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ISOLATION AND CHARACTERIZATION OF VARIOUS FUNGAL STRAINS FROM TEXTILE EFFLUENT FOR THEIR USE IN BIOREMEDIATION

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Abstract

Bioremediation is an inexpensive mean to remove hazardous metal ions from the contaminated effluent. Effluent from the local textile industry was characterized for the incidence of fungal strains and its physiochemical properties were investigated. Sampling was done spanning a distance of five km from the effluent's discharge point. Effluent was analyzed and highest colony forming units per mL were found at the end point ($5x10^3$). *Penicillium*, *Rhizopus* and *Candida* were the three main fungal genera found in such alkaline pH where as *Drechslera* sp., and Rhodotorula sp., were in low number. Effluent was also analyzed for Mn, Mg, Zn, Fe, Cu, Cd, Ni and Cr using Atomic Absorption Spectrophotometry. Concentration of all these metal ions in the effluent were above the recommended N.E.Q.S. There was increase in TSS, TDS, BOD,COD,EC, colour and metal ions concentration.

Introduction

Industrial effluents entering into the surface waters are perhaps the most important sources of toxic contaminants in the environment. Textile is one of the largest industries of Pakistan, and results in water pollution contributed by untreated effluent discharge, which contains high concentrations of consumed metal based dyes, phenol, aromatic amines etc. The presence of metal based coloured dyes and foaming chemicals in textile waste water not only retards biological activity by reducing the light penetration but also causes metal toxicity to both aquatic and terrestrial life (Sarnaik & Kanekar, 1995). Mobilization of toxic metals from these sites leads to spread of the metal contamination via ground and surface water, posing health and environmental problems. In Pakistan, Indus river has accumulated enormous amounts of metal ions such as As, Hg, Pb and Mn (Tariq *et al*., 1996).

Bioremediation constitutes the use of natural biota and their processes for pollution reduction; it is a cost effective process and the end products are non-hazardous (Ahmedna *et al*., 2004). Microbial communities are of primary importance in bioremediation of metal contaminated soil and water, because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization. Heterotrophic fungi (*Mucor*, *Aspergilus*, *Penicillium* and *Yarrowta*) can remove both soluble and insoluble metal species from solution and are also able to leach metal cations from solid waste (White *et al*., 1997). *Trametes versicolor* and *Bjerkandera adusta* degrade nickel based dyes, phthalocyanine, and copper containing azo-dye (Heinfling *et al*., 1997).

Due to such remediative capability of fungi, and scarcity of data from Pakistan, a study was carried out to determine the extent of pollution through physiochemical and metal concentration analysis in effluents of textile industry alongwith isolation, enumeration and identification of indigenous fungal flora. This study may serve to provide a base for further attempts on bioremediating contaminated soils.

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Materials and Methods

Location: The study area characterized in this study was Kohinoor Textile Mills, Peshawar Road, Rawalpindi, located at the populated peri-urban outskirts of the city. The 6 sampling sites were different points spanning from the specific point where the effluent is discharged from the factory to a distance of 1 km. The uncontaminated reference site was that of a nearby area stream.

Sampling: Effluent and stream water were sampled in dry, sterile, polypropylene bottles, which were kept in ice during transportation. Samples were stored in the refrigerator (4^oC) till the isolation of fungi.

The samples were preserved for analysis of heavy metals (Cu, Cd, Cr, Fe, Ni, Mn and Zn) by acidification with concentrated HNO_3 (1.5 mL HNO_3/L of sample, and then stored at 4^oC till analyzed.

Fungal identification and enumeration: Fungal population of effluent and stream water was determined by serial dilution and plating on Sabouraud Dextrose agar as described by Harley & Prescott, (1993). Malt extract and cellulytic media were used for further identification (Kirk *et al*., 1978). Identification of fungal isolates was carried out using fungal taxonomic identification key at the Pakistan Museum of Natural History, Islamabad.

Physiochemical analysis: Temperature, pH, colour and odour of the samples was recorded on the spot. Electrical conductivity, total dissolved solids, total suspended solids, chemical oxygen demand and biochemical oxygen demand was carried out according to standardized methods (Clesceri *et al*., 1989).

Metal analysis: All the 8 metals were analyzed by Solar Unicam atomic absorption spectrophotometer using Air-Acetylene flame, as recommended by Chrislarsen (1982). The detectable limits for the various metals were 0.03 mg/L (Cu), 0.02 mg/L (Cd), 0.02 mg/L (Cr), 0.005 mg/L and 0.05 mg/L (Zn). Na and K were analysed using a flame photometer.

Results

The physiochemical characterization of effluent from various sites is given in Table 1. Textile effluent was highly alkaline in nature with pH values ranging between 8.06 to 12.44, whereas temperatures were generally below recommended NEQS (40^oC). Wastewater was highly coloured, showing presence of high concentrations of unused dye. Total suspended solids were extremely high (upto 15343.2 mg/l at site number 5 and 6), whereas total dissolved solids were high at sites number 2 and 3. BOD levels at various sites were 70.38 – 552.88 mg/L.

The results of the triplicate digestion of waste water samples of various sites are shown in Table 2. Cu concentrations in different samples were within the NEQS range, except at site 5 (9.70 ppm) and 6 (8.60 ppm). Cd, Cr, Ni and Zn at different sites show similar pattern to Cu. Fe and Mn exhibited high concentrations (ppm) at sites 5 and 6, which were 110.2 and 112.0 for Fe, and 7.20 and 7.40 for Mn, respectively.

Table 1. Physiochemical characterization of effluent

Parameter	NEQS	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Ref site
Temperature (°C)	upto 40.0	33.3	51.5	30.5	42.0	32.0	32.0	28.0
pH	6-9	11.21	12.44	11.47	11.79	8.15	8.06	7.23
Electrical conductivity (mS/cm)		2.32	5.81	3.83	4.91	1.08	1.07	0.20
Colour	Colourless	Brown	Brown	Brown	Dark mauve	Dark grey	Black	Transparent
Odour	Odourless	Fishy	Fishy	Fishy	Fishy	Pungent	Pungent	-
Total suspended solids (mg/L)	upto 200	415.6	759.6	625.2	619.2	15343.2	15343.2	40.0
Total dissolved solids (mg/L)	upto 3500	2453	4975	4948	3856	1278	1230	
Chemical oxygen demand (mg/L)	upto 150	1728	448	2080	1696	1088	1030	30
Biochemical oxygen demand (mg/L)		552.88	70.38	547.88	370.38	242.88	230.08	20

Table 2. Concentrations (mg/L) of metals in effluent.

METAL	NEQS	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Ref site
Cu	1.0	0.26	0.32	0.12	0.19	9.70	8.60	0.12
Cd	0.1	0.03	0.04	0.03	0.01	0.63	0.69	0.03
Zn	5.0	0.63	0.78	1.08	0.48	7.87	7.76	0.13
Fe	2.0	0.46	0.75	0.32	0.41	110.2	112.0	0.121
Cr	1.0	1.14	1.13	1.40	1.32	2.14	2.14	0.00
Ni	1.0	0.58	0.18	0.04	0.01	1.11	1.11	0.00
Mn	1.5	0.31	0.38	0.24	0.09	7.20	7.40	0.45
Pb	0.5	0.17	0.30	0.32	0.17	0.29	0.29	0.00
Na		154.00	258.0	180.0	218.0	84.0	84.0	0.00
K		52.00	111.0	93.0	40.0	62.0	62.0	1.00
P		0.104	0.135	1.21	0.85	2.15	1.35	0.002

Table 3. Fungal genera identified in the effluent.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Reference site
Strains	*Rhizopus* spp. *Penicillium* spp. *Rhodotorula rubra* *Candida tropicalis* *Drechslera* spp.	*Rhizopus* spp.	*Aspergillus* spp. *Aspergillus flavus* *Aspergillus fumigatus*	*Aspergillus niger* *Rhizopus* spp. Unidentified white mycelial mass	*Rhizopus* spp. Unidentified white mycelial mass	Unidentified white mycelial mass	No fungal population
CFU/mL	3.8×10^3	0.001×10^3	0.15×10^3	4×10^3	5×10^3	5×10^3	

Fungal species from effluent samples: The total viable count at various sampling sites ranged between 0.001 x 10^3 to 5 x 10^3 at pH values between 8.06 to 12.44. The fungal strains isolated from water samples revealed the presence of *Rhizopus* spp, *Aspergillus* spp., *Penicillium* spp., *Candida* spp., Drechslera spp., and *Rhodotorula spp*., *Aspergillus* spp., and *Rhizopus* spp., were predominant genera. *Aspergillus* species identified were *A. niger*, *A. fumigatus* and *A. flavus*. Two types of yeast, viz., *Candida* spp., and *Rhodotorula* spp., were found. Lowest incidence of *Drechslera* spp., was observed. Samples from site number 5 and 6 contained fungal isolates having white septate mycelium and did not yield fruiting body on either Sabouroud Dextrose agar, Malt extract agar or cellulytic media. Therefore, these fungal isolates remained unidentified. These unidentified white fungi were most abundant, having a CFU of 5 x 10^3.

Discussion

Textile effluent from Kohinoor Mills was highly coloured, alkaline and foul smelling in nature, which is in agreement with previous findings regarding textile effluent (Sarnaik & Kanekar, 1995). Increased pH is due to excessive use of carbonate, bicarbonate, H_2O_2 and NaOH during bleeching process (Wood & Kellog, 1988).

The last two sites had the least electrical conductivity, pH and total dissolved solids, whilst the COD and BOD levels at sink were lower than the source. Total suspended solids were highest in the sink and lowest at the point of origin of the effluent. This fact is attributable to the process of floc formation which leads to deterioration in water quality, thus affecting aquatic life (Buckley, 1992).

Decrease in BOD and COD levels is suggestive of the fact that the process of bioremediation is in progress, as earlier studies have shown that high BOD and COD levels are often indicated in waste water containing substances that can be biologically degraded (Ademorotti *et al*., 1992; Pathe *et al*., 1995).

Total dissolved and suspended solids are much higher than the NEQS upper limits. High BOD and COD levels are another indicator of an increased load of organic pollutants in the effluent. Cd, Cr, Cu,Fe, Mn, Ni, Zn, K and Na were much higher than permissible under the NEQS (Malik & Ahmed, 2002). Several fungal strains were isolated and identified from the effluent, while one isolate remained unidentified.

High metal ion concentration is another problem associated with textile effluent, which arises from usage of higher amount of metal containing dyes in dyeing process (Correia *et al.*, 1994; Sabour *et al*., 2001). Levels of Pb in the samples from all the sites were within permissible limits, while those of Cr in all the sites were beyond the upper level of NEQS. The high level of Cr in all the samples can be comprehended due to the use of metal based dyes in the textile industry, which mainly contain chromium (Banat *et al*., 1996).

In case of other metals, Cu, Cd, Fe, Mn, Ni and Zn, only the last two sites had a concentration beyond the acceptable limits of the NEQS. This increase of metal ion concentration from source to sink is attributable to the fact that the waste carried in the effluent flows towards, and concentrates, in the sink. The source of metal in the effluent being the dyes used in textile industry (Marks & DeLeo, 1997), as about 10-15% of dyes are lost into the waste water during the dyeing process (McMullan *et al*., 1995).

Rhizopus spp., and *Aspergillus* spp., were two predominant genera found in highly alkaline effluent. *Aspergillus terreus* and *Aspergillus niger* have demonstrated nickel uptake capability from aqueous solution (Dias *et al*., 2002; Keshinkan *et al*., 2004)).

Alkali extracted mycelial biomass of *A. niger* is reported to have removed Zn (9 g/L) and Cr (420 g/L), by recycling biosorbent (Akhter & Mohan, 1995). Cu removal reported by *Sargassum* has also been documented (Volesky *et al.*, 2003).

A. niger was the most prevalent fungal strain in the waste water. *A.niger* have bioremediative potential as reported in by Sayer *et al.*, (1997), where *A. niger* solubilized insoluble compounds, such as CuO_2, PbS, $CaCO_3$ and $Mn(CO_3)_2$ in solid media. Price *et al*., (2001) also observed similar capabilities of this strain, where *A. niger* removed 91% copper and 70% zinc from swine waste water. The presence of *A. niger* in all the samples of the study under discussion, probably, indicates its role in tolerance and detoxification of metal ions in the effluent.

Based upon these findings, it can be concluded that fungi present in the vicinity of discharged effluent possess a great potential for use in decontamination of soils. These fungal strains can be studied for bioaccumulation of heavy metal ions from textile and other metal containing waste waters, and have a potential for use in bioreactor for industrial discharge treatment, through the application of biotechnology.

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