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Research Article

MICROBE ASSISTED PHYTOREMEDIATION OF FLUORIDE BY *BRACHIARIA DISTACHYA* (L.), A NATIVE HYPER ACCUMULATOR OF FLUORIDE CONTAMINATED SITE, VISAKHAPATNAM

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Key words: Fluoride accumulation, Hyper accumulators, *Brachiaria distachya* (L.), *Bacillus cerus*, *Providencia vermicola.*

ABSTRACT

Fluoride is the most phytotoxic element among the trace elements. Fluoride pollution is a serious problem worldwide. Fluoride emissions from industries are main sources of air and soil pollution. *Brachiaria distachya* (L.), a native hyper accumulator (779.9 mg/kg of F) growing in the phosphate dumping site of Coromandel fertilizer factory, was studied to test the accumulating capacity of the plant in 3 different concentrations of (50 mg/kg, 75 mg/kg, 100 mg/kg NaF) in small scale pot experiments for 60 days by amending the bacteria (*Bacillus cerus, Providencia vermicola*) isolated from the rhizosphere soil of same plant in the contaminated site. The results show an increase in the biomass from 0.8 ± 0.3 to 5.9 ± 1.7 in *bacillus* treated to 3.1 ± 0.05 in *P. vermicola* treated. The chlorophyll increased in the bacterial amendments when compared to fluoride treated, the TF was 2.1 ± 0.03 in *Bacillus* treated to 1.4 ± 1.9 in *P. vermicola* at 100 mg/kg NaF, the BF ranges from 0.4 ± 0.2 to 3.4 ± 0.1 in different bacterial treatments when compared to fluoride treated ones. Comparative results (biomass, chlorophyll, mineral content, TF and BF) between *B. cerus* and *P. vermicola* indicated the application of *Bacillus cerus* significantly increased the uptake of fluoride by *Brachiaria distachya* (L.), which showed a good tolerance level at 100 mg.kg NaF. In conclusion the *Brachiaria distachya* (L.) and the bacteria both can be potentially used in fluoride removal from soil.

INTRODUCTION

Fluoride is the most phytotoxic element among the trace elements. Fluoride pollution is a serious problem worldwide. Fluoride emissions from industries are main sources of air and soil pollution. *Brachiaria distachya* (L.), a native hyper accumulator (779.9 mg/kg of F) growing in the phosphate dumping site of Coromandel fertilizer factory, was studied to test the accumulating capacity of the plant in 3 different concentrations of (50 mg/kg, 75 mg/kg, 100 mg/kg NaF) in small scale pot experiments for 60 days by amending the bacteria (*Bacillus cerus, Providencia vermicola*) isolated from the rhizosphere soil of same plant in the contaminated site. The results show an increase in the biomass from 0.8 ± 0.3 to 5.9 ± 1.7 in bacillus treated to 3.1 ± 0.05 in *P. vermicola* treated. The chlorophyll increased in the bacterial amendments when compared to fluoride treated, the TF was 2.1 ± 0.03 in *Bacillu*s treated to 1.4 ± 1.9 in *P. vermicola* at 100 mg/kg NaF, the BF ranges from 0.4 ± 0.2 to 3.4 ± 0.1 in different bacterial treatments when compared to fluoride treated ones. Comparative results (biomass,

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chlorophyll, mineral content, TF and BF) between *B. cerus* and *P. vermicola* indicated the application of *Bacillus cerus* significantly increased the uptake of fluoride by *Brachiaria distachya* (L.), which showed a good tolerance level at 100 mg.kg NaF. In conclusion the *Brachiaria distachya* (L.) and the bacteria both can be potentially used in fluoride removal from soil.

Fluoride the lightest member of the halogen group was first discovered by Henri Moissan in 1886. It is the 13th most abundant element in the Earth's crust with an atomic number of 9 and a molecular weight of 18.998 g mol^{-1}. Fluorine occurs as a diatomic molecule F_2 in its elemental form It is the most electronegative element in the periodic table and is the most reactive of all elements (Jha, *et al*., 2009). Fluorine does not occur as elemental form in nature; hence no chemical substance is capable of freeing fluorine from its compounds, making it extremely difficult for scientists to isolate. Fluoride, unlike sulphur, nitrogen and chlorine, is not an essential element for plants. It is a non-biodegradable element that accumulates in soil, plants, and humans and is the most phytotoxic pollutant (Cronin, *et al*., 2000).

Excessive fluoride concentrations have been reported in ground waters of more than 20 developed and developing countries including India. All countries of South Asia such as India, Pakistan and Sri Lanka are highly affected. India contributes 12 million out of 80 million deposits of Fluoride in earth crust (Teotia and Teotia, 1984). In India, almost all the states are known to have fluoride contamination in water. The most seriously affected states are Rajasthan, Andhra Pradesh, Punjab, Haryana, Rajasthan, Gujarat, Tamil Nadu, and Uttar Pradesh. Andhra Pradesh and Telangana are the second most affected states in India after Rajasthan. The Nalgonda district of Andhra Pradesh is most extensively studied part in India for fluoride problem. Fluoride levels are in the range of 0.5–4.5 mg/L in groundwater in Ranga Reddy district. In Anantapur district, fluoride as high as 5.8 mg/L was reported from groundwater and it has been linked to fluorine-bearing minerals in the strata (Sujatha and Reddy, 2003). Higher Fluoride concentration was reported in Varaha River basin during post-monsoon season (Rao, 2009).

Fluoride pollution is now recognized as a global problem. The reason fluorides are considered as serious contaminants even when they are present at low levels is that they persist for a long time in air, soil, and water and exert negative effects at all levels of an ecosystem. Thus, immediate attention is the need of the hour to remediate the environment from F pollution. There are various physical and chemical methods for Defluorination of drinking water (adsorption & biosorption, ionexchange, electrocoagulation, flotation and reverse osmosis) are too expensive so it is important to develop eco-friendly and more effective method to decontaminate soils. Phytoremediation uses green plants and their associated micro biota for the treatment of contaminated soil and ground water (Sadowsky, 1999). Plant selection is a crucial step in the process of phytoremediation. Grasses with their fibrous root system that increases the root surface area are said to be excellent candidates for phytoremediation (Kulakow, *et al.,* 2000). Tolerant bacteria could survive in contaminated habitats and could be isolated and selected for their potential application in the bioremediation of contaminated sites (Rajbhansi, 2008; Wasi, *et al*., 2008). Microbes and higher organisms may play a bioremediative role to make fluoride less available and less dangerous (Chouhan, *et al*., 2012). Successful phytoremediation depends on the interaction of plants and soil and microbes (Glick, 2003).

The objective was to study a) effect of fluoride on the chlorophyll, biomass, growth factors of *B. distachya*, b) effect of microbial consortium on plant biomass, chlorophyll, mineral content of plant organs (c) check efficacy of phytoremediation in small scale of pot experiment under given microbial treatment.

MATERIALS AND METHODS

Identification of plant species suitable for study

22 plants were identified present as vegetation patches in study area. Fluoride concentration was measured in all the plants using alkali fusion ion selective electrode (Edye, 1982). The highly accumulating plant growing abundantly was selected for the study.

Isolation and identification of bacteria from rhizosphere soils

10 g of soil close to the roots of plant was collected and brought to the laboratory for isolation of bacteria by soil dilution plate method (Bergey, *et al*., 1984). The bacteria were identified by molecular method by 16s rRNA sequencing (Murmur, 1961). The strains were provided with accession numbers MG674654, MG674655 for *P. vermicola, B. cerus.*

Pot experiments: Set: I

Young plants of *B. distachya* from low fluoride areas with 1.2 mg/kg of fluoride in soil were brought and sown in plastic pots containing 1kg of sterilized soil sterilized at 121°C temperature and 15 lbs pressure for one hour. The pots were laid in controlled conditions

(25°C temperature and 60% humidity). The pots were given treatment with different concentrations of Fluoride as 50, 75, 100 mg/kg NaF, and garden soil was taken for control pots. The potted plants were left undisturbed for one week after treating with fluoride for acclimatization to fluoride. Three replicates were taken for each set and for each treatment. Data were collected on physico chemical parameters of the soil on the starting day of the experiment, and also at the time of harvest.

Set II and III

Another set of experiments was subjected to different type of inoculation with isolated bacteria along with NaF to all three plants. Bacterial strain suspension of 10 ml with B. *cerus* strains in set II, and *P. vermicola* in set III were added to the pots, by spraying them on soil surfaces, few days after acclimatization with fluoride. To the control pots, 10 ml of sterile distilled Millipore water was added. Plants after harvesting were washed with tap water and deionized sterile water for further analysis. Half of the plants were used to determine the biomass and biochemical parameters, where as other half of plants were separated in to roots and shoots to measure the fluoride levels. The bioaccumulation and translocation factor were determined according to (Niu, *et al*., 2007). Total F content in plant organs and remaining soil was calculated by alkali fusion-ion selective technique, (Edye, 1982). Soil in each set was air dried and sieved and measured for physico chemical parameters. Garden soil as such was taken for Control.

Soil characteristics

The physiochemical characteristics like conductivity, pH, moisture content, bulk density, N, P, K, were calculated according to the standard method of (Marc and Jacques, 2003).

Photosynthetic pigments

Total chlorophyll (Total Chl), Chlorophyll a (Chl a) and Chlorophyll b (Chl b) (Arnon, 1949).

The leaves were cut into tiny pieces; and homogenized 100 mg material in 5 ml of chilled 100% acetone by grinding the leaves of control and stressed seedlings in a pre-cooled mortar and pestle until the powdered materials becomes completely non-green. The homogenate was centrifuged for 5 min at 3000 rpm at 4 in a cooling centrifuge. The pellet was discarded and the supernatant was re-adjusted to 5 ml acetone. To 1.6 ml of the supernatant, 0.4 ml double distilled water was added. The chlorophyll content absorbance of the resulting supernatant was recorded at 645 and 663 nm using a double beam UV-Vis spectrophotometer.

Chl a (mg/l) = 12.7 (A663) - 2.69 (A645)

Chl b (mg/l) = 22.9 (A645) - 4.68 (A663)

Total Chl (a and b) (mg/l) = a + b

Statistical analysis

Statistical parameters like, mean, Standard deviation, Standard error, ANOVA were calculated for each parameter. Post significance tukey's test was done.

RESULTS AND DISCUSSION

Soil parameters

The conductivity was increased in all the concentrations of fluoride compared to control. The pH which is the most important factor is slightly acidic in nature in all the bacterial treated ones. The moisture content was decreased in all the 3 concentrations of fluoride when compared to control. zero day data in Table 1. The N,P,K levels are almost same in all the fluoride treated plants. quite variation is changed in all the parameters after addition of bacteria, seen in Table 2.

Chlorophyll content

The chlorophyll contents decreased when exposed to fluoride the highest total chlorophyll value of 1.81 ± 0.006 is obtained in 100 mg/kg concentration of F + *P. vermicola* treated plants (Table 3, Fig 1). The chlorophyll a, b values were high (0.91 mg/L 0.41 mg/L) in 75 mg/kg of F+ *Bacillus cerus* with significant level of at 0.05 P value. Similar results were obtained by (Khushboo and Suphiya, 2016).

Growth parameters

The root length and shoot length in all the concentrations increased after addition of bacteria The root length and shoot length is depicted in Table 4 with high shoot length in 100 mg/kg of both *B. cerus* and *P. vermicola* treated plants and higher compared to control. The shoot length in fluoride treated plants is not higher than control ($p>0.05$). The root length is highest in 75 mg/kg of *Bacillus* treated

Table 1. Zero-day data analysis of soil.

S. No	Parameters	Values
1	Conductivity (µmhos/cm)	0.29 ± 0.1
2	p^H (10% w/v suspension)	6.8± 1.6
3	Bulk density (gm/cm^3)	0.98 ± 0.01
4	Moisture Content (% w/w)	4.2 ± 0.3
5	Potassium as K (% w/v)	1.1 ± 0.1
6	Phosphorous as P (% w/v)	24.9 ± 0.7
7	Nitrogen as N (% w/v)	9.8 ± 0.1

Table 2. The effect of fluoride on physico chemical parameters of *Bracharia distachya* L. soil after harvest.

S. No	Treatment	Set I			Set II			SET III			C
		50 mg/kgF	75 mg/kgF	100 mg/kgF	50 mg/kgF	75 mg/kgF	100 mg/kgF	50 mg/kgF	75 mg/kgF	100 mg/kgF	
1	Conductivity	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.005	0.16 ± 0.003	0.17 ± 0.005	0.18 ± 0.003	0.15 ± 0.005	0.13 ± 0.005	0.11 ± 0.003
2	pH	7.63 ± 0.13	7.1 ± 0.03	6.9 ± 0.03	6.33 ± 0.08	6.8 ± 0.03	6.2 ± 0.12	6.76 ± 0.06	6.3 ± 0.03	7.1 ± 0.08	7.0 ± 0.014
3	Bulk density	1.08 ± 0.03	1.03 ± 0.01	0.97 ± 0.01	1.49 ± 0.03	1.50 ± 0.01	1.64 ± 0.01	1.40 ± 0.003	1.34 ± 0.003	1.29 ± 0.008	1.0 ± 0.01
4	Moisture Content	4.1 ± 0.05	4.63 ± 0.1	4.60 ± 0.1	6.4 ± 0.08	5.90 ± 0.1	5.96 ± 0.06	5.7 ± 0.06	5.70 ± 0.05	5.76 ± 0.03	5.1 ± 0.1
5	Potassium	1.2 ± 0.1	0.8 ± 0.08	1.3 ± 0.1	1.5 ± 0.2	1.8 ± 0.05	1.9 ± 0.03	1.7 ± 0.05	1.4 ± 0.05	2.0 ± 0.05	1.2 ± 0.03
6	Phosphorous	21.5 ± 0.1	21.6 ± 0.1	23.2 ± 0.2	29.7 ± 0.1	30.8 ± 0.2	31.0 ± 0.4	24.4 ± 0.08	23.7 ± 0.08	25.2 ± 0.2	22.4 ± 0.30
7	Nitrogen	10.4 ± 0.1	10.0 ±0.2	9.8 ±0.2	11.6 ±0.1	12.5 ±0.06	12.8 ±0.08	11.8 ±0.05	12.0 ±0.05	11.7 ±0.1	9.6 ±0.08
The values denote Mean ± Standard Error values											

Table 3. The Chlorophyll a, b and total chl content of *Brachiaria distachya* L. stapf

S.no	Treatment	Chl a mg/L	Chl b mg/L	Total mg/L
1	Control	0.67 ± 0.03	0.11± 0.08	0.75 ± 0.02
2	BD1 (50 mg/kgF)	0.33 ± 0.02	0.15 ± 0.005	0.48 ± 0.02
3	BD2 (75 mg/kgF)	0.36 ± 0.005	0.15 ± 0.02	0.51 ± 0.03
4	BD3 (100 mg/kgF)	0.40 ± 0.006	0.12 ± 0.003	0.52 ± 0.008
5	BD4 (50 mg/kgF + *B.cerus*)	1.06 ± 0.03	0.39 ± 0.01	1.10 ± 0.0
6	BD5 (75 mg/kgF + *B. cerus*)	1.09 ± 0.03	0.41 ± 0.01	1.24 ± 0.02
7	BD6 (100 mg/kgF+ *B.cerus*)	1.21 ± 0.008	0.35 ± 0.008	1.31 ± 0.03
8	BD10 (50 mg/kgF+P. *vermicola*)	0.62 ± 0.008	0.19 ± 0.003	1.81 ± 0.006
9	BD11 (75 mg/kgF+ P. *vermicola*)	0.62 ± 0.008	0.20 ± 0.005	0.82 ± 0.01
10	BD 12 (100 mg/kgF+ P. *vermicola*)	0.64 ± 0.01	0.23 ± 0.01	0.84 ± 0.008

plants, when compared to *P. vermicola the B. cerus* treated plants showed much growth parameters. The biomass increased from 1.6 ± 0.4 to 1.8 ± 0.6 g in fluoride treated and further in *Bacillus* treated 5.9 ± 1.7 and 3.1 ± 0.2 in *P. vermicola* treated plants.

Fluoride accumulation

The bioaccumulation factor (BF) values ranges from 0.4 ± 0.2 to 3.4 ± 0.1 at different microbial consortium amendments (B.C and P.V) with various F concentrations. The B. distachya showed

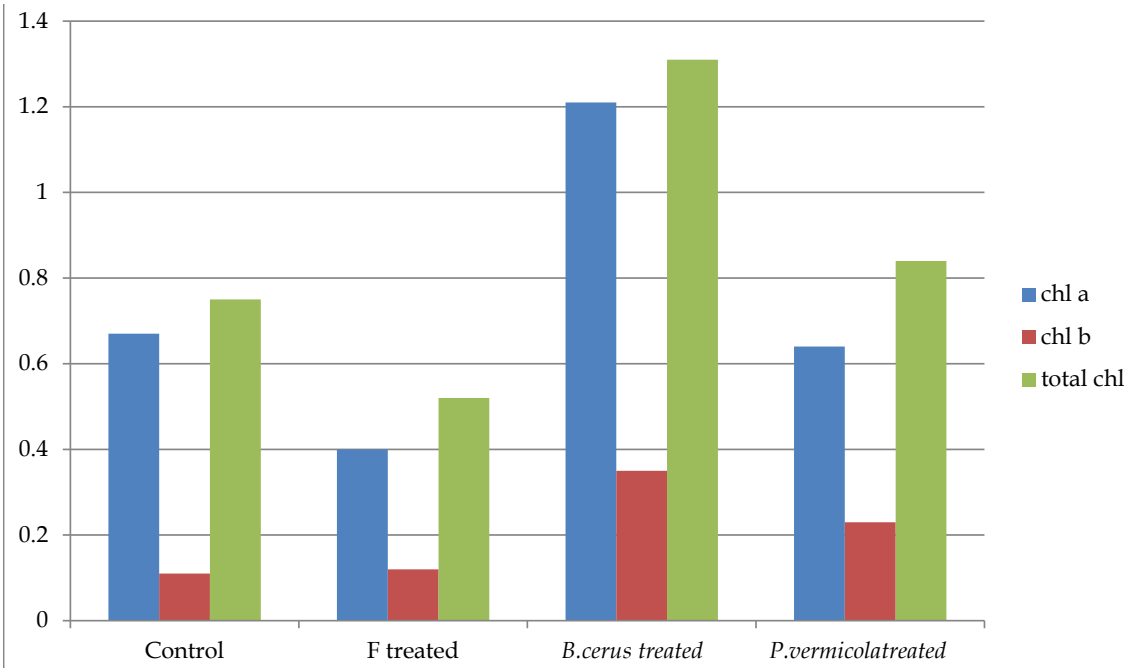

Fig. 1 The chlorophyll contents in different amendments of *B. distachya* L.

Table 4. The root length and shoot length of *B. distachya* L.

S. No	Treatment	Root length	Shoot length
1	C	9.7 ± 0.2***	5.0 ± 0.1
2	BD1(50 mg/kgF)	4.9 ± 0.6	3.6 ± 0.3
3	BD2 (75 mg/kgF)	7.0 ± 0.5	4.8 ± 0.6
4	BD3 (100 mg/kgF)	6.5 ± 0.3	4.2 ± 0.3
5	BD4 (50 mg/kgF + *Bacillus* group)	9 ±.0.3	5.4 ± 0.2
6	BD5 (75 mg/kgF + *Bacillus* group)	9.2 ± 0.5	5.5 ± 0.1
7	BD6 (100 mg/kgF + *Bacillus* group)	10 ± 0.3	5.9 ± 0.08
8	BD10 (50 mg/kgF + *P. vermicola*)	5.3 ± 0.5*	4.5 ± 0.2**
9	BD11 (75 mg/kgF + *P. vermicola*)	9.4 ± 0.2*	4.6 ± 0.2**
10	BD 12 (100 mg/kgF + *P. vermicola*)	10 ±.03 ***	5.1 ± 0.1**

Table 5. The TF and BF of *B. distachya* L.

S.no	Treatment	TF	BF
1	C	-	-
2	BD1 (50 mg/kgF)	0.39 ± 0.4	0.10± 0.08
3	BD2 (75 mg/kgF)	0.14 ± 0.03	0.43 ± 0.18
4	BD3 (100 mg/kgF)	0.09 ± 0.1	0.4 ± 0.2
5	BD4 (50 mg/kgF + *Bacillus* group)	1.7 ± 0.1	1 .1 ± 0.08
6	BD5 (75 mg/kgF + *Bacillus* group)	2.5 ± 0.2	1.1 ± 0.1
7	BD6 (100 mg/kgF+ *Bacillus* group)	2.4 ± 0.1	2.1 ± 0.2
8	BD10 (50 mg/kgF+ *P. vermicola*)	1 ± 0.1	1.5 ± 0.05
9	BD11 (75 mg/kgF+ *P. vermicola*)	1.4 ± 0.2***	0.5 ± 0.08
10	BD12 (100 mg/kgF+ *P. vermicola*)	1.4 ± 1.9	1 ± 0.1

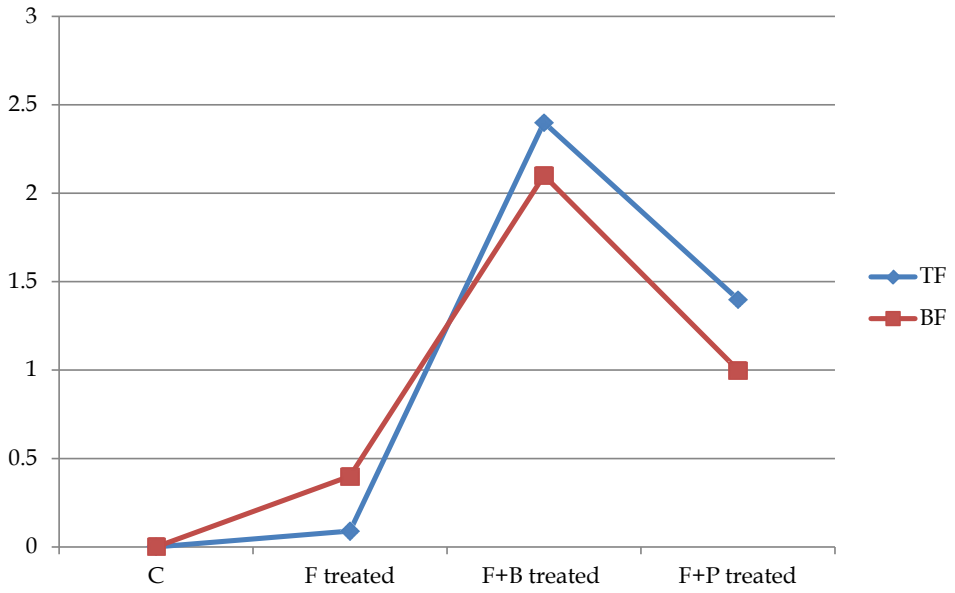

Fig. 2 The TF and BF of *B. distachya* L. *at 100 mg/kg*.

a translocation factor (T.F) (Table 5) (Fig. 2) which ranges from 0.09 ± 0.1 in fluoride treated plants to 2.4 ± 1.0 in *Bacillus* treated to 2.1 ± 0.03 in whole genome and 1.4 ± 1.9 in *P. vermicola* treated plants in 100 mg/kg F. The ratio of translocation factor was greater than 1 mean hyper accumulator plant (Gupta, 2009). The result of the present investigation showed that microbial consortium had high ability and increased the efficiency of F accumulation. Plant growth promoting bacteria deserve a special attention because they can directly improve the phytoremediation process by increasing the metal solubility through altering soil pH, release of chelators (e.g. organic acids, siderphores), and they also help to increased metal mobility for high accumulation in plant organ (Rajkumar, *et al*., 2010).

CONCLUSION

Recent study demonstrated that *Bacillus cerus* improve abilities of *B. distachya* L phytoremediation efficiency, mineral content and increase plant biomass as compare to the control. Results suggested that F had negative impact on chlorophyll pigments and mineral content of *B. distachya* L. Based on the results soil nutrients contents are sensitive to F concentration in the soil. *B. distachya* L needs the microbial treatment in soil to decline F toxicity. However, this work could be applied in the field scale and can be commercialized using microbial treatment with F. *Bacillus cerus* (B.C) for the use for remediation, counter balance nutrients content and could contribute to sustainable agriculture especially in areas where F contaminated problem in soil and water.

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