

Long Term Impact of Irrigation with Textile Waste Water and an Ecofriendly Approach for Heavy Metal Degradation

M.N. Abubacker and B. Kirthiga

Abstract The major environmental pollution encountered by the textile effluent is the presence of color, dissolved solids and refractory materials such as detergents, organic pollutants, heavy metal ions which might arise from dyes. Discharge of the textile effluent to the nearby water body alters the physical, chemical and biological nature of the water bodies, seep into the aquifer, decreases the oxygen level in water leading to death of the aquatic biota and biomagnification. The present study was developed to comprehend the pollution caused by the local textile mill to the environment and their remediation. The physicochemical analyses of discharged effluent, adjacent soil and the cultivated crop near the discharge point at Tirupur, Tamil Nadu, India were analyzed and found to be altered. The organic matter and total available nitrogen content in the soil was found to be reduced, the high concentration of heavy metal in the seeds of *Pennisetum typhoides* characterized with SEM–EDX indicates soil lost its fertility. The 16S rRNA sequence of the predominant bacterial isolates exhibiting maximum resistant level towards heavy metal was deposited in NCBI Genebank and designated as *Bacillus cereus*, *Pseudomonas putida*. Further, the efficacy of the immobilized strains with *Luffa aegyptiaca* to accumulate/uptake heavy metals (Cr and Zn) under optimized conditions and their subsequent toxicity assessment in response to waste water irrigation were analyzed.

Keywords Textile effluent • Ecofriendly approach • Heavy metal • Degradation

M.N. Abubacker (✉)

Department of Biotechnology, National College, Tiruchirappalli, Tamil Nadu, India
e-mail: abubacker_nct@yahoo.com

B. Kirthiga

Department of Microbiology, D G Vaishnav College, Chennai, Tamil Nadu, India

© Springer International Publishing AG 2017

M. Prashanthi et al. (eds.), *Bioremediation and Sustainable Technologies*

for *Cleaner Environment*, Environmental Science and Engineering,

DOI 10.1007/978-3-319-48439-6_12

1 Introduction

The rapid economic growth achieved after globalization has adversely affected the quality of the environment and has become a major threat to sustainable development (World commission on environment and development 1987). The pollution load discharged into the environment has exceeded the assimilative capacity and affects sectors like agriculture, domestic water supply, fisheries, public health and biodiversity. Among the entire, industrial processing units textile processing units use huge quantities of water, the effluents discharged by these units are generally hot alkaline, strong smelling, coloured with large amount of toxic substances and they accumulated into the soil. Most of the small scale industries practice traditional processing technology is not treating their effluents properly (Nelliyat 2007). The use of metals and chemicals in dyeing industries generates large quantities of liquid effluent loaded with high levels of heavy metals, often as bioavailable forms (Calderon et al. 2003). For these reasons, interest has arisen recently in the investigation of some alternative methods involving only ecofriendly materials, that leads to metal recovery with minimal impact on the environment and are cost effective. Potentially bioremediation is cheaper than the chemical and physical methods and can deal with even lower concentration of contaminants more effectively although the process may take longer. Microbial populations in metal polluted environments adapt to toxic concentrations and become metal resistant. Different species of *Aspergillus*, *Pseudomonas*, *Bacillus* have been reported as efficient metal reducers and their detoxifying ability can be manipulated (Rajbanshi 2008; World commission on environment and development 1987). This biological process for removal of metal ions from solution can be divided into biosorption and bioaccumulation. Bioaccumulation is the uptake of metal by species means of metabolism dependent processes which may involve both transport into cell and partitioning into intracellular components.

2 Materials and Methods

In 2011, water, soil and plant (*Pennisetum typhoides*) samples from an irrigation field receiving discharge from small scale textile industries of Tirupur, located on the banks of the Noyyal River were collected (Fig. 1). A portion of the effluent and soil samples were acidified to $\text{pH} \leq 2$ with Conc. HNO_3 , refrigerated to prevent the volume change due to evaporation and the concentration of various heavy metal present in all the samples. Crops were washed, air dried, crushed and ground to a powder using mortar and pestle for metal analysis. Soil samples from the rhizosphere region to recover the *Arbuscular mycorrhizal* spores. The samples were processed in 24 h for isolation, enumeration, identification and characterization of microbial flora.



- (A) Main effluent discharge from small scale dyeing unit
 (B) 100 meters away from the effluent discharge point
 (C) 200 meters away from the effluent discharge point
 (D) 500 meters away from the discharge point
 (E) 1km away (treated effluent mix with irrigation water)
 (F) Reference site

Fig. 1 Sample collection area

2.1 Physico-chemical Characterization of Effluent, Soil and Crop

The physico-chemical parameters like color, odour, pH, temperature, TS, TSS, TDS, Chlorides, BOD and COD of the effluent, soil texture (Bouyoucos 1962),

water holding capacity (Rayment and Higginson 1992), electrical conductivity, cation exchange capacity, organic carbon, free calcium carbonate, organic matter, nitrogen content, bioavailable metal ions (Atomic Absorption Spectrophotometer), sulphide (acidified ammonium acetate extract) whereas bio available potassium, nitrate and phosphorus (Spectronic 20 flame photometer) were analyzed according to standard methods (APHA 2005). Rhizosphere was analyzed for the number of AM spores per 50 g by wet sieving and decanting technique using a series of sieves (50, 75, 100 μm mesh size). Spores were counted on filter paper under a dissection microscope and grouped according to their morphological characteristics (Abubacker et al. 2014).

Detection of metal concentration: The effluent (Babushakila 2009), soil (Banytan et al. 2000) and the crop (Patel et al. 2004) were digested and analyzed using hollow cathode lamps for chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn) and nickel (Ni) at 357.9, 228.8, 217.0, 213.9 and 232.0 nm respectively at Sophisticated Analytical Instrument Facility (SAIF), in Indian Institute of Technology Madras (IITM), Chennai using Inductively Coupled Plasma Atomic Emission Spectroscopy.

2.2 Characterization of *Pennisetum typhoides* Seeds Elemental Analysis

Elemental analysis and Scanning Electron Microscopic images coupled with energy dispersive Xray consisting 3.5 and 2.5 nm resolution for tungsten filament and LaB6 and EDX detector resolution 133 eV of *Pennisetum typhoides* seeds was assessed (Make: SEM—TESCON—Czechoslovakia). The seeds were dried in hot air oven at 50 °C for 1 h and then powdered using mortar and pestle. The powdered material was placed in steel stab with carbon tape and sputter coated with gold particle for 50 s in high vacuum conditions (Vinod and Sasidhar 2010).

2.3 Enumeration, Isolation and Identification of Bacteria

The soil and effluent samples were serially diluted and plates yielding 30–300 colonies were selected for enumeration. Further, isolation and identification was carried by following standard procedures (Cappuccino and Sherman 2002) and numbered as TEAK 01–TEAK 13 and identified at generic level using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt et al. 1994). Upon the percentage of distribution, the predominant isolates from soil and effluent sample were chosen and characterized. CTAB method was used to extract large quantities of heavy molecular weight DNA from bacteria. The pellet obtained

was washed with 70% ethanol, dried and dissolved in 50 µl TE buffer with pH 7 or water (Prabha et al. 2013).

For sequencing of 16S rRNA gene region of the selected bacterial isolates, standard PCR amplification was carried out using Ready-To-Go PCR Beads (Sigma Aldrich) containing 5 µl of 10X MTP Taqbuffer, 1 µl of 10 Mm dNTP mix, 0.5 µM of each primer (5' AACGGCTCACCAAGGCGACG 3' and 5' GTACCGTCAAGGTGCCGCCC 3'), 0.05 µl of MTP Taq DNA polymerase and 10 ng of template DNA in a 50 µl of reaction mixture in a thermal cycler. The cycling profile included an initial denaturation of 94 °C for 1 min followed by 30 cycles of denaturation (94 °C for 1 min), annealing (5 °C for 60 s) and Extension (72 °C for 65 s) and cooled at 4 °C. 10 µl of the amplified products were subjected to electrophoresis, visualized under UV transilluminator. The amplified PCR products were purified using QIA quick PCR purification kit (Qiagen GmbH, Hilden, Germany) as recommended by the manufacturer. The DNA sequences were run on an ABI 310 automated sequencer using the chain-termination method with big-dye terminators (Applied Biosystems, Foster, CA, USA). Automated base calls were checked by manual inspection of the electrophorogram of both forward and reverse sequences. The base call conflicts were resolved by alignment and comparison of both strands using the Seq Scape® software v 2.5 (Applied Biosystems, Foster, CA, USA) and blasted in the GenBank database (www.ncbi.nlm.nih.gov) for similarity search.

The Phylogenetic tree was constructed using the BLAST tree tool to demonstrate the evolutionary relationship between species, a group of genes related through a process of divergent evolution from a common ancestor or the result of convergent evolution was demonstrated.

2.4 Maximum Resistance Levels (MRL) of Bacterial Isolates Against Cr and Zn

MRL of the purified and characterized bacterial isolates against the HM was determined by preparing nutrient agar medium amended with varying concentrations (mg/L) of the heavy metals Zn (100–3600) and Cr (25–3000). The surface of the heavy metal incorporated agar plates were streaked with pure isolates and incubated at 37 °C for 48–72 h. The highest concentration of heavy metal that allows growth after 48 h of incubation was designated as MRL (Rajbanshi 2008). Textile effluent *Bacillus* sp. MTCC 5342 and *Pseudomonas* sp. MTCC 2847 were used as positive control.

2.5 Bioaccumulation Potential of Bacterial Isolates

A stock metal solution of (4000 mg L⁻¹) Zn and Cr was prepared by dissolving AR grade salts of ZnSO₄ · 7H₂O and K₂Cr₂O₇ in double distilled water. The working concentration (100, 500, 1000 mg L⁻¹) were prepared from the stock metal solution.

2.5.1 Optimization of the Accumulation Process in Bacteria

Investigation of process dependency on pH: 2% inoculum concentration of bacterial isolates (2 days old cultures) were inoculated separately into 5 sets of 250 ml Ehrlimayar's flask contained 100 ml of minimal salt medium containing 100 mg L⁻¹ of chromium or 100 mg L⁻¹ of zinc. The pH varied from 6.5 to 8.5 (6.5, 7.0, 7.5, 8.0 and 8.5). The pH of the medium was adjusted using dilute HCL or NaOH and incubated on a rotary shaker in 150 rpm speed at 37 °C with control containing cells inoculated medium without heavy metal. Every 6 h interval, the residual metal concentration was analyzed. Cells were harvested by centrifugation at the end of 48 h incubation. The biomass was weighed, digested and analyzed for accumulation of chromium and zinc ions (Congeevaram et al. 2007).

Dependency of the metal concentration for nourishment: Minimal salt medium (pH 7.5) containing varying concentrations of metal ions ranging from 100 to 3000 mg L⁻¹ was incubated with 2% inoculum at 37 °C. Every 6 h interval, a flask of varying metal concentration was analyzed for the residual metal concentration. At the end of 48 h, the cells were harvested and the biomasses were weighed and the concentrations of heavy metals were analyzed (Congeevaram et al. 2007).

After centrifugation, the filtrate and the pellet were digested with conc. HNO₃ in slow boiling, evident by brownish fume (Babyshakila 2009). The concentration of chromium was determined using diphenyl carbazide assay method and concentration of zinc was analyzed using inductively Coupled Plasma Atomic Emission Spectroscopy as described earlier.

Compatibility analysis of the Screened, adapted Bacterial strains: The Compatibility among the isolates, i.e. the antagonistic effect of bacterial isolate on the other bacterial strain was studied by standard well-cut method. Nutrient agar was prepared and poured into sterilized petri dishes and allowed to solidify. Two wells of 6 mm diameter were punctured at equidistant positions adjacent to one another in 9 cm petri dish containing the culture media using sterile cork borers. Hundred µl of each bacterial suspension was added to the wells and the plates were incubated at 37 °C. The diameter of the zone was measured in mm (Chin et al. 2001).

2.6 Bioremoval of Cr (VI) and Zn (II) Ions from Waste Water Through Accumulation Process

Immobilization: An inexpensive matrix *Luffa aegyptiaca*, commonly known as bath sponge was used for immobilization of bacteria. The dry matrix was sliced into pieces, washed with distilled water and dried in oven at 105 °C. The dried matrices weighing approximately 0.1 g were inoculated with 1 ml of bacterial suspension in petri plates containing 20 ml of sterilized nutrient broth and incubated. The Immobilized material was removed after 48 h and tried for remediation studies (Iqbal and Edyvean 2005).

Bioaccumulation of Cr (VI) and Zn (II) from textile dye effluent waste water: 100 ml of filtered Textile effluent aliquots were taken in six 250 ml Erlenmeyer flasks and their initial chromium and zinc ion concentrations were assayed. To this effluent, growing cells of bacterial isolate TEAK 03 and TEAK 06, bacterial consortium immobilized on *Luffa aegyptiaca* were added separately, enriched with 0.01% glucose and incubated under the optimized condition respectively. After incubation, the immobilized bacterial cells were harvested and the aliquots were digested using 10 ml of triple acid solution (HNO₃, H₂SO₄ and HClO₄ in 9:2:1 proportion respectively) till the effluent becomes colorless (Ameer Basha and Rajaganesh 2014). Filtered and unfiltered effluent sample without inoculum were used as control. Later, the digested sample was analyzed for the residual metal ions Cr and Zn as per the standard methods.

2.7 Toxicity Assessment of Treated Waste Water—Bioaccumulation Process

The Physicochemical parameters like pH, EC, total suspended solids, total solids, total dissolved solids, chlorides, BOD, COD, metal concentration of the treated effluent was analyzed as per standard procedures (APHA 2005). Seeds of *Pennisetum typhoides* were surface sterilized with 95% ethanol and then treated with 0.2% mercuric chloride and washed three times with sterile distilled water and blot dried under aseptic conditions. Seeds for the study were obtained from the Institute of Agricultural Research, Tamil Nadu. The seeds were soaked in 40 ml of treated wastewater for 30 min and dried under aseptic condition for 4 h to assess the toxic effect of treated waste water where as the seeds soaked in distilled water and untreated wastewater was used as a control. A total of six seeds were placed in each petri plate containing a moist filter paper and the plates were maintained in a growth chamber for 7 days, the germination percentage was calculated (Prashanth and Mathivanan 2009). The concentration of chromium and zinc was analyzed by digesting the germinated seeds and the filtrate samples were analyzed for Cr and Zn concentration using Inductively Coupled Plasma Atomic Emission Spectroscopy (Patel et al. 2004).

3 Results and Discussion

3.1 Physico-chemical Characterization of Effluent, Soil and Crop

Various parameters *viz* colour, smell, pH, temperature, electrical conductivity, total dissolved solids, metal concentration of the collected effluent samples, soil samples and VAM spores were analyzed (Tables 1, 2, 3, 4, 5, 6, 7 and 8). The color of the effluents at the sites 1–6 are blackish green, greenish black, slight greenish black, slight greenish, slight greenish, slight greenish and colourless respectively. The offensive odour was found at site 1, pungent odour at site 2, 3 and effluent at site 4–6 were odourless. Because of the effluent pollution, the colour of the soils were dark brown at site 1, reddish brown at site 2–3, brown at site 4, light yellow and site 5 and site 6 remains colourless. There was pungent odour in site 1, fishy in site 2 and rests were muddy in odour. The site 1 is clay loam in texture, whereas in remaining sites were sandy.

The study clearly demonstrates that soil adjacent to the effluent discharge point experiences a change in physicochemical parameters from site to site, well above the CPCB limits (Asfaw 2014; Furaha et al. 2015; Mohabansi et al. 2011; Rohilla et al. 2012). The elevated metal concentrations may be due to high content of metal ions present in the dye and mordants (HRB 38, Nickle-phthalocyanine complex, HRV5 copper containing azo dye, zinc yellow pigment and chrome yellow), and discharge of unbounded metal ions into the waste water and thus direct and continuous exposure alters the physicochemical properties and accumulation of metal ions in the soil (Jayashree et al. 2011). Thus, waste water being used for crops cultivation results in built up toxic substances in the soils (Joshi and Santani 2012; Joshi et al. 2011; Mahawar and Akhtar 2015) and affects the fertility. Reduced agricultural production due to accumulation of heavy metals is concern of regional global scale (Muhammad et al. 2015). Long term irrigation with heavy metal contaminated soil, accumulation of heavy metal in the root and shoot system of the plant (Jayashree et al. 2011).

Significant variation in AMF spore density was observed in all the sites (Table 9). Over all 22 different AMF species were isolated from the rhizosphere of *Pennisetum typhoides*, commonly cultivated near the contaminated sites. *Acaulospora elegans*, *Acaulospora morrowiae*, *Sclerocystis rubiformis* were not present in the contaminated soil (Fig. 2).

Thus, the results conclude plants grown well with heavy metal contaminated soil on long term irrigation reduces the AM spore population and changes its diversity. The symbiotic association formed by the mycorrhiza fungi in the roots of plant responsible for nutrient transfer is affected in the polluted site (Abubacker et al. 2014).

Table 1 Physico-chemical characterization of effluent

S. No.	Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Reference	CPCB limit (APHA 2005)
1	pH	11.3 ± 0.2	10.4 ± 0.1	9.7 ± 0.2	8.3 ± 0.1	7.9 ± 0.2	7.6 ± 0.2	6.8 ± 0.1	6.0-9.0
2	Temperature (°C)	32 ± 1.0	32 ± 1.0	30 ± 1.0	29 ± 1.0	30 ± 1.0	30 ± 1.0	20 ± 1.0	28-30
3	Total solids (mg/L)	4432 ± 15.0	4168.6 ± 13.4	3868.8 ± 16.9	3347.6 ± 14.1	2744.8 ± 22.9	2238.4 ± 23.5	1986.3 ± 18.0	NA
4	Total suspended solids (mg/L)	283 ± 5.0	211.6 ± 3.4	179.8 ± 6.9	158.6 ± 4.1	127.8 ± 2.9	100.4 ± 3.4	92.3 ± 2.8	100
5	Total dissolved solids (mg/L)	4194 ± 0.0	3957 ± 10.0	3689.0 ± 10.0	3189 ± 10.0	2617 ± 20.1	2138 ± 20.1	1894 ± 10.0	500-2000
6	Chlorides (mg/L)	1134.2 ± 8.0	1094.1 ± 7.0	1003 ± 6.0	989.6 ± 7.2	956.3 ± 5.6	934.8 ± 4.9	928.5 ± 5.6	250-1000
7	BOD (mg/L)	108 ± 10.1	98 ± 20.0	86 ± 20.0	72 ± 10.0	64 ± 20.0	41 ± 10.0	20 ± 10.0	30
8	COD (mg/L)	997 ± 30.1	864 ± 10.1	791 ± 20.1	698.4 ± 10.1	564.3 ± 20.1	498.6 ± 30.1	184 ± 10.1	250

*NA—Not Applicable

Table 2 Concentration of metal ions present in the effluent

S. No.	Metal (mg/L)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Reference	WHO std (mg/L)	Permissible limit (mg/L)
1	Cr	24.5 ± 0.37	19.6 ± 0.42	14.8 ± 0.11	11.2 ± 0.48	9.41 ± 0.89	7.32 ± 0.03	0.03 ± 0.01	0.05 ± 0.01	0.05
2	Cu	17.7 ± 0.91	13.2 ± 0.16	9.75 ± 0.54	6.49 ± 0.21	4.63 ± 0.21	2.47 ± 0.94	0.72 ± 0.02	1 ± 0.02	0.5
3	Zn	36.1 ± 1.27	31.2 ± 1.01	26.3 ± 2.1	19.8 ± 2.35	15.6 ± 2.36	11.2 ± 0.62	3.97 ± 0.97	5 ± 1.2	0.2
4	Ni	0.78 ± 0.54	0.61 ± 0.01	0.53 ± 0.02	0.41 ± 0.01	0.29 ± 0.02	0.17 ± 0.01	0.06 ± 0.01	0.01 ± 0.00	-
5	Cd	7.28 ± 0.42	6.08 ± 0.02	4.94 ± 0.01	3.96 ± 0.03	2.21 ± 0.01	1.08 ± 0.03	0.02 ± 0.00	0.05 ± 0.23	0.01
6	Pb	1.79 ± 0.13	0.99 ± 0.85	0.29 ± 0.01	0.07 ± 0.01	ND	ND	0.74 ± 0.01	0.07 ± 0.01	0.065

*ND—Not Detected

Table 3 Exchangeable form of metal ions in effluent sample

S. No.	Metal	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
1	Cr	19.32 ± 0.41	16.36 ± 0.21	11.29 ± 0.42	09.49 ± 0.36	8.94 ± 0.81	7.58 ± 0.01
2	Cu	14.84 ± 0.87	11.24 ± 0.20	8.54 ± 0.19	5.68 ± 0.21	4.12 ± 0.21	1.34 ± 0.26
3	Zn	30.42 ± 1.42	28.62 ± 1.31	23.45 ± 2.2	18.81 ± 2.41	13.00 ± 2.01	9.88 ± 0.81
4	Ni	0.57 ± 0.54	0.41 ± 0.02	0.37 ± 0.01	0.28 ± 0.02	0.09 ± 0.01	0.04 ± 0.01
5	Cd	6.13 ± 0.62	5.13 ± 0.03	3.27 ± 0.02	2.54 ± 0.01	1.98 ± 0.02	0.97 ± 0.02
6	Pb	0.99 ± 0.13	0.69 ± 0.12	0.54 ± 0.02	0.32 ± 0.02	ND	ND

*ND—Not Detected

Table 4 Physico-chemical characterization of effluent discharged soil

S. No.	Parameters soil	Site 1	Site 2	Site3	Site4	Site 5	Site 6	Reference site	Permissible limit (CPCB)
1	pH	11.24 ± 0.05	10.31 ± 0.01	9.87 ± 0.02	9.68 ± 0.07	8.9 ± 0.03	8.4 ± 0.04	7.61 ± 0.02	7-8.5
2	EC (µS/cm)	63.8 ± 3.0	61.7 ± 2.1	59.36 ± 2.9	57.7 ± 3.0	54.28 ± 1.0	51.68 ± 2.0	46.7 ± 2.0	0-1.5
3	Holding capacity (%)	42.94 ± 2.0	51.26 ± 2.0	59.26 ± 2.0	64.98 ± 1.0	70.25 ± 1.0	74.26 ± 2.0	80.21 ± 1.0	NA
4	Organic carbon (%)	1.71 ± 0.1	1.6 ± 0.2	1.2 ± 0.1	0.91 ± 0.2	0.93 ± 0.1	0.89 ± 0.2	0.71 ± 0.2	0.5-0.75
5	Free CaCO ₃ (%)	8.700 ± 0.12	8.13 ± 0.22	7.56 ± 0.24	6.87 ± 0.14	6.26 ± 0.11	5.12 ± 0.23	2.89 ± 0.31	NA
6	Organic matter (%)	0.41 ± 0.01	0.49 ± 0.01	0.54 ± 0.01	0.63 ± 0.02	0.69 ± 0.01	0.74 ± 0.03	0.91 ± 0.02	0.8-1.30
7	Nitrogen content (%)	0.19 ± 0.009	0.11 ± 0.001	0.09 ± 0.001	0.09 ± 0.001	0.08 ± 0.001	0.07 ± 0.001	0.097 ± 0.006	0.043-0.065
8	Potassium (mg/Kg)	589.3 ± 1.3	574.03 ± 1.0	556.09 ± 2.0	543.02 ± 3.0	517 ± 3.0	503 ± 1.0	494.06 ± 1.0	NA
9	Nitrate (mg/Kg)	0.91 ± 0.03	0.83 ± 0.01	0.76 ± 0.02	0.67 ± 0.01	0.54 ± 0.01	0.42 ± 0.02	0.30 ± 0.02	NA
10	Phosphorus (mg/Kg)	19.34 ± 0.24	17.26 ± 0.34	15.41 ± 0.01	11.28 ± 0.12	10.28 ± 0.04	9.24 ± 0.17	7.26 ± 0.07	NA
11	Sulphate (mg/Kg)	29.7 ± 3.0	26.1 ± 2.0	21.4 ± 2.0	19.5 ± 2.1	17.3 ± 3.0	15.1 ± 2.0	12.18 ± 1.0	200
12	Calcium (mg/Kg)	309 ± 30.0	301 ± 10.0	294 ± 9.0	281 ± 08.0	269 ± 10.0	257 ± 10.0	149 ± 11.0	200
13	Magnesium (mg/Kg)	0.97 ± 0.02	0.86 ± 0.03	0.079 ± 0.01	0.071 ± 0.02	0.065 ± 0.04	0.061 ± 0.01	0.56 ± 0.03	NA
14	Sodium (mg/Kg)	41.38 ± 1.0	57.02 ± 3.0	61.08 ± 2.0	67.04 ± 3.0	71.14 ± 1.0	84.03 ± 2.0	118.07 ± 2.0	200

*NA—Not Applicable

Table 5 Concentration of metal ions present in the soil

S. No.	Metal (mg Kg ⁻¹)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Reference site	WHO standard (mg Kg ⁻¹)
1	Cr	29.89 ± 1.23	21.86 ± 0.77	18.61 ± 0.94	13.47 ± 0.03	11.21 ± 0.55	9.45 ± 0.43	0.03 ± 0.03	0.05
2	Cu	21.55 ± 0.57	19.27 ± 0.27	12.43 ± 0.31	9.51 ± 0.27	7.61 ± 0.21	4.97 ± 0.25	0.98 ± 0.12	1
3	Zn	47.83 ± 3.14	41.32 ± 1.98	33.28 ± 2.40	24.78 ± 1.69	20.03 ± 1.87	14.98 ± 2.11	4.52 ± 1.01	5
4	Ni	1.27 ± 0.97	0.99 ± 0.03	0.78 ± 0.01	0.61 ± 0.02	0.43 ± 0.01	0.31 ± 0.02	0.24 ± 0.01	-
5	Cd	9.97 ± 0.69	8.77 ± 0.21	6.28 ± 0.31	5.89 ± 0.71	4.37 ± 0.42	2.17 ± 0.22	0.91 ± 0.02	0.05
6	Pb	2.31 ± 0.72	1.87 ± 0.51	0.97 ± 0.24	0.77 ± 0.14	0.58 ± 0.05	0.27 ± 0.04	0.84 ± 0.03	0.1

Table 6 Exchangeable form of metal ions in soil sample

S. No.	Metal (mg Kg ⁻¹)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
1	Cr	26.23 ± 1.54	19.87 ± 0.81	15.28 ± 0.23	10.68 ± 0.01	8.68 ± 0.21	7.42 ± 0.49
2	Cu	19.23 ± 0.57	16.73 ± 0.15	11.84 ± 0.26	7.88 ± 0.32	5.96 ± 0.33	3.01 ± 0.07
3	Zn	40.08 ± 2.98	36.41 ± 0.21	29.62 ± 0.31	21.02 ± 1.02	16.47 ± 1.42	09.27 ± 3.84
4	Ni	0.94 ± 0.02	0.68 ± 0.01	0.54 ± 0.02	0.31 ± 0.02	0.15 ± 0.02	0.09 ± 0.03
5	Cd	8.41 ± 0.21	6.95 ± 0.34	5.87 ± 0.42	3.94 ± 0.81	2.88 ± 0.62	1.42 ± 0.31
6	Pb	1.23 ± 0.11	0.85 ± 0.62	0.43 ± 0.26	0.26 ± 0.21	0.09 ± 0.01	0.07 ± 0.01

Table 7 Concentration of metal ions present in the plant

Crop cultivated	Plant part	Irrigated near to effluent discharge point		Irrigated 1 km away from discharge point		Reference	
		Cr	Zn	Cr	Zn	Cr	Zn
<i>Pennisetum typhoides</i>	Root	0.73 ± 0.02	6.97 ± 0.83	0.59 ± 0.08	5.61 ± 0.51	0.001 ± 0.00	1.03 ± 0.01
	Seed	0.49 ± 0.04	4.87 ± 0.06	0.37 ± 0.02	3.46 ± 0.05	ND	0.04 ± 0.01

*ND—Not Detected

Table 8 AMF Spore density associated with plantation near the effluent discharge point (Total No. of spores/100 g of soil)

S. No.	AMF Spores	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Reference
1	<i>Acaulospora elegans</i>	ND	ND	ND	ND	02	09	18
2	<i>Acaulospora morrowiae</i>	ND	ND	ND	ND	ND	11	29
3	<i>Glomus amphispurum</i>	96	101	117	139	146	154	194
4	<i>Glomus albidium</i>	29	37	49	56	63	74	91
5	<i>G. citricolon</i>	19	25	34	48	53	61	73
6	<i>G. claroideum</i>	ND	ND	ND	ND	ND	ND	19
7	<i>G. deserticola</i>	ND	ND	ND	ND	ND	ND	61
8	<i>G. dimorphicum</i>	ND	ND	ND	ND	ND	ND	29
9	<i>G. etunicatum</i>	ND	ND	ND	ND	ND	ND	34
10	<i>G. geosporum</i>	17	29	35	47	61	73	79
11	<i>G. macrocarpum</i>	ND	ND	ND	ND	ND	ND	52
12	<i>G. melanosporum</i>	29	42	51	68	91	86	113
13	<i>G. microcarpum</i>	18	31	48	67	74	83	96
14	<i>G. mossae</i>	62	87	93	114	128	153	263
15	<i>G. multisubstensum</i>	ND	ND	ND	ND	ND	ND	22
16	<i>G. oculatum</i>	ND	ND	ND	ND	ND	ND	39
17	<i>G. pulvinatum</i>	ND	ND	ND	ND	ND	ND	28
18	<i>G. radiatum</i>	ND	ND	ND	ND	ND	ND	35
19	<i>G. tortuosum</i>	ND	ND	ND	ND	ND	ND	33
20	<i>G. fulvum</i>	ND	ND	ND	ND	ND	ND	47
21	<i>Sclerocystis rubiformis</i>	ND	ND	ND	ND	ND	07	26

*ND—Not Detected

3.2 Scanning Electron Microscopy—Energy Dispersive X-Ray (SEM–EDX) of *Pennisetum typhoides* Seeds Elemental Analysis

SEM–EDX elemental analysis confirms the presence of Cr and Zn in the seeds of *Pennisetum typhoides* irrigated near the effluent discharge point and the concentration was found to be 0.22 and 1.30% and the seeds cultivated at 1 km distance from the discharge point showed decreased concentration (0.08 and 0.44%) whereas the control seeds does not show the occurrence of metals (Figs. 3, 4 and 5).

Table 9 Biochemical characterization of isolated bacterial stains from effluents

Test	ETEAK1	ETEAK2	ETEAK3	ETEAK4	ETEAK5	ETEAK6	ETEAK7	ETEAK8	ETEAK9	ETEAK10	ETEAK11	ETEK12	ETEAK13
Gram stain	+	+	-	-	+	+	-	+	+	+	-	+	+
Motility	+	+	+	-	+	+	+	+	-	-	-	+	-
Spore	+	-	-	-	-	+	-	-	+	-	-	-	+
Indole	-	-	-	-	-	-	-	-	-	-	+	-	-
MR	+	+	+	+	-	-	-	+	-	+	+	+	+
VP	+	+	+	+	-	+	+	+	-	+	+	+	+
Citrate	+	+	+	+	+	+	-	+	-	-	+	+	+
Catalase	+	+	+	+	+	+	+	-	+	+	-	-	+
Oxidase	+	+	-	-	-	+	+	-	+	+	-	+	+
TSI	∓	∓	+	∓	+	-	∓	∓	±	±	∓	-	+
H2S	-	-	-	+	-	-	-	-	-	-	-	-	-
Urease	-	-	-	+	-	-	-	-	+	-	+	-	-
Starch	+	-	+	-	-	-	-	-	+	-	+	-	+
Gelatin	-	-	+	-	-	+	+	-	-	+	±	-	-
Nitrate	+	-	+	+	-	+	+	+	-	+	+	-	+

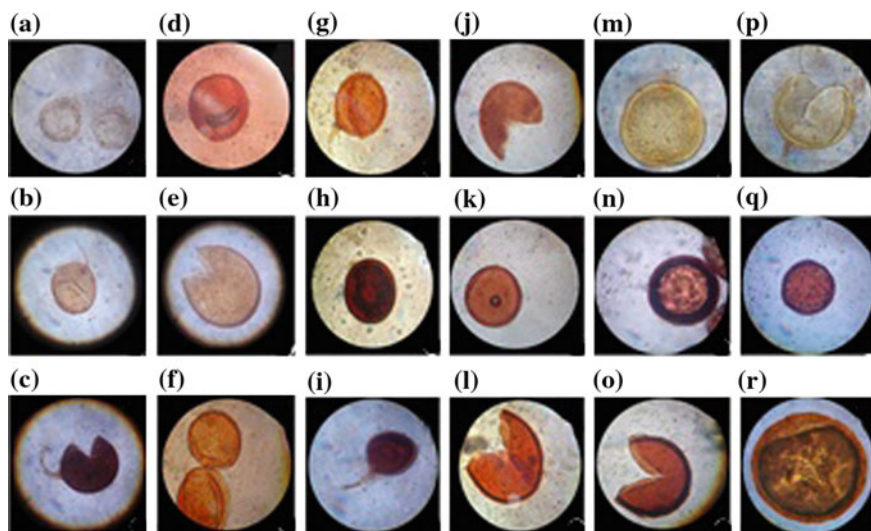


Fig. 2 AM spore (400X) identified in contaminated site. **a** *Sclerocystis rubiformis*; **b** *Acaulospora elegans*; **c** *Acaulospora morrowiae*; **d** *Glomus albidum*; **e** *G. citricolon*; **f** *G. claroideum*; **g** *G. deserticola*; **h** *G. dimorphicum*; **i** *G. geosporum*; **j** *G. occulatum*; **k** *G. melanosporum*; **l** *G. etunicatum*; **m** *G. mossae*; **n** *G. fulvum*; **o** *G. radiatum*; **p** *G. pulvinatum*; **q** *G. microcarpum* and **r** *G. macrocarpum*

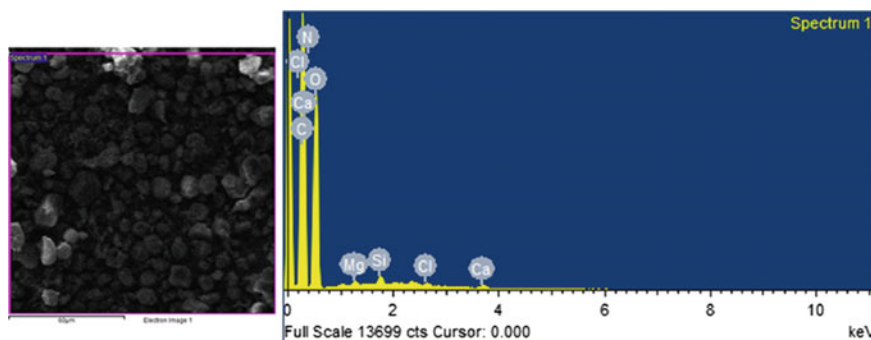


Fig. 3 SEM-EDX: Seeds of *Pennisetum typhoides* irrigated with borewell water (control)

3.3 Enumeration, Isolation and Identification of Bacteria

Bacterial load was calculated in all the sites (Soil and effluent). The soil sample contained more cfu/mg in site 1 to site 6 ($3.70 \pm 0.01 \times 10^6$ to $2.99 \pm 0.02 \times 10^6$) than the reference sample ($2.40 \pm 0.02 \times 10^5$). Similarly the effluent sample has more cfu/ml varied from $5.81 \pm 0.03 \times 10^3$ to $4.21 \pm 0.01 \times 10^3$ and the reference site showed $3.29 \pm 0.01 \times 10^3$. The colony of the bacterial isolates from effluent ETEAK 1–13 were round/irregular, undulate margin; circular; small

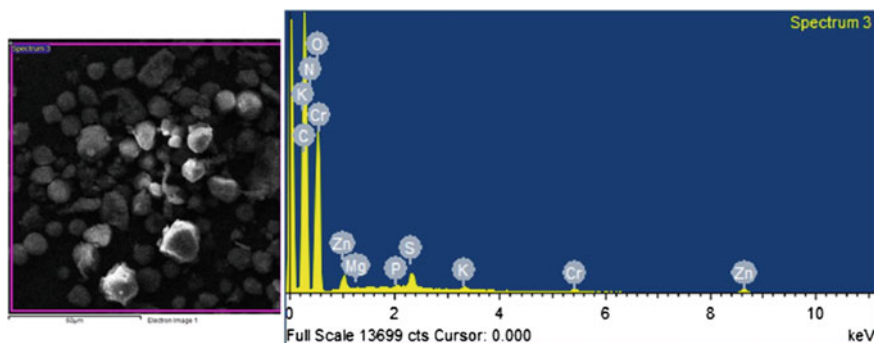


Fig. 4 SEM-EDX: Seeds of *Pennisetum typhoides* irrigated with effluent near discharge point

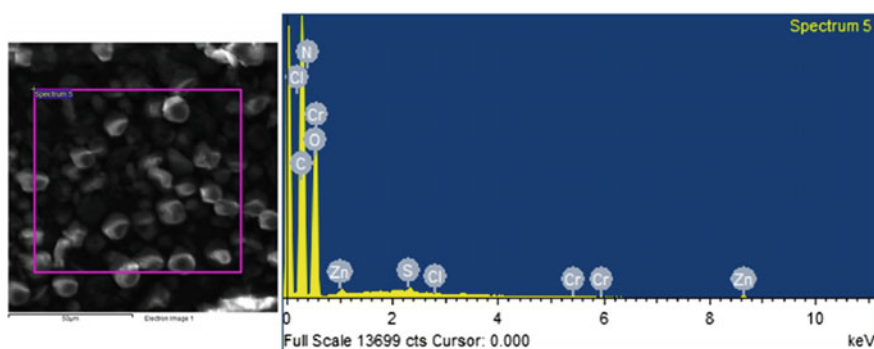


Fig. 5 SEM-EDX: Seeds of *Pennisetum typhoides* irrigation field 1 km away from discharge point

mucoid, entire umbonate; small circular; small circular flat, entire, powdery; large, irregular flat; circular convex, smooth entire; small, pinpoint convex; irregular round, medusa head; circular, pinhead, convex, entire; mucoid, umbonate elevation; circular, pinpoint, entire raised depressed center and circular, flat, undulated margin respectively. The ETEAK 1–13 were yellow; bright yellow non-diffusible pigment; red pigment; No pigment; light yellow green with black centre; smooth waxy whitish to cream mousy smell; tan to buff-color; grey color; golden-brown color; diffusible green pigment, fruity odor; white and cream colour, whereas the shapes were rods, diplo cocci, rods thin, diplo rods, diplo rods, rods in chain, short rods, coccirods, diplo coccic, rods, diplo cocci and rods respectively. Based on the morphological and biochemical characteristics the isolates were identified as *Lactobacillus* sp., *Agrobacterium* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Enterococcus* sp., *Aerococcus* sp. (Adesemoye et al. 2006; Malekzadeh et al. 2010; Das et al. 2010) (Table 9).

The colony morphology of soil bacterial isolates STEAK 1–13 isolated from soil contaminated by textile effluent were formed the colony and seemed as small, round, convex, entire; small, round, convex, entire; small, circular, convex, entire; small, circular elevated entire; small round, biconvex spreading edge; small circular, flat undulate margins; small irregular round, medusa head; small irregular, wrinkled, opaque; pinhead circular, convex, entire; pinhead circular, convex, entire; small, mucoid, umbonate elevation, entire; small, pinpoint convex entire and small, circular, convex, entire respectively. The colour and pigment of them were as follows off-white to cream, shiny; watery white cream no pigment; shiny wet, diffusible green fluorescent pigment; pale yellow white; greyish pigment; translucent, colorless, fruity smell no pigment; Diffusible cream no pigment; Smooth grayish pigment; slightly yellow colour, no pigment; bright yellow, non-diffusible pigment; Whiteno pigment; diffusible green pigment, fruity odor; Grey, no pigment and semi-transparent, white grey, surrounded by narrow zone of green color. The shape of the colony were rods, except for STEAK 5, 10, 12 (Diplococci), STEAK 9, 13 (Cocci). From the morphological and biochemical characteristics (Table 10), the genus semi-transparent, white grey, surrounded by narrow zone of green color were identified as *Lactobacillus* sp., *Agrobacterium* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Bacillus subtilis*, *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Enterococcus* sp. and *Aerococcus* sp. respectively.

Long term exposure to heavy metals microbial community adapts to the polluted environment. In the present investigation, bacterial isolates *Pseudomonas putida* STEAK 03, *Bacillus cereus* ETEAK 06 exhibited their survival and tolerance towards heavy metal polluted environment. Results imply that heavy metals promote the selection of resistant species and decrease the other microbial diversity (Ezzouhri et al. 2009; Hemamalini and Sneha 2014; Singh et al. 2013).

The soil isolates STEAK 03 (29%) and effluent isolate ETEAK 06 (21%) has the highest frequency of occurrence and they belong to *Pseudomonas* and *Bacillus* genus. The evolutionary history of *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 was inferred using the Neighbor-Joining method. The optimal tree is drawn to scale with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The 16s rRNA sequence (*Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06) determined in this study was deposited in NCBI Genbank under accession number KP731884 and KP731883 (Figs. 6 and 7).

3.4 Maximum Resistance Levels (MRL) of Bacterial Isolates Against Cr and Zn

Genotypically characterized *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 were assessed for their maximum tolerance level towards chromium

Table 10 Biochemical characterization of isolated bacterial stains from soil contaminated with textile effluent

Test	STEAK1	STEAK2	STEAK3	STEAK4	STEAK5	STEAK6	STEAK7	STEAK8	STEAK9	STEAK10	STEAK11	STEAK12	STEAK13
Gram stain	+	-	-	-	-	+	+	+	+	+	-	+	+
Motility	-	-	+	-	-	-	-	-	-	-	-	+	-
Spore	-	-	-	-	-	-	+	+	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	+	+	-
MR	-	+	-	+	-	-	+	-	+	+	+	-	+
VP	-	+	-	+	-	+	+	+	+	+	+	+	+
Citrate	+	-	-	+	-	-	-	-	-	-	+	-	+
Catalase	+	+	+	+	+	+	+	+	+	+	-	-	-
Oxidase	+	+	+	-	+	+	-	+	+	+	-	+	-
TSI slant	±	±	+	-	+	+	±	±	±	±	±	±	±
H2S	-	-	-	-	-	-	-	-	±	-	-	-	-
Urease	-	+	-	-	-	-	-	-	+	-	+	-	-
Starch	-	-	-	-	-	+	+	+	+	-	+	-	-
Gelatin	-	-	-	-	-	-	-	+	+	+	±	+	-
Nitrate	+	+	-	-	-	-	+	-	+	+	+	+	-

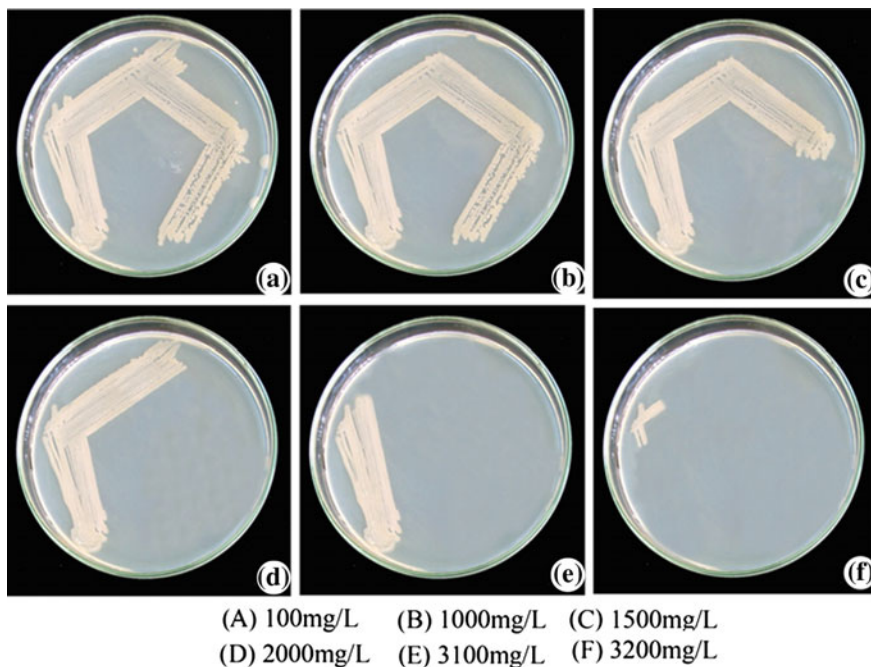


Fig. 6 MRL of *Bacillus cereus* ETEAK 06 against zinc

and zinc. *Pseudomonas putida* STEAK 03 exhibited a greater tolerance level of $2,400 \text{ mg L}^{-1}$ of Cr (VI) (Figs. 6 and 7). *Bacillus cereus* ETEAK 06 showed moderate growth in chromium concentration as high as 2100 mg L^{-1} with good growth at 1900 mg L^{-1} . For Zn, both the strains showed the highest degree of tolerance, 3200 and 3100 mg L^{-1} .

The time-course data for heavy metal removal and cellular growth were observed for each isolate under its optimal pH. The bacterial isolates removed Cr and Zn at pH 7.5. In the present study, the accumulation of bacterial isolates peaked at 2000 and 3000 mg L^{-1} of Cr and Zn and no growth at 3000 mg L^{-1} of Cr and lowered at 3000 mg L^{-1} of Zn. Thus, the viable cells used in shake flasks for removal of metal ions from aqueous solution offered several advantages including self replenishment, continuous metabolic uptake of metals after physical adsorption (Qazilbash 2004; Leung et al. 2000; Tam et al. 2001).

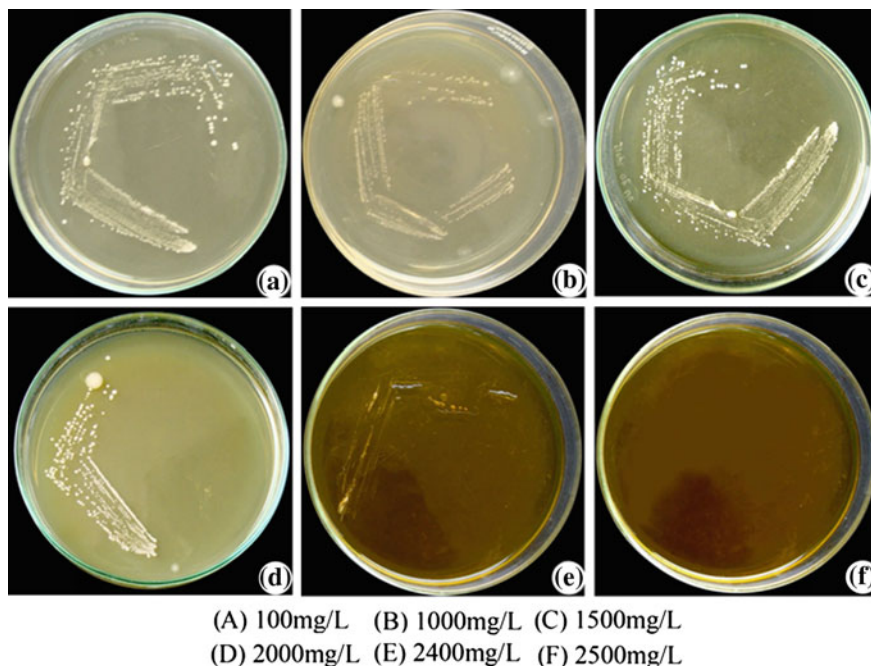


Fig. 7 MRL of *Pseudomonas putida* STEAK 03 against chromium

3.5 Bioaccumulation Potential of Bacterial Isolates

3.5.1 Optimization of the Accumulation Process in Bacteria

A series of experiments was designed to determine the tolerance/resistance levels of the *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 and indeed these strains remove Cr and Zn ions from the minimal salt media. Both the strains were cultured on minimal salt media with varying pH and varying metal concentration and observed for 2 days at the interval of 6 h (Figs. 8 and 9) represents the optimal pH and metal concentration.

The maximum uptake, of Cr and Zn by *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 was observed at pH 7.5 and complete accumulation was absorbed at 1000 mg L⁻¹ with the maximum biomass ranges between (0.36–0.42 g) Cr and (0.49–0.51 g) Zn by *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 respectively.

To build a consortium both bacterial isolates should be compatible, *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 was analyzed for their compatibility in the nutrient agar medium and the isolates were found to be compatible.

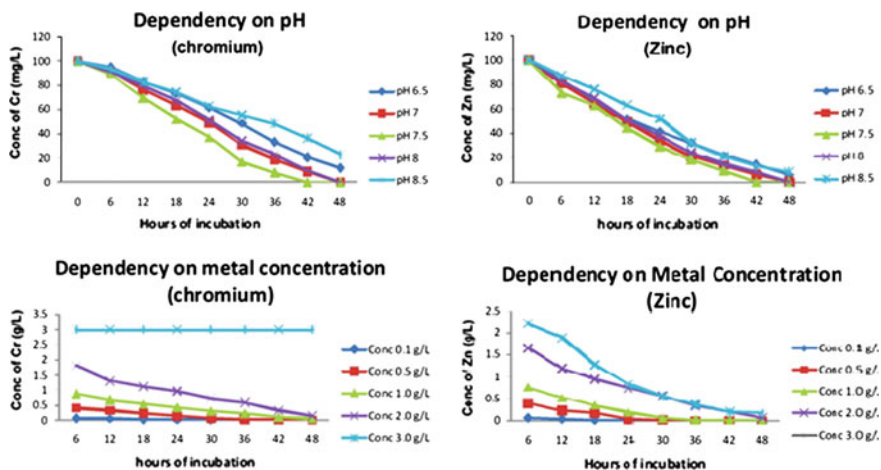


Fig. 8 Optimization of resistance level of *P. putida* STEAK 03 against chromium and zinc

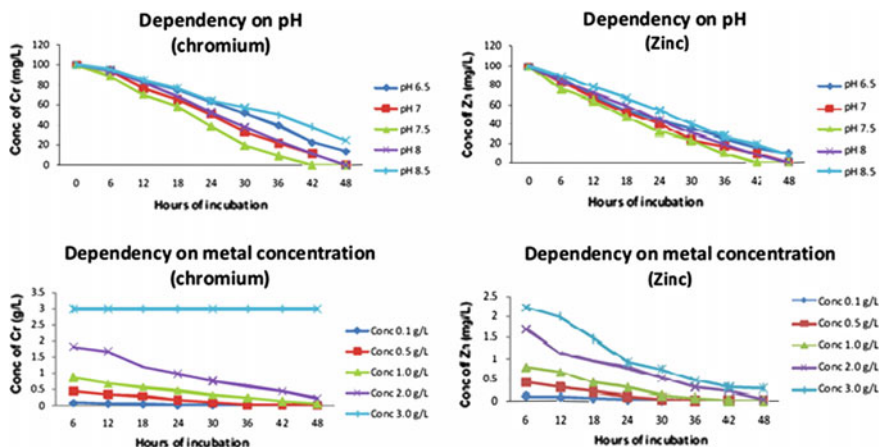


Fig. 9 Optimization of resistance level of *B. cereus* ETEAK 06 against chromium and zinc

3.6 Bioremoval of Cr (VI) and Zn (II) Ions from Waste Water Through Accumulation Process

Immobilization: Improvement in the removal of heavy metal using live cells depends on contact between the effluent and the isolates. Thus, immobilized growth was preferred to increase the contact time. Matrix with better holding capacity like *Luffa aegyptiaca* (bath sponge) was chosen and found to be most efficient. *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 and consortium of bacterial isolates were immobilized on *Luffa aegyptiaca* separately (Fig. 10a, b and

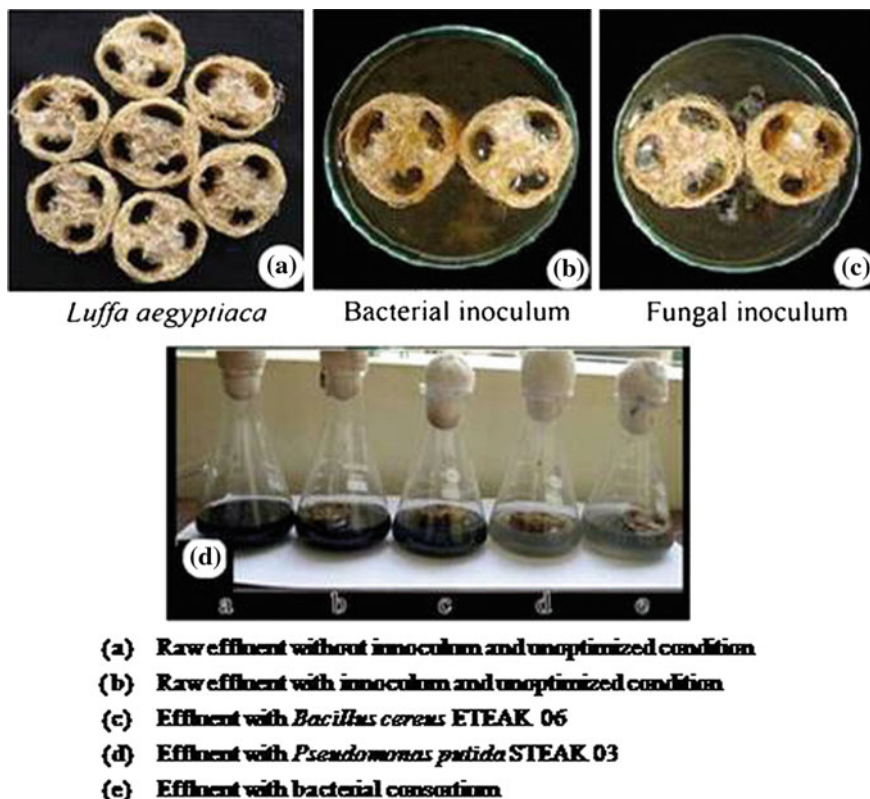


Fig. 10 Bioremoval of Cr (VI) and Zn (II) through bioaccumulation

c). Obtained results highlighted the potential use of immobilization with *Luffa aegyptiaca* for the removal of heavy metals from textile effluents.

Bioaccumulation of Cr (VI) and Zn (II) from textile dye effluent waste water: Over the stipulated period of incubation time, the results of the accumulation studies with immobilized consortium under optimized conditions showed noticeable changes in reduction of heavy metals and cellular growth than the individual bacterial isolates (Fig. 10d). 29.7% of Cr and 44.5% of Zn was adsorbed with *Bacillus cereus* ETEAK 06 and *Pseudomonas putida* STEAK 03 removed 32.2% of Cr and 48.5% of Zn, whereas Bacterial consortium removed 38.5 and 51.2% of Cr and Zn (Table 11). The results prove the accumulation by adsorption over the cell wall of microorganisms. However, it is to be noted that, more interestingly, the unfiltered effluent (raw effluent) which contained a mixture of microorganisms worked more effectively and removed (37.23% of Zn and 23.6% of Cr). This translated to the fact, bioremediation occurs naturally in the polluted environment, results also indicate consortium of microorganisms were doing equally good on the removal of heavy metal from the contaminated sites.

Table 11 Physicochemical parameters of the bacterial consortium treated effluent

S. No.	Parameters	Raw effluent	Bacterial consortium treated effluent	Reference	CPCB limit
1	Colour	Blackish green	Light yellow	Colourless	Absent
2	Smell	Offensive	Odourless	Absent	Absent
3	Ph	11.3 ± 0.2	8.1 ± 0.2	6.8 ± 0.1	6.0–9.0
4	Temperature (°C)	32 ± 1.0	30	20 ± 1.0	28–30
5	Total solids	4432 ± 15	1779.5 ± 20.0	1986.3 ± 18.0	Not applicable
6	Total Suspended solids (mg/L)	283 ± 5.0	101.5 ± 5.0	92.3 ± 2.8	100
7	Total dissolved solids (mg/L)	4194 ± 10	1678 ± 15.0	1894.0 ± 10.0	500–2000
8	Chlorides (mg/L)	1134.2 ± 8	896 ± 4.0	928.5 ± 5.6	250–1000
9	BOD	108.0 ± 0.1	30.0 ± 2.0	20.0 ± 10.0	30
10	COD	997.0 ± 30.1	213.9 ± 15.0	184.0 ± 10.1	250

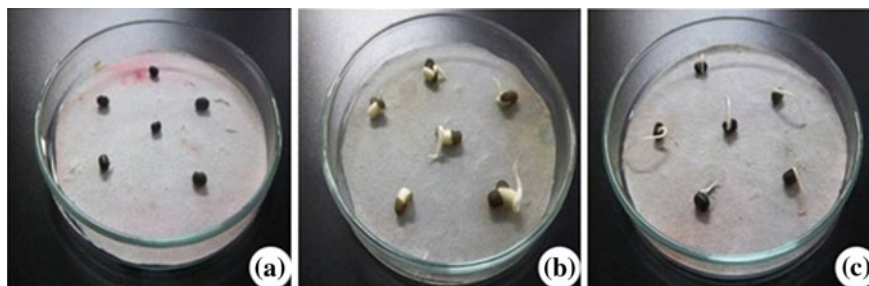
Metal concentration

S. No.	Metals	Raw effluent	Bacterial biosorbent treated effluent	WHO standards	Permissible limit for irrigation (mg/L)
11	Cr	24.51 ± 0.37	10.59 ± 0.02	0.05 ± 0.01	0.05
12	Zn	36.13 ± 1.27	7.23 ± 0.05	5.0 ± 1.2	0.2

3.7 Toxicity Assessment of Treated Waste Water—Bioaccumulation Process

The Physicochemical parameters like pH, EC, total suspended solids, total solids, total dissolved solids, chlorides, BOD, COD, metal concentration of the treated effluents was analyzed. The results reveal all the physicochemical parameters are within the permissible limit of central pollution control board. Observed average concentration (56.7 and 79% of Cr and Zn ions) was removed from the effluent sample.

Seed germination test: Phytotoxic studies on *Pennisetum typhoides* and *Vigna radiata* was carried out with consortium treated effluent and untreated effluent from industry. In seed germination study, there was no inhibition of germination by the bacterial consortium treated effluent and with the seeds soaked in bore-well water devoid of effluent where as 100% inhibition was observed with untreated effluent on 7 days of incubation and the germination was observed after 14 days of incubation and the metal concentration in the crop was also less and within the permissible limits for irrigation (Fig. 11).



(A) Seeds soaked with treated effluent discharged from industry
(B) Seeds soaked with distilled water
(C) Seeds soaked with treated effluent

Fig. 11 Seed germination test of treated water on *Vigna radiate*

The reduction in germination percentage with untreated textile effluent might have been due to presence of high concentration of metals and other toxic organic compounds that cause a range of cellular toxicities (Kadar and Kastori 2003) and the presence of high amounts of salts and organic compound in untreated textile effluent reduces the availability of water thereby resulting in reduced germination (Ashraf 2004).

4 Conclusion

Thus, *Pseudomonas putida* STEAK 03 and *Bacillus cereus* ETEAK 06 strains sufficiently supported the process of bioremediation. This technology may indeed be labeled as the panacea for the removal of toxic substances from the environment. Bioremediation is without doubt an innovative technique that needs to be adapted to help cleanse the environment of such toxic pollutants such as Cr and Zn, save the biodiversity of AM Spores, conserve them. Based on the above results, it is concluded the biological treatment of textile dye effluent with *Luffa aegyptiaca* immobilized bacterial isolates for the recovery of heavy metals are proven to be the best and the effluent treated is safe for irrigation.

References

- Abubacker MN, Visvanathan M, Srinivasan S (2014) Impact of pesticides on AMF spore population and diversity in banana (*Musa spp.*). *Plant soils* 2(4):1279–1286
- Adesemoye AO, Opere BO, Makinde SCO (2006) Microbial content of abattoir waste water and its contaminated soil in Lagos Nigeria. *Afr J Biotechnol* 5(20):1963–1968

- Aklilu A (2014) Heavy metals concentration in tannery effluents associated surface water and soil at Ejersa area of East Shoa Ethiopia. *Herald J Geogr Reg Plann* 3(3):124–130
- Ameer Basha S, Rajaganesh K (2014) Microbial bioremediation of heavy metals from textile industry dye effluents using isolated bacterial strains. *Int J Curr Microbiol App Sci* 3(5):785–794
- APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association American Water Works Association Water Environment Federation, Washington
- Ashraf M (2004) some important physiological selection criteria for salt tolerance in plants. *Flora* 199:361–376
- Ateeq M, Khurshid R, Khan I, Shaheen A (2015) Effect of heavy metals from tannery effluent on the soil and groundwater using multivariate analysis in district Sheikhpura Pakistan. *Res J Chem Environ* 19(1):48–55
- Babysyakila P (2009) Effect of diluted effluent on soil properties and plant growth. *Adv Stud Biol* 1(8):391–398
- Banytan SC, Sahu RK, Bhargava K, Chatterjee C (2000) Distribution of heavy metals in wheat, mustard and weed grown in field irrigated with industrial effluents. *Bull Environ Contam Toxicol* 64:489–496
- Bouyoucos GJ (1962) Hydrometer method improved for making particle size analysis of soils. *Agron J* 54:464–465
- Calderon J, Ortiz-Perez D, Yanez L, DiazBarriga F (2003) Human exposure to metals: Pathways of exposure, biomarkers of effect, and host factors. *Ecotoxicol Environ Saf* 56(1):93–103
- Cappuccino JG, Sherman N (2002) Microbiology. A laboratory manual. 6th edn. Pearson Education, Inc. San Francisco, pp 215–224
- Chin HS, Shim JS, Kim JM, Yang R, Yoon SS (2001) Detection and antibacterial activity of a bacteriocin produced by *Lactobacillus plantarum*. *Food Sci Biotechnol* 10(5):461–467
- Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi K (2007) Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J Hazard Mater* 146(1):270–277
- Das M, Ahmed K, Begum F, Parveen S, Islam M, Begum M (2010) Microbial load in tannery and textile effluents and their receiving rivers of DHAKA. *Biol Sci* 19(1):73–81
- Ezzouhri L, Castro E, Moy M, Espinola F, Lairini K (2009) Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier Morocco. *Afr J Microbiol Res* 3(2):35–48
- Furaha MC, Kelvin MM, Karoli NN (2015) Assessment of heavy metals in treated wastewater used for the irrigation of vegetable plants in Arusha City. *Int J Res Chem Environ* 5(1):54–60
- Hemamalini V, Sneha S (2014) Biodiversity characterization of bacterial and fungal isolates from gold electroplating industry effluents. *J Appl Environ Microbiol* 2(5):212–219
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) *Bergey's manual of determinative bacteriology*, 9th edn. Williams and Williams, Baltimore
- Iqbal M, Edyvean RGJ (2005) Loofa sponge immobilized fungal biosorbent: a robust system for cadmium and other dissolved metal removal from aqueous solution. *Chemosphere* 61:510–518
- Jayashree S, Rathinamala J, Lakshmanaperumalsamy P (2011) Determination of heavy metal removal efficiency of *Chrysopogon zizanioides* (Vetiver) using textile wastewater contaminated soil. *J Environ Sci Technol* 4:543–551
- Joshi VJ, Santani DD (2012) Physicochemical characterization and heavy metal concentration in effluent of textile industry. *Univers J Environ Res Technol* 2(2):93–96
- Joshi PK, Swarup A, Maheshwari S, Kumar R, Singh N (2011) Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. *Indian J Microbiol* 51:482–487
- Kadar I, Kastori R (2003) Mikroelem-terhelés hatása a repccére karbonátos csernozjom talajon. *Agrokémiaes Talajtan* 52:331–346
- Leung WC, Wong MF, Chua H, Lo W, Yu PH, Leung CK (2000) Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Water Sci Technol* 44:233–240

- Mahawar P, Akhtar A (2015) Physico-chemical characterization of soil and effluent of dye industries in Kaithun region of Kota Rajasthan. *Int J Pure Appl Biosci* 3(2):419–422
- Malekzadeh E, Alikhani HA, Savaghebi GR, Zarei M (2010) Resistance to nickel and cadmium of indigenous and non-indigenous plant growth promoting rhizobacteria (PGPRs) to heavy metal contaminated soils. *Iran J Soil Water Res* 2(41):257–263
- Mohabansi NP, Tekade PV, Bawankar SV (2011) Physico-chemical parameters of textile mill effluent Hinganghat Dist. Wardha (M.S.). *Current World Environment* 6(1):165–168
- Nelliyat P (2007) Public private partnership in urban water management: the case of Tiruppur. In: Mitra B, Okonski K, Satyanand M (eds) *Keeping the water flowing: understanding the role of institutions, incentives, economics and entrepreneurship in ensuring Access and optimizing utilization of water*. Academic foundation, New Delhi
- Patel KP, Pandya RR, Maliwal GL, Patel KC, Ramani VP, George V (2004) Heavy metal content of different effluents and their relative availability in soils irrigated with effluent waters around major industrial cities of Gujarat. *J Indian Soc Soil Sci* 52(1):89–94
- Prabha TR, Revathi K, Vinod MS, Shanthakumar SP, Bernard P (2013) A simple method for total genomic DNA extraction from water moulds. *Curr Sci* 104(3):345–347
- Prashanth S, Mathivanan N (2009) Optimization and production of salicylic acid by rhizobacterial strain *Bacillus licheniformis* MML2501. *Int J Microbiol* 6:1–15
- Qazilbash AA (2004) Isolation and characterization of heavy metal tolerant biota from industrially polluted soils and their role in bioremediation. *Pak Res Repos* 192
- Rajbanshi A (2008) Study on heavy metal resistant bacteria in Guheswori sewage treatment plant. *Our Nat* 52–57
- Rayment GE, Higginson FR (1992) Electrical conductivity. In: *Australian laboratory handbook of soil and water chemical methods*. Inkata Press, Melbourne, pp 24–26
- Rohilla SK, Salar RK, Kumar J (2012) Optimization of physicochemical parameters for decolorization of reactive black HFGR using soil fungus, *Aspergillus allhabadii* MTCC 9988. *J Bioremediat Biodegradation* 3:153–159
- Singh HP, Kaur G, Batish DR, Kohli RK (2013) Lead (Pb)-induced biochemical and ultrastructural changes in wheat (*Triticum aestivum*) roots. *Protoplasma* 1:53–62
- Tam NFY, Ke L, Wang XH, Wong YS (2001) Contamination of polycyclic aromatic hydrocarbons in surface sediments of mangrove swamps. *Environ Pollut* 114:255–263
- Vinod VTP, Sasidhar RB (2010) Bioremediation of industrial toxic metals with gum Kondagogu (*Cochlospermum gossypium*): a natural carbohydrate biopolymer. *Indian J Biotechnol* 10:113–120
- World commission on environment and development 1987