas, and the roots enter to large depths in the soil. The

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objective was to investigate (a) the effect of plant growth promoting bacteria on biochemical parameters and antioxidant enzyme activity (b) and improvement of accumulation by F hyperaccumulator plant *P. juliflora.*

MATERIAL AND METHODS

Pot experiment

Prosopis juliflora seeds collected from Central Arid Zone research institute (CAZRI) Jodhpur (Rajasthan) India. Seeds were surface sterilized with 20% H_2SO4 for 15 min and were rinsed with deionized sterile millipore water. Seeds were germinated in plastic pots (7-cm diameter and 12-cm height) with 1 kg of the testing soils. Each pot received six seeds which were placed at 4-cm depth. Pots were rearranged in the greenhouse chamber. Treatment has given of F at 5 concentrations, 0, 25, 50, and 75 and 100 mg kg^{-1}. Each treatment was subjected to a different type of inoculation: PF 8904 (*Pseudomonas fluorescence*), and PA 1934 (*Pseudomonas aeuroginosa*) strains, obtained from (MTCC) Chandigarh. Three replicates were used for each F level inoculation type treatment. Bacterial strain suspension (10^8 cfu mL^{-1}) in nutrient broth was used for the inoculation, by spraying soil surfaces (Marques *et al*., 2010), 10 days after germination. To the control pots, 10 mL of sterile distilled millipore water was added. Plant parts (roots and shoots) were harvested after 120 days and washed with tap water and deionized sterile water.

Antioxidant activity

Extraction of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), 1 g of plant tissue was homogenized with 3 ml of 0.1 M of sodium phosphate (NaPO4) buffer (pH 7) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C to collect supernatant for estimation of SOD, CAT, and POD. The activity was measured at 560 nm absorbance of SOD (Beauchamp and Fridivich, 1971), CAT was determined by measuring the change of absorbance at 240 nm (Luck *et al*., 1974) and POD activity was determined by H_2O_2 at 420 nm absorbance through spectrophotometer (Putter *et al*., 1974).

Determination of F

After 120 days of treatment, root/ shoots were harvested for studying the bioaccumulation and translocation factor were measured by Niu *et al*. (2007). A total F content in plant sample and remaining soil was calculated by alkali fusion-ion selective technique (McQuaker and Gurney, 1977).

BF = {F concentration in shoot}/ {F concentration in soil}
TF = {F concentration in shoot}/ {F concentration in root}

Statistical analysis

Standard deviation (SD) and Spearman's correlation were calculated by statistical package (SPSS 17.0) to examine difference between each treatment at ($P \leq 0.05$).

RESULT AND DISCUSSION

Growth parameters

The plants grown on different treated with *P. fluorescence* showed the best growth, (fig. 1) the values were even greater than the control as compare to *P. aeuroginosa* . Fluoride cause reduction in root length (12.76 cm) at 100 $mgkg^{-1}$ NaF soil and shoot length (10.43) cm at 100 $mgkg^{-1}$ NaF soil due to unbalanced nutrient uptake by seedlings in the presence of F in soil (Sabal *et al*., 2006). The increasing concentration of NaF showed phytotoxic effects on physiology and growth parameters. *P. fluorescence* increased as the rate of (34.3- 24.9 cm at 25 $mgkg^{-1}$ NaF) root and shoot respectively and the largest reduction was observed in plants treated with 100 $mgkg^{-1}$ F.

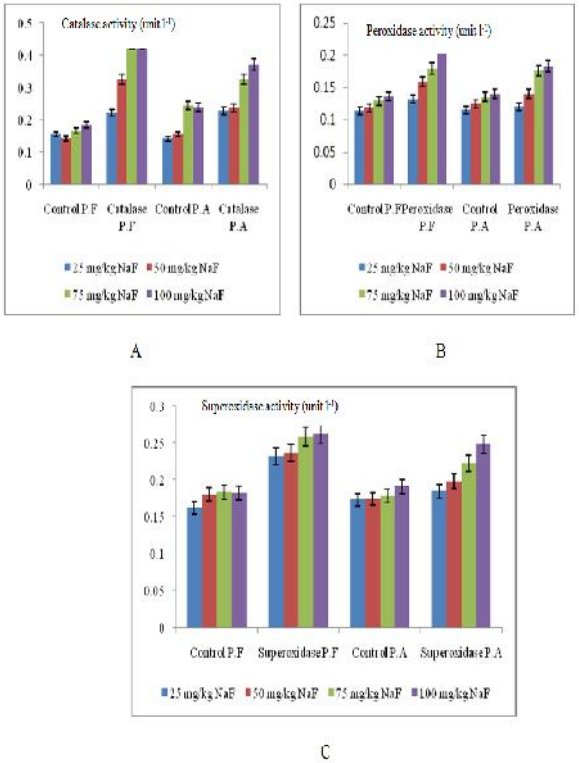

Figure 1 Antioxidant activity of Catalase (A) Peroxidase (B) and Superoxidase (C) in *P. juliflora* after 120 days of NaF with microbial treatment (P.F = *Pseudomonas fluorescence* and P.A = *Pseudomonas aeuroginosa*).

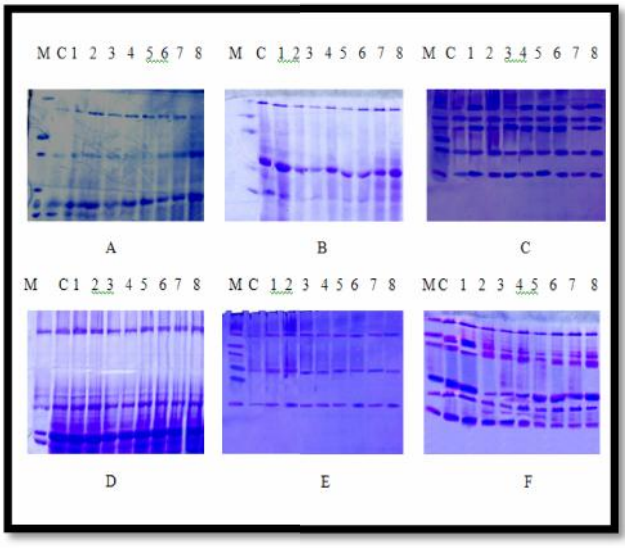

Figure 1: Efeect of microbial treatment on isoenzyme patterns of CAT, SOD and POD in *P. juliflora* leaves after 120 days treatment with F. In fig(A-CAT, B-SOD and C-POD) M-Broad range marker14.3KDa-205KDa , C-control, 1- 25$mgkg^{-1}$ F, 2- 50 $mgkg^{-1}$ F+, 3- 75 $mgkg^{-1}$ F, 4- 100 $mgkg^{-1}$ F, 5- 25 $mgkg^{-1}$ F+P.F, 6-50 $mgkg^{-1}$ F+P.F, 7-75 $mgkg^{-1}$ F+P.F and 8-100 $mgkg^{-1}$ F+P.F. In fig (D-CAT, E-SOD and F-POD) M- marker, C-control, 1-25 $mgkg^{-1}$ F, 2-50 $mgkg^{-1}$ F+, 3-75 $mgkg^{-1}$ F, 4- 100 $mgkg^{-1}$ F, 5- 25 $mgkg^{-1}$ F+P.A, 6-50 $mgkg^{-1}$ F+P.A, 7-75 $mgkg^{-1}$ F+P.A and 8-100 $mgkg^{-1}$ F+P.A.

Antioxidant enzyme mechanism

P. juliflora treated of plants to different rhizobacteria (both with and without treatment) led to an increase in the activity of CAT, POD and SOD. The hydrogen peroxide is toxic for plants but it behaves both as oxidant and reductant. The hyperactivity of POD under metal stress indicated the scavenging activity of H_2O_2 generated through the activity of photorespiration in plant cells. Therefore, an increase in POD activity prevents plants from toxic effects (Ali *et al*., 2003). Catalase activity scavenges H_2O_2 by breaking it directly to form water and oxygen. Similar trends increases in CAT activity were also obtained with increase in salinity amount in *Triticum aestivum* (Heidari, 2009).

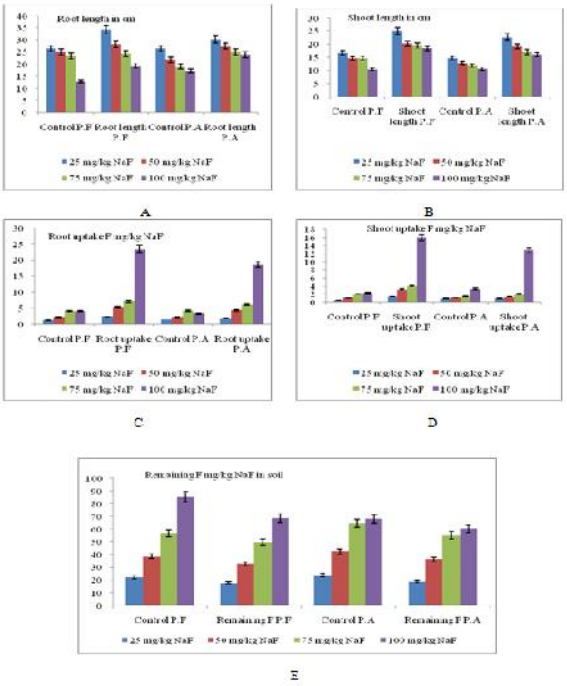

Figure 3 Effect of microbial treatment on growth (A and B) and organ-wise accumulation (C, D and E) of F hyperaccumulator plant *P. juliflora* (F concentration + *P.fluoresence* (P.F) and *P. aeuroginosa* (P.A) treatment) for 120 days (control, 25, 50, 75 and 100 $mgkg^{-1}$ NaF soil).

as doses of rhizobacteria. Among these enzymes, superoxide dismutase (SOD) is the first line of defense against ROS, dismutating O^{-2} to oxygen molecule and H_2O_2 (Gill *et al*., 2011).

There was a significant correlation between F concentration and peroxidase activity, catalase activity and superoxidase activity ($r = 0.99$, $p<0.05$), ($r = 0.96$, $p<0.05$) and ($r = 0.99$, $p<0.05$) was observed between the given of microbial treatment in the leaves of *P.juliflora* and activity of CAT, POD and SOD (fig. 1). Three isoenzymes of CAT were observed after non denatured electrophoresis (Fig. 1). All bands appeared at 100 $mgkg^{-1}$ F + *P. fluorescence* and its intensity increased with F amount. Five isoenzymes of POD observed after native PAGE in leaves extract of *P. juliflora* (Fig. 1). It showed that an increase in band intensity correlated with F-dose at 100 $mgkg^{-1}$ F + *P. fluoresence* $mgkg^{-1}$ then decreased at 25 $mgkg^{-1}$ F + *P. fluoresence* $mgkg^{-1}$. SOD activity staining on a gel after native PAGE, three isoenzymes band in leaves, its band intensity increased at 100 $mgkg^{-1}$ F + *P. fluoresence* $mgkg^{-1}$ then decreased at 25 $mgkg^{-1}$ F + *P. fluoresence* $mgkg^{-1}$. In comparison basis *P. fluorescence* +100 $mgkg^{-1}$ show high intensity bands as compare to *P. aeuroginosa* + F $mgkg^{-1}$. Similar results are obtained in plants stressed by the same metal, Cu (Demirevska- Kepova *et al*., 2004), or other metals such us Mn, Pb, Ni and Cd (Gomes-Junior *et al*., 2006).

Oragn-wise F uptake

P. juliflora seedlings accumulated (23.5 $mgkg^{-1}$ NaF soil) root dry tissue, (15.9 NaF $mgkg^{-1}$ soil) shoot dry tissue and remaining F in soil at (68.7 NaF soil $mgkg^{-1}$). In the present study, we reported higher accumulation of F in roots. The higher accumulation of F in roots, could be due to the fact that the soluble fluoride fraction in soil is taken up passively by roots as the non-ionic form at low pH (Horner and Bell, 1995). A significant positive correlation ($r = 0.86$) was found between F concentration with microbial treatment and uptake of F at ($P \leq 0.05$) level of significance. According to Jha *et al*. (2009) concluded in onion (*Allium cepa L*.) that there is high amount of accumulation in roots than shoots. The bioaccumulation factor (BF) at different microbial treatment

Table 1 Effect of microbial treatment (*P. fluorescence and P. aeuroginosa* suspension 10^{-8} cfu/ml in nutrient broth) on translocation and bioaccumulation factor of F hyperaccumulator plant *P. juliflora* grown in soil under different concentration of F for 120 days.

F-Treatment	Translocation factor (TF)	Bioaccumulation factor (BF)
Control	0.733	2.513
Control (25 $mgkg^{-1}$)	0.759	24.512
25 $mgkg^{-1}$+ *P. fluoresence*	0.974	25.550
Control 50 $mgkg^{-1}$	0.756	23.302
50 $mgkg^{-1}$ + *P. fluoresence*	1.089	25.340
Control 75 $mgkg^{-1}$	0.756	21.212
75 $mgkg^{-1}$ + *P. fluoresence*	1.091	27.06
Control 100 $mgkg^{-1}$	0.805	24.244
100 $mgkg^{-1}$ + *P. fluoresence*	1.104	25.000
Control 25 $mgkg^{-1}$	0.661	14.751
25 $mgkg^{-1}$ + *P. aeuroginosa*	0.789	26.790
Control 50 $mgkg^{-1}$	0.767	19.249
50 $mgkg^{-1}$ + *P. aeuroginosa*	0.783	25.26
Control75 $mgkg^{-1}$	0.696	17.244
75 $mgkg^{-1}$ + *P. aeuroginosa*	0.994	26.12
Control 100 $mgkg^{-1}$	0.796	18.273
100 $mgkg^{-1}$ + *P. aeuroginosa*	1.005	26.08

Although H_2O_2 take part in several important functions in plant cells such as signal transduction, protein cross linking and cell wall lignifications (Low and Merida, 1996). The activity of enzymes increased with increase in duration of F stress as well

(*P. fluoresence and P. aeuroginosa*) with F concentration was in the range of 2.51-27.06. The *P. juliflora* showed a translocation factor (T.F) of (0.66-1.10) at different microbial treatment (*P. fluorescence and P. aeuroginosa*) with different

concentration of F (Table 1). If the value of ratio greater than 1 means Shuod higher of accumulation efficiency(Gupta and Banerjee, 2009). The result of the present investigation showed that microbial treatment has high ability and increase the efficiency of F accumulation. Among the microorganisms bacteria deserve special attention because they can directly improve the phytoremediation process by changing the metal bioavailability through altering soil pH, release of chelators (e.g., organisc acids, siderphores), oxidation and reduction reaction (Rajkumar *et al*., 2010).

CONCLUSION

Present study demonstrated that, *Pseudomonas fluorescence* and *Pseudomonas aeuroginos* improves F accumulation abilities of *P. juliflora*. The increase in shoot and root biomass observed for inoculated plants grown in the F-contaminated soils enhances the application of the produced biomass for energy purposes after harvest of the above ground tissues. Higher antioxidant activities with increase in F concentrations suggest a role in tolerance to fluoride stress in *P. juliflora*. This work showed increased hyperaccumulation efficiency of F in P. *juliflora*,that could be utilized for phytoremediation purpose of fluoride contaminated soil. Further, results of this could be treated in field scale.

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Reference

1. Ahemad, M. and Khan, M. S. (2011). Functional aspects of plant growth promoting rhizobacteria recent advancements. Insight Microbiology, 1:39-54.
2. Ali, M. B., Vajpayee, P., Tripathi, R. D., Rai, U. N., Singh, S. N., and Singh, S. P. (2003). Phytoremediation of lead, nickel and copper by Salix acmophylla Boiss; Role of antioxidant enzymes and antioxidant substances. Bulletin of Environment Contamination Toxicology, 70:462-469.
3. Bhattacharyya, P. N. and Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology Biotechnology, 28:1327-1350.
4. Chakrabarti, S. and Patra, P. K. (2013). Effect of fluoride on superoxide dismutase activity in four common crop plants. Fluoride, 46(2):59-62.
5. Chaudhary, V., Sharma, M., and Yadav, B. S. (2009). Elevated fluoride in canal catchment soils of northwest Rajasthan, India. Fluoride, 42(1):46-49.
6. Chookhampaeng, S. (2001). The effect of salt stress on growth, chlorophyll content proline content and antioxidant enzymes of pepper (Capsicum annuum L.) seedling. European Journal of Science Research, 49:103-109.
7. Choubisa, S. L. (2012). Status of fluorosis in animals. Proceedings of National Academy of Science, India Section B, Biology Science 82(3):331-339.
8. Choubisa, S. L., Choubisa, L., and Choubisa, D. K. (2001). Endemic fluorosis in Rajasthan. Indian Journal Environment Health, 43(4):177-89.
9. Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Hölzer, R., Feller, U. (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. Environment and Experimental Botany, 52:253-266.
10. Gill, S. S., Khan, N. A., and Tuteja, N., (2011). Differential cadmium stress tolerance in five Indian mustard (Brassica juncea L.) cultivars, an evaluation of the role of antioxidant machinery. Plant Signaling and Behaviour, 6:293-300.
11. Glick, B. R. (2010). Using soil bacteria to facilitate phytoremediation. Biotechnology Advance, 28:367-374.
12. Gomes-Juniora, R. A., Moldes, C. A., Delite, F. S., Gratãoa, P. L., Mazzaferab, P., Lea, P. J., Azevedoa, R. A. (2006). Nickel elicits a fast antioxidant response in Coffea arabica cells. Plant Physiology Biochemistry, 44:420-429.
13. Gupta, S., Banerjee, S. (2009). Fluoride accumulation in paddy (Oryza sativa) irrigated with fluoride contaminated ground water in an endemic area of the Birbhum District. West Bengal, Fluoride, 42:224-227.
14. Gupta, S., Banerjee, S., and Mondal, S. (2009). Phytotoxicity of fluoride in the germination of paddy (Oryza sativa) and its effect on the physiology and biochemistry of germinated seedlings. Fluoride, 42(2):142-146.
15. Heidari, M. (2009). Antioxidant activity and osmolyte concentration of sorghum and wheat genotypes under salinity stress. Asian Journal Plant Science, 8:240-244.
16. Jha, S. K., Nayak, A. K., Sharma, Y. K. (2009). Fluoride toxicity effect in onion (Allium cepa L) grown in contaminated soils. Chemosphere, 76:353-356
17. Kuiper, I., Lagendijk, E. L., Bloemberg, G. V., and Lugtenberg, T. J. J. (2004). Rhizoremediation: a beneficial plant-microbe interaction. Molecular Plant Microbiology Interact, 17:6–15.
18. Low, P. S. and Merida, J. R. (1996). The oxidative burst in plant defense function and signal transduction. Physiologia Plantarum, 96:533-542.
19. Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S., and Castro, P. M. L. (2010). Assessment of the plant growth promotion abilities of six bacterial species using Zea mays as indicator plant. Soil Biology and Biochemistry, 42:1229-1235.
20. McQuaker, N. R. and Gurney, M. (1977). Determination of total fluoride in soil and vegetation using an alkali fusion-selective ion electrode technique. Analytical Chemistry, 49:53-86.
21. Niu, Z. X., Sun, L. N., Sun, T. H., Li, Y. S., Wang, H. (2007). Evaluation of phytoextracting cadmium and Lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. Journal of Environment Science, 19:961-967.
22. Petriccione, M., Di Patre, D., Ferrante, P., Papa, S., Bartoli, G., Fioretto, A., and Scortichini, M. (2013). Effects of Pseudomonas fluorescens Seed Bioinoculation on Heavy Metal Accumulation for Mirabilis jalapa Phytoextraction in Smelter-Contaminated Soil. Water Air Soil Pollution, 224:1645-1647.
23. Rajkumar, M. Ae. N., Prasad, M. N. V., and Freitas, H. (2010). Potential of siderophore-producing bacteria for improve heavy metal phytoextraction. Trends Biotechnology, 28:142-149.
24. Sabal, D., Khan, T. I., and Saxena, R. (2006). Efeect of fluoride on cluster bean (Cyamopsis Tetragonoloba) seed germination and seedling growth. Fluoride, 39:228-230.

25. Shilev, S. I., Ruso, J., Puig, A., Benlloch, M., Jorrin, J., and Sancho, E. (2001). 'Rhizospheric bacteria promote sunflower (Helianthus annus L.) plant growth and tolerance to heavy metals. Minerva Biotechnology, 13:37–39.
26. Vaidyanathan, H., Sivakumar, P., Chakrabarty, R., and Thomas, G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (Oryza sativa L.) differential responses in salt tolerant and sensitive varieties. Plant Science, 165:1411-1418.
27. Valls, M., de Lorenzo, V., Gonzalez-Duarte, R and Atrian, S.(2000). Engineering outer membrane proteins in Pseudomonas putida for enhanced heavy-metal bioadsorption. Journal of Inorganic Biochemistry, 79:219-223.
28. Wasi, S., Tabrez, S., and Ahmad, M. (2011). Suitability of immobilized Pseudomonas fluorescens SM1 strain for remediation of phenols, heavy metals, and pesticides from water. Water Air and Soil Pollution, 220:89-99.Weinstein, L. H. and Davison, A. W. (2004). Fluorides in the environment: effects on plants and animals. Wallingford, Oxon, UK: CABI Publishing.

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