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BIOREMEDIATION OF TEXTILE EFFLUENT BY INDIGENOUS BACTERIAL CONSORTIA AND ITS EFFECTS ON *Zea mays* L. CV C1415

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ABSTRACT

Bacterial consortia BMP1 (containing six isolates), BMP2 (three isolates) and BBP (three isolates) were isolated from textile affected soil, sludge and effluent of Hudiara drain in vicinity of Nishat Mills Limited 5Km Off - 22Km Ferozepur Road Lahore, Pakistan. These consortia were capable of degrading red, green, black and yellow dyes and were resistant to heavy metals (Cu, Cd, Cr, Ni, Mn & Pb). They were equally beneficial for the reduction of other pollution parameters like colour, pH, EC, nitrogen, phosphorus, chloride, COD, BOD, TDS and TSS from textile effluents. The physicochemical characterization of textile effluents was carried out before and after treatment by consortia to sort out most efficient consortium. The results indicated that the consortium BMP1 showed maximum reduction of EC (52.98%), pH (11.85%), nitrogen (79.02%), phosphorus (68.78%), chloride (46.42%), BOD (59.49%), COD (61.35%), TDS (44.93%) and TSS (52.58%). It was also helful in reduction of heavy metals such as Cu (92.3%), Cd (89.46%), Cr (83.52%), Ni (80.7%), Mn (88.3%) and Pb (93.5%). Effect of untreated and treated textile effluents was investigated on maize (*Zea mays* L. CV C1415) and it was observed that effluent treated by consortium BMP1 was not toxic to maize crop in comparison to untreated effluent. The current study revealed that utilization of bacterial consortium for textile effluent treatment could be an effective method.

Key words: Textile effluent, bioremediation, maize, bacterial consortia.

INTRODUCTION

Textile industries consume large quantities of water and generate huge amounts of effluents which contain considerable amount of suspended solids, additives, detergents, surfactants, carcinogenic amines, aldehydes, heavy metals and dyes. Fluctuating pH, high temperature, COD and complex coloration are main physiochemical characters of textile effluents; they pose serious environmental threats to receiving water bodies (Jadhav *et al.,* 2010; Phugare *et al.,* 2011). Colour is the first contaminant to be recognized in textile effluent as the major contributor of biological oxygen demand (Murugalatha *et al.,* 2010). Among commonly used dyes azo dyes are difficult to decolorize because of their complex structure and synthetic origin. The azo anthraquinone, sulfur, indigoid, triphenylmethyl and phthalocyanine derivatives are the most frequently used dyes on industrial scale. Biodegradation of azo dyes is possible if the azo bond is first reduced. Permanent decolorization of the azo compounds occurred on cleavage of azo bond but the intermediates can be re oxidized to colored byproducts. Majority of these dyes are toxic, mutagenic, carcinogenic and stable to light and temperature. These resistant properties inhibit the attack of microorganisms. Removal of such dyes is a matter of serious concern (Barka *et al.,* 2011; Ong *et al.,* 2012).

The process of biodegradation is relatively inexpensive, running costs are low and the end products of complete mineralization are non toxic. Most reduction occurs during active bacterial growth. There is a growing concern regarding adverse effects on aquatic biota and humans due to the contamination of water by textile effluents (Saratale *et al.,* 2009; Carneiro *et al.,* 2010). Important component of dyes is heavy metals, which are frequently found in textile effluent in free ionic or complex compound state. When these heavy metals reached the water bodies they settle down in the sediment and accumulate in bodies of aquatic organisms on inhalation thus causing serious health impacts leading to the death of those organisms (Phugare *et al.,* 2011). Land irrigated with textile effluents causes serious impacts on plant growth as soil can act as a sink for heavy metals and other resistant chemicals results in the reduction of productivity. Metals leaching cause contamination of ground water. These contaminants entered in the food chain and become health risk for plants, animals and human (Ross, 1994). However treated textile effluent can be a good source of irrigation water. Investigation of pollutants and their impacts on plant growth could be of prime importance as plants are commercial products of agrarian society (Jadhav *et al.,* 2010).

The removal of pollutants is the major task of bioremediation. Mostly a simple one step process is not possible; usually a sequence of biological transformations is needed to eliminate particular substance. These sequenced steps are possible by developing a microbial community called consortium comprising populations with particular bio-transformation activities. It may happen with other processes of bioremediation that in the chain of reactions the intermediate can be more harmful than pollutant. If the rate of transformation is slower, the harmful intermediate will be accumulated and the process fails to meet expected results (Drobnõk, 1999).

After wheat and rice, maize is the third most important cereal in Pakistan. It is used for both human and animal consumption. Due to photo-thermo-insensitive character, it is grown round the year; this quality makes it 'queen of cereal'. In Pakistan, area of 1.016 million hectares is under cultivation of maize. Approximately 66% of the maize cultivation is carried out in irrigated area and the remaining in rain-fed area (Tariq and Iqbal, 2010; Verma *et al.*, 2012).

In the current study isolation and screening of the indigenous bacteria was carried out from textile effluent contaminated sites and bacterial consortia were developed to reduce the pollution parameters from textile effluents. Effect of untreated and treated textile effluents was investigated on *Zea mays* L. (Maize).

MATERIALS AND METHODS

Sample collection: Wastewater (Textile effluents), sludge and affected soil samples were collected in screw capped sterilized bottles from Hudiara drain near Nishat Mills Limited 5Km Off - 22Km Ferozepur Road Lahore, Pakistan. One sample of soil, four sludge and four samples of waste water were collected from 0, 5,500 and 1000 meter away from the main outlet following standard procedures (APHA, 2005).

Isolation, screening and identification of the indigenous bacteria: The isolation of indigenous bacteria was carried out through serial dilution method from affected soil, sludge and textile effluent on nutrient agar medium (Jadhav *et al.,* 2010; Kaur *et al.,* 2010). Isolated bacterial strains were screened out by incubating them on nutrient agar medium containing red (Carmine) green (Light Green) black (Erichrome Black T) and yellow (Metanil Yellow) dyes 200ppm of each. They were also incubated on nutrient agar containing 50 ppm of Cu, Cd, Cr, Ni, Mn and Pb each. The stock cultures of screened bacterial isolates were maintained routinely on the nutrient agar medium and stored at 4 ^{o}C. The screened bacterial strains were identified on morphological, biochemical and physiological properties using the protocol given in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

Decolorization: Decolorization ability of bacterial isolates was analyzed by using spectrophotometer (SpectroScan 80D UV–VIS) at optimum wavelength. The decolorization activity was expressed in terms of percentage decolorization using formula of Cheriaa *et al.* (2012):

$$\text{Decolorization \%} = \frac{t_0 - t_f}{t_0} \times 100$$

Where;
(t_0) = initial absorbance
(t_f) = absorbance at incubation time

Development of bacterial consortia: The isolates for the consortium development were selected based on criteria; ability to degrade the dyes efficiently above 60% within 3 days and also with ability to degrade red, green, black and yellow dyes, and also can grow on media containing at least 50ppm of Cu, Cd, Cr, Ni, Mn and Pb. Following three consortia were developed using combinations of six (Consortium BMP1) and three isolates (Consortium BMP2, Consortium BBP). A loopful of selected isolates was individually inoculated for 24 h to form a consortium (Tony *et al.*, 2009). 1. Consortium BMP1: *Bacillus subtilus, Bacillus cereus, Bacillus mycoides, Bacillus sp, Pseudomonas sp. and Micrococcus sp.* 2. Consortium BMP2: *Bacillus sp Micrococcus sp., Pseudomonas sp* 3. Consortium BBP: *Bacillus cereus, Bacillus mycoides, Pseudomonas sp.*

Physicochemical characterization of textile effluents: Characterization of the effluent for various parameters such as temperature, colour, pH, EC, nitrogen (N), phosphorus (P), chloride (Cl), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved soilids (TDS), total suspended solids (TSS) and heavy metals (Cu, Cd, Cr, Ni, Mn & Pb) was carried out before and after treatment by bacterial consortia to measure reduction in these parameters (Ali *et al.,* 2009).

Maximum resistant limits to heavy metals: The maximum resistant limits to heavy metals of bacterial isolates was determined by using solution of 50ppm, 100ppm, 150ppm, 200ppm 400ppm, 500ppm, 1000ppm, 1200ppm, 1400ppm, 1600ppm and 1800ppm solutions of deferent metal salts (copper sulphate, cadmium chloride, potassium dichromate, nickel**,** manganese chloride and lead nitrate). The maximum resistant limits for the bacterial isolates were checked by increasing the concentration of respective metal stepwise (Guo *et al.*, 2010).

Effect of treated and untreated textile effluent on maize: In order to assess the effect of treated and untreated textile effluent on maize (common agricultural crop of Pakistan), the tests were performed on *Zea mays* L. CV C1415. The textile effluent treated by consortium BMP 1 was used. The study was carried out at room temperature (20 seeds were placed in each germination plate) by watering 5 ml of untreated and treated textile effluents. Control set was carried out using irrigation water at the same time. The trial was laid out in

Completely Randomized Design (CRD) with three replications. Germination (%) as well as the length of plumule and radical was recorded after 7 days (Saratale *et al.,* 2009).

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) using software package Co-stat version 3.03 (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Physicochemical characterization of textile effluents: The effluents collected from Nishat Mills Limited were brownish black in color, pungent smell, high temperature (35.6^{O}C) and alkaline pH (8.66). Electric conductivity of effluents was 5.78 ms/cm, indicating the high amount of dissolve salts. TSS (3920 mg/l) and TDS (4960 mg/l) were quite high. Nitrogen, phosphorus, chloride and surface tension of the textile effluents were 16mg/l, 5.4mg/l, 273mg/l and 71.16mN/m, respectively. A high load of COD (1219 mg/l) and BOD (370 mg/l) was also observed. The analysis for heavy metals showed high amount as compared to National Environmental Quality Standards (NEQS 2000). The average amount (ppm) of metal like Cu, Cd, Cr, Ni, Mn and Pb was 8.54, 0.59, 2.34, 1.12, 3.87 and 0.62, respectively (Table 1).

The high levels of COD in the textile effluents indicated the toxicity level of pollution (Dawkar *et al.,* 2010) which is very harmful for the whole ecology of aquatic ecosystem of the receiving bodies. The reduction in COD and BOD after treatment with consortium BMP1 was consequent of the removal of organic load from effluent and ultimately the toxicity (Ong *et al.,* 2012). The exceeded permissible limits of pollution parameters reduced the natural process of bioremediation. The concentration of the solids in textile effluent was another matter of concern and the carcinogenic effect of the dyes adds to it. The results of the present study indicated that the consortium BMP1 can be used for the treatment of such waters. Reduction in the concentration of TDS and TSS were near fifty percent due to degradation of these solids by the bacteria present in the consortium. The results were in correlation with those as observed in other studies (Ali *et al.,* 2009; Kumar *et al.,* 2010).

Decolorization and maximum resistant limits of bacterial isolates against heavy metals: It was observed that bacterial isolates showed remarkable behavior for the degradation of dyes present in textile effluents. The maximum decolorization for red, green, black and yellow dyes by *Bacillus mycoides* was 74%, 76%, 73% and 76% respectively while for *Bacillus cereus* was 76%, 75%, 74% and 74% respectively. *Bacillus subtilus* decolorized (%) red, green, black and yellow dyes up to 77, 78, 79 and 79. The *Micrococcus sp.* was responsible of decolorizing the red, green, black and yellow dyes to 75%, 71%, 73% and 77% respectively. The *Pseudomonas sp.* showed decolorizing of red, green, black and yellow dyes up to 79%, 76%, 77% and 78% respectively (Table 2). A great deal of resistance was shown by the bacterial isolates, when incubated on nutrient agar having different concentrations of heavy metals. *Bacillus mycoides* showed maximum resistant limit 150ppm, 100ppm, 200ppm, 100ppm, 50ppm and 500ppm for Cu, Cd, Cr, Ni, Mn and Pb respectively. Similarly *Bacillus cereus* showed 200ppm for Cr and Pb while *Bacillus sp.* and *Micrococcus sp.* were maximum resistant at 1000ppm Pb. While *Bacillus subtilus* showed maximum resistant limit at 1000 ppm for Cr. The *Pseudomonas sp.* had maximum resistant limit (1600ppm) for Cr which was higher than other heavy metals (Table 2). This remarkable difference in maximum resistant limit is due to variation in their enzymatic activities (Ali *et al.,* 2009).

Effect of selected consortia on reduction of pollutants: The results indicated that consortium BMP1 show remarkable behavior towards the reduction of pollution parameters than other two consortia. The consortium BMP1 reduced EC, pH, nitrogen, phosphorus, chloride, BOD, COD, TDS and TSS up to 52.98%, 11.85%, 79.02%, 68.78%, 46.42%, 59.49%, 61.35%, 44.93% and 52.58% respectively. While consortium BMP2 exhibited 39.41%, 8.03%, 73.19%, 64.321%, 41.01%, 48.53%, 51.15%,35.79% and 43.22% reduction for EC, pH, nitrogen, phosphorus, chloride, BOD, COD, TDS and TSS respectively. Similarly consortium BBP also exhibited 46.23%, 9.58%, 75.37%, 67.11%, 44.12%, 54.71%, 56.28%, 40.31% and 48.88% reduction for EC, pH, nitrogen, phosphorus, chloride, BOD, COD, TDS and TSS respectively (Figure 1). The bacterial consortia BMP1, BMP2 and BBP were developed from isolates of textile effluents, sludge and affected soil of Nishat Mills Ltd. Lahore, Pakistan. The bacterial consortia BMP1, BMP2 and BBP found to have significant potential on decolorization of the textile effluent. The venture of present study was to evaluate the use of a biological treatment by developing bacterial consortia for decolorization and reduction of pollution parameters from textile effluent. The reason for effective decolorization of the effluent by consortia might be associated with the combined metabolic activities and interactions of these bacterial isolates. The results of the current study were comparable to the research conducted by Joshi *et al.* (2010) and Kaur *et al.* (2010).

Heavy metal reduction ability of various consortia in textile effluents: The consortium BMP1 reduced Cu, Cd, Cr, Ni, Mn and Pb up to 92.3%, 89.46%, 83.52%, 80.7%, 88.3% and 93.5% respectively. Consortium BBP was also able to reduce 77.5%, 64.6%, 71.2%, 68.9%, 81.7% and 81.1% of Cu, Cd, Cr, Ni, Mn and Pb respectively. While consortium BMP2 showed low % reduction than both consortia discussed above. It reduced (%) Cu, Cd, Cr, Ni, Mn and Pb up to 66.4, 54.8, 60.2, 51.3, 67.2 and 62.4, respectively (Fig. 2).

Among the three consortia only consortium BPM 1 was able to reduce the Cu, Cd, Cr, Ni, Mn and Pb up to permissible limits (Table 3). The consortium BPM 1 can be exploited for the bioremediation of Cu, Cd, Cr, Ni, Mn and Pb containing wastes, since it seems to have a potential to reduce up to permissible limits. The usage of higher amount of metal containing dyes for dying process contribute high metal concentration in textile effluent, which causes a lot of problems associated with human and ecosystem health. A variety of mechanisms exist for the removal of heavy metals from aqueous solution by bacteria, fungi, algae, macrophytes and higher plants. The bacterial response to the presence of metals includes processes such as biosorption, active cell transport and enzymatic binding **(**Rehman *et al.,* 2008, Zahoor and Rehman, 2009, Shakoori *et al.*, 2010). Efficiency of consortia might be associated with different specificity of the metals towards biding sites present on the bacterial cells. Hence all the bacterial consortia not only exhibited the ability to survive in contaminated textile effluent (Guo *et al.,* 2010) but also demonstrated remediation of toxic heavy metals (Cu, Cd, Cr, Ni, Mn & Pb). The consortium BMP1 represented a marked increase in bioremediation of Cu, Cd, Cr, Ni, Mn & Pb. It might be due to the proteins induced during stress period (Nies, 2003). Therefore bacterial consortium BMP1 can be used for the bioremediation of Cu, Cd, Cr, Ni, Mn & Pb containing effluent, since it seems to have a potential to reduce these toxic heavy metals.

Effect of treated and untreated textile effluent on Maize: The water bodies used for irrigation purposes contain untreated effluent from dyeing industry. This practice is of great environmental concern as it associated with biotic and ecosystem health. Soil fertility is directly and indirectly dependent on irrigation water. Biodegradation of effluent leads to generation of various products. Therefore it is virtually important to study the toxicity impact of these degradation products on plants. Treated and untreated textile effluents were applied on *Zea mays* L. to study their effect on seed germination and seedling health. The plumule and radical length for control were 20±0.64 and 6±0.96 cm, respectively, whereas germination was 100% (Figure 3). The germination was inhibited to 39%, while plumule and radical length was up to 6cm and 2cm respectively (Figure 4). When textile effluent treated by consortium BMP1 was applied, the germination was 94% while plumule and radical length was 13cm and 5cm respectively, it was very near to control. The results of the present study illustrated the significant difference in lengths of plumule and radical of the *Zea mays* L. CV C1415 grown in the irrigation water (control) and textile effluent. Plant germination and growth are the techniques used to evaluate the effect of treated and untreated textile effluent. Germination has been regarded as a less sensitive method than plumule and radical length

Table 1: Characteristics of textile effluent.

Parameters	Concentration	NEQS (2000)
Temperature	35.50 ±1.50 ^{0}C	40.00 ^{0}C
Colour	Brownish black	-
EC (ms/cm)	5.78 ±0.13	-
pH	8.66±0.26	6.00-10.00
Nitrogen (mg/l)	16.00±0.65	-
Phosphorous(mg/l)	5.40±0.05	-
Chloride (mg/l)	273.00±3.23	1000.00
Surface Tension (mN/m)	71.36±1.12	-
DO (ppm)	0.14±0.02	-
BOD(mg/l)	370.00±2.56	80.00
COD(mg/l)	1219.00±3.78	150.00
TDS(mg/l)	4960.00±3.54	3500.00
TSS(mg/l)	3920.00±2.95	150.00
Cu (ppm)	8.54±0.15	1.00
Cd (ppm)	0.59±0.10	0.10
Cr (ppm)	2.34±0.12	1.00
Ni (ppm)	1.12±0.09	1.00
Mn (ppm)	3.87±0.11	1.50
Pb (ppm)	0.62±0.08	0.50

Detection limits: Cu, Cd, Pb& Ni = 0.05ppm
Mn & Cr = 0.01ppm

Table 2: Comparison of dyes decolonization and maximum resistant limits for heavy metals by different bacterial isolates from textile industry effluent.

Bacterial isolates	Dyes				Heavy metals					
	Red	**Green**	**Black**	**yellow**	**Cu**	**Cd**	**Cr**	**Ni**	**Mn**	**Pb**
	Decolorization %				**Maximum resistant limits (ppm)**					
Bacillus mycoides	74	76	73	76	150	100	200	100	50	500
Bacillus cereus	76	75	74	74	100	50	200	150	100	200
Bacillus subtilus	77	78	79	79	50	50	1000	200	100	50
Bacillus sp.	77	78	79	79	50	50	200	150	50	200
Micrococcus sp.	75	71	73	77	50	50	200	150	50	1000
Pseudomonas sp.	79	76	77	78	100	50	1600	200	200	150

(Kalyani *et al.*, 2008; Gomare *et al.*, 2009). The results of the present study suggested that textile effluents were toxic and treated with consortium BMP1 were non-toxic to a common crop of Punjab, Pakistan such as maize (*Zea mays* L. CV C1415). The ultimate aim of bioremediation and biodegradation was to reduce the concentration of environmental pollutant as well as its toxicity. The present study and literature indicated that the textile effluent exert strong effects germination, plumule and radical length of maize (Saratale *et al.*, 2009; Phugare *et al.*, 2011).

Table 3: Heavy metal reduction by consortium BMP1 in textile effluents.

Heavy metals	Concentration in effluents (ppm)	Concentration remaining (ppm)	% Reduction
Cu	8.54	0.65	92.30
Cd	0.59	0.06	89.46
Cr	2.34	0.38	83.52
Ni	1.12	0.21	80.70
Mn	3.87	0.45	88.30
Pb	0.62	0.04	93.50

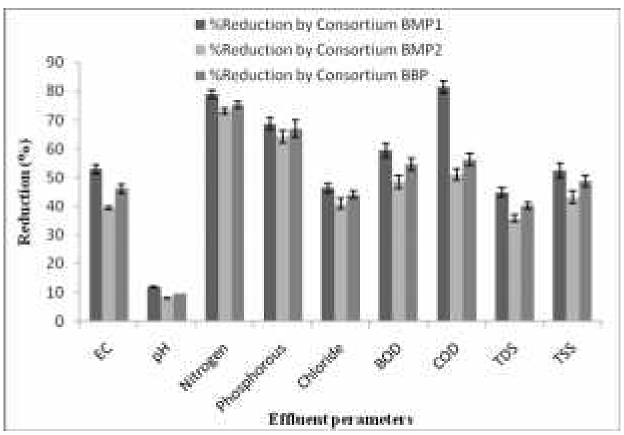

Figure 1: Reduction of pollution parameters from textile effluents by various consortia

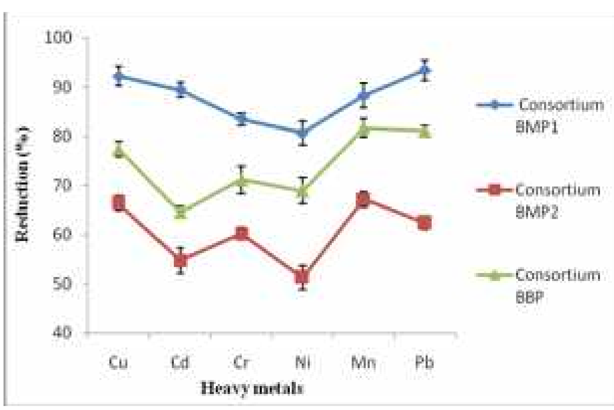

Figure 2: Reduction of heavy metals in textile effluents by various consortia

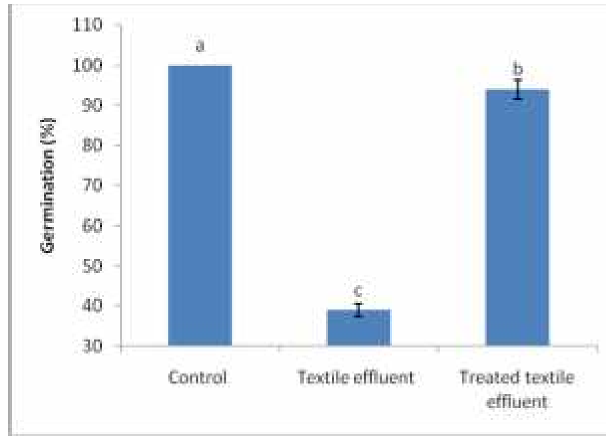

Figure 3: Effect of treated and untreated textile effluent on maize germination

Different letters in the bar differ significantly at P=0.05 whereas Least Significant Difference is 3.19

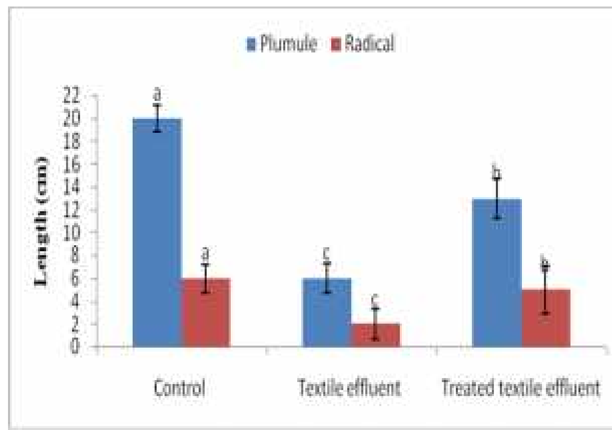

Figure 4: Effect of treated and untreated textile effluent on plumule and radical length of maize

Different letters in the bar differ significantly at P=0.05 whereas Least Significant Difference for plumule is 0.74 and 0.73 for radical

Conclusions: Nishat Textile Mills Limited effluents are clearly imparting a high load of dyes and heavy metals into Hudiara drain. The bacterial consortia were used for the bioremediation of heavy metals (Cu, Cd, Cr, Ni, Mn & Pb), dyes removal and other effluent parameters (colour, pH, EC, N, P, Cl, COD, BOD, TDS, TSS). Significant reductions in all these parameters were found with BMP1 consortium. The study also revealed that effluents treated by consortium BMP1 was not toxic to maize (*Zea mays* L. CV C1415). Hence, present work demonstrates that consortium BMP1 (*Bacillus subtilus*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus sp*, *Pseudomonas sp. and Micrococcus sp.*) can be the best biological tool for textile effluent treatment.

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