Environmental Engineering and Management Journal

March 2018, Vol.17, No. 3, 545-550
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

DECOLORIZATION AND DETOXIFICATION OF TANNERY WASTEWATER BY *Trichoderma viride* SPFT1

Smiley Sharma[1], Piyush Malaviya[1,2*]

[1]Department of Environmental Sciences, University of Jammu, Jammu-180006 (J&K) INDIA
[2]The Institute of Environmental and Human Health (TIEHH), Department of Environmental Toxicology, Texas Tech University, Box 41163, Lubbock, Texas 79409-1163, USA

Abstract

This study deals with the decolorization and detoxification of tannery wastewater by an indigenous fungal isolate, *Trichoderma viride* SPFT1 isolated from untreated tannery wastewater. The results showed that treatment with *Trichoderma viride* SPFT1 reduced chemical oxygen demand (COD) (74.20%), nitrate (60.83%) and color (45.24%) of the tannery wastewater after six days of the incubation period. The toxicity assessment of the tannery wastewater on *Phaseolus mungo* L. var. PU-19 showed 70% seed germination in treated wastewater as compared to untreated wastewater (30%). Moreover, untreated tannery wastewater also inhibited seedling growth and reduced chlorophyll content.

Key words: chlorophyll, decolorization, fungi, germination, wastewater

Received: January, 2014; Revised final: June, 2014; Accepted: July, 2014; Published in final edited form: March 2018

1. Introduction

Pollution of the aquatic environment by industrial wastewaters is one of the growing problems all over the world (Malaviya and Rathore, 2001; Pant and Adholeya, 2010). Tanning industry is globally considered as highly polluting industry. Leather, the prime product of tanneries is manufactured in a number of steps, involving a wide range of processes and chemicals that include sodium chloride, lime, sodium sulphate, fat liquor ammonia, sulphuric acid, chromium sulphate and a number of dyes (Kankaria et al., 2011). Processing one metric ton of raw hide generates 200 kg of final leather product (containing 3 kg of chromium), 250 kg of non-tanned solid waste, 200 kg of tanned waste (containing 3 kg of chromium), and 50,000 kg of wastewater (containing 5 kg of chromium) (Huffer and Taeger, 2004). After every step, wastewater is generated and final effluent contains high Biochemical oxygen demand (BOD), COD, Total Dissolved Solids (TDS), chromium and residual dyes (Sharma and Malaviya, 2013).

The tannery wastewaters are frequently toxic and persistent and affect both human health and the environment. Moreover, untreated or partially treated wastewater can introduce a huge amount of inorganic and organic contaminants into agricultural lands (Lonigro et al., 2015; Wang and Tao, 1998), the continual use of which over a period of time can exert adverse impacts on quality of soil and plants grown on it (Sinha et al., 2006).

The different approaches developed for tannery wastewater treatment include membrane processes (De Gisi et al., 2009), adsorption (Begum et al., 2006), coagulation and flocculation (Aziz et al., 2007; Malaviya and Singh, 2011) and bacterial treatment (Ingole et al., 2012). Even though bioremediation is considered as an alternative to tannery wastewater detoxification and recycling, little information is available on the indigenous microorganisms present in

* Author to whom all correspondence should be addressed: e-mail: piyushmenv@gmail.com; piyushmalaviya@rediffmail.com

the tannery wastewater and their possible use for biodegradation of the wastewater.

Therefore, the objective of this study was to evaluate the bioremediation potential of *Trichoderma viride* SPFT1 isolated from the tannery wastewater. As phytotoxicity assessment is an indirect measure of the impact of the wastewater on natural and manipulated ecosystems, therefore, an attempt has also been made to determine the toxicity of raw and bioremediated tannery wastewater on an economically important and widely cultivated legume, *Phaseolus mungo* L. var. PU-19.

2. Materials and methods

2.1. Isolation of fungal strain

The tannery wastewater for the isolation of fungus was collected in acid-rinsed polyethylene containers from the final discharge section of a tannery located in Central Leather Research Institute (CLRI) complex, Kapurthala Road, Jalandhar, India. The collected samples were brought to the laboratory and stored in a refrigerator at 4 °C till their utilization.

For isolation of fungus, 1 mL of tannery wastewater was inoculated in a 250 mL Erlenmeyer flask containing 100 mL of Lee's minimal medium amended with 1 g tannery sludge and pH maintained at 5.3. The flasks were incubated at 28 °C and 150 rpm for three days. This process was repeated several times with fresh sludge amended minimal salt medium (MSM). Finally, 0.1 mL of serial dilutions were spread on sludge amended MSM plates (Singh et al., 2013). The fungal colonies appearing on MSM were picked and inoculated on Potato Dextrose Agar (PDA) plates.

The isolated and purified fungal strain was identified as *Trichoderma viride* by National Center of Fungal Taxonomy (NCFT), New Delhi.

2.2. Fungal inoculum preparation and bioremediation experiments

For bioremediation studies, the fungal inoculum was prepared in the form of mycelial pellets. Erlenmeyer flasks (250 mL capacity) containing 100 mL potato dextrose broth (PDB) and streptopenicillin (100 ppm) were inoculated with mycelial discs (Srivastva and Thakur, 2006).

These flasks were incubated at 30 ºC for 5 days in orbital shaker at 150 rpm. The mycelium thus obtained was filtered by cheesecloth and air-dried on sterilized petriplates. Fungal pellets were prepared by cutting in approximately 1.5-2.0 mm size. The fungal pellets (2% w/v) were inoculated in combined tannery effluent amended with 0.1% glucose and 0.1% ammonium nitrate. The pH value was maintained at 5.3 and the flasks were incubated at 30 ^{0}C in a shaker for six days at 150 rpm. The wastewater samples were collected at different time intervals (2d, 4d and 6d) and reduction in COD, color and other pollution parameters was measured.

2.3. Analytical methods

Chemical oxygen demand (COD) and total suspended solids (TSS) were determined according to American Public Health Association (APHA) methods (Greenberg et al., 1995). Color was measured spectrophotometrically (465 nm) according to the method of Bajpai et al. (1993). Other parameters of the wastewater e.g. pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured using Multi Parameter Water Analyzer Kit (WTW, Germany). Sodium, chloride and nitrate ions were measured by Thermo Scientific Orion DUAL STAR ion meter while turbidity was measured by Digital Turbidity Meter (Environmental and Scientific Instruments Co., India). The data obtained in the study were analyzed by Duncans' Multiple Range Test using SPSS Inc. (v 17.0) software. The differences between means were considered significant at values of $p \leq 0.05$.

2.4. Phytotoxicity studies

The phytotoxicity study was carried out on *Phaseolus mungo* L. var. PU-19, an agriculturally important crop. Twenty seeds of *Phaseolus mungo* L. var. PU-19 were surface sterilized with 2.0% $HgCl_2$ solution (Ahsan et al., 2007), and were placed in glass petri dishes lined with two Whatman No.1 filter paper discs. These filter discs were moistened with 5 mL of tap water for control and with the same volume of untreated and treated tannery wastewater samples followed by incubation at 28°C in a BOD incubator for a period of fourteen days. The visible protrusion of radical from seed coat was taken as criterion of seed germination. Various germination indices adopted from Czabator (1962), Rao et al. (1979) and Zucconi et al. (1981) were used to record the germination parameters and seedling growth (Kohli and Malaviya, 2013; Malaviya et al., 2012). For the estimation of pigment content, the leaves were extracted in 80% acetone and the absorbance of pigment extract was measured spectrophotometrically at wavelength 663 and 645 nm for chlorophyll content (Arnon, 1949) and 480 and 510 nm for carotenoid content (Duxbury and Yentsch, 1956).

3. Results and discussion

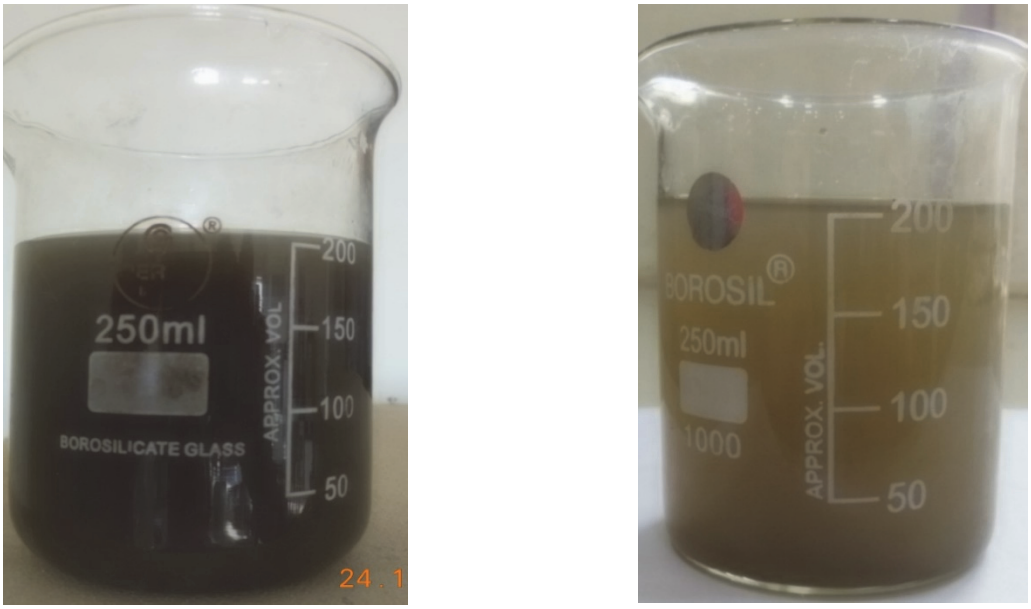
3.1. Tannery wastewater characterization before and after fungal treatment

The physico-chemical characteristics of the tannery wastewater before and after different durations of treatment with *Trichoderma viride* SPFT1 are shown in Table 1. After six days of fungal treatment, there was significant reduction in COD, color (Fig. 1a-b) and other pollution parameters of the tannery wastewater. The order of reduction in different physicochemical parameters i.e. COD, color, TSS, turbidity and TDS, was 74.20, 45.24, 35.78, 22.57 and 14.45%, respectively after six days of treatment with *Trichoderma viride*.

Table 1. Physico-Chemical characteristics* of untreated tannery wastewater (0d) and treated tannery wastewater after different durations of treatment (2d, 4d, and 6d) with *Trichoderma viride* SPFTI
(Figures in parenthesis show % reduction)

Parameters	***Treatment duration***			
	0d	***2d***	***4d***	***6d***
pH	$9.16^{a} \pm 0.20$	$5.30^{b} \pm 0.29$	$5.50^{a} \pm 0.15$	$7.44^{c} \pm 0.19$
COD (mg L^{-1})	$5776^{a} \pm 30.10$	$2581.70^{b} \pm 10.69$ (55.30)	$1490^{c} \pm 18.17$ (74.20)	$1490^{c} \pm 14.00$ (74.20)
Color (CU)	$1984.85^{a} \pm 12.80$	$1391.10^{b} \pm 9.57$ (29.91)	$1155.60^{c} \pm 7.30$ (41.78)	$1086.90^{d} \pm 6.60$ (45.24)
TDS (mg L^{-1})	$17650^{a} \pm 20.10$	$15801^{b} \pm 6.02$ (10.47)	$15799^{b} \pm 1.73$ (10.48)	$15100^{c} \pm 3.037$ (14.45)
TSS (mg L^{-1})	$1694^{a} \pm 11.20$	$1121.70^{b} \pm 4.51$ (33.78)	$1096.60^{c} \pm 3.97$ (35.26)	$1088.60^{c} \pm 5.00$ (35.78)
Turbidity (NTU)	$505^{a} \pm 2.00$	$480.33^{b} \pm 4.51$ (4.88)	$430.55^{c} \pm 5.50$ (14.74)	$391^{d} \pm 4.58$ (22.57)
EC (mS cm^{-1})	$35.30^{a} \pm 0.25$	$31.33^{b} \pm 1.41$ (11.25)	$31.26^{b} \pm 1.42$ (11.44)	$29.86^{b} \pm 1.13$ (15.41)
Na^{+} (mg L^{-1})	$3080^{a} \pm 35.60$	$3041^{b} \pm 4.58$ (1.27)	$2979^{c} \pm 6.55$ (3.28)	$2871^{d} \pm 3.60$ (6.78)
Cl^{-} (mg L^{-1})	$4700^{a} \pm 40.10$	$3743^{b} \pm 6.08$ (20.36)	$3731^{b} \pm 4.58$ (20.62)	$3608.70^{c} \pm 6.11$ (23.22)
NO_3^{-} (mg L^{-1})	$600^{a} \pm 5.00$	$389^{b} \pm 5.57$ (35.17)	$284^{c} \pm 3.46$ (52.67)	$235^{da} \pm 9.64$ (60.83)

**=Mean±SD (n=3); *Within each row, values not followed by same superscript are significantly different at p≤0.05.*



(a) (b)

Fig. 1. (a) Untreated tannery wastewater, (b) Treated Tannery wastewater

Similarly, ions like nitrate, chloride and sodium have shown 60.83, 23.22 and 6.78% reduction on sixth day of the treatment.

Contrary to findings of the present study, Mohamed et al. (2011) reported only 19.42% COD removal in tannery wastewater after three-day treatment with *A. niger*. The high COD reduction (55.30%) within two days in the present study might be attributed to organics degrading capability of *Trichoderma viride* SPFT1 as it was directly isolated from the tannery wastewater and was being naturally adapted to utilize the organics present in the tannery wastewater.

In the present study, 45.24% decolorization of tannery wastewater was achieved after six days of fungal treatment. The decolorization was possibly achieved by oxidative degradation of the dye molecules (Mohorcic et al., 2006). After six days of fungal treatment there was 35.78% and 22.57% reduction in TSS and turbidity, respectively because filamentous fungi entrap the suspended solid particles in the wastewater (Alam and Fakhrul-Razi, 2001; Fakhrul-Razi and Molla, 2007). The reduction in NO_3^{-}, Na^{+} and Cl^{-} ions might be attributed to utilization of these ions for growth by the fungal isolate (Mert and Dizbay, 1977).

3.2. Effect of treated and untreated tannery wastewater on germination parameters of Phaseolus mungo L. var. PU-19

Data regarding the effect of treated and untreated tannery wastewater on germination parameters of *Phaseolus mungo* L. var. PU-19 has been depicted in Table 2. The values for positive germination parameters i.e. Seed germination (%), Germination index, Speed of germination, peak value, Germination value and Seedling Vigour Index (SVI) have shown significant increase in the seeds irrigated with treated wastewater over the untreated wastewater. Whereas, the negative germination parameters i.e. delay index and percent inhibition have shown an increase in values in seeds treated with untreated wastewater.

The inhibitory effect of untreated tannery wastewater on seed germination was ascribed to the presence of high content of total solids, total nitrogen, phosphate, sulfates, and heavy metals, which induces high osmotic pressure and anaerobic conditions (Chandra et al., 2011). It has also been reported that high osmotic pressure of the germination solution makes imbibition more difficult and create anaerobic conditions which impairs various physiological and biochemical processes essential for seed germination (Malaviya and Sharma, 2011; Rodger et al., 1957).

3.3. Effect of treated and untreated tannery wastewater on seedling growth and pigment content of Phaseolus mungo L. var. PU-19

Data regarding the effect of treated and untreated tannery wastewater on seedling growth and pigment content of *Phaseolus mungo* L. var. PU-19 has been shown in Table 3 and 4. There has been a significant increase in root length, shoot length, root shoot ratio, fresh weight and pigment content in the seedlings irrigated with treated wastewater than the untreated wastewater. The reduction of seedling growth in the untreated wastewater was due to the toxic effect of various ions and unbalanced nutrient uptake by seedlings.

These deleterious effects may result in a decrease in photosynthesis and increase in respiration rate leading to a shortage of assimilate to the developing organs, thus slowing down growth. Accordingly, percentage inhibition in root length and shoot length of *Phaseolus mungo* L. var. PU-19 was observed as 177.40 and 129.10%, respectively in untreated wastewater as compared to treated wastewater. The root length showed more reduction in the untreated wastewater because the root continuously remains in direct contact with the highly polluted wastewater which retards cell multiplication (Kannan et al., 2008).

Compared to treated wastewater, the untreated wastewater significantly reduced the foliar content of chlorophyll 'a' (chl-a), chlorophyll 'b' (chl-b) and total chlorophyll whereas, carotenoids have shown an increase. There has been 60.72 and 43.78% decrease in chlorophyll 'a', respectively in untreated wastewater as compared to treated wastewater. More decrease in chl-a was attributed to higher sensitivity of chl-a than chl-b (Singh and Malaviya, 2013; Vajpayee et al., 2000).

However, carotenoid has shown 20.75% increase in untreated wastewater as compared to treated wastewater because it acts as a non-enzymatic antioxidant and protect chlorophyll pigments under stress conditions (Kenneth et al., 2000).

Table 2. Effect of treated and untreated tannery wastewater on germination parameters in *Phaseolus mungo* L. var. PU-19

Treatments	*Seed germination (%)*	*Germination index*	*Speed of germination*	*Delay index*	*Peak value*	*Germination value*	*Percent inhibition*	*Seedling Vigour Index (SVI)*
Control	95.00^{a} ±3.00	-	10.00^{a} ±1.00	-	23.75^{a}±2.24	2256.25^{a} ±8.51	-	2516.55^{a} ±14.86
TW	70.00^{b} ±4.00	57.63^{a} ±1.20	6.00^{b} ±1.00	2.00^{b} ±1.00	14.00^{b} ±3.38	980.00^{b} ±14.52	25.00^{b} ±2.00	1586.90^{b} ±21.20
UTW	30.00^{c} ±5.00	8.90^{b} ±1.48	0.73^{c} ±0.07	7.00^{a} ±2.00	3.75^{c} ±1.24	112.50^{c} ±3.30	65.00^{a} ±3.00	371.10^{c} ±15.55

**=Mean±SD (n=3); TW=treated wastewater; UTW=untreated wastewater*
**Within each column, values not followed by same superscript are significantly different at p≤0.05*

Table 3. Effect of treated and untreated tannery wastewater on seedling growth in *Phaseolus mungo* L. var. PU-19*

Treatments	*Root length (cm)*	*Shoot length (cm)*	*Root/Shoot ratio*	*Fresh weight(g)*	*Dry weight (g)*	*Moisture (%)*
Control	5.85^{a}±0.929	20.66^{a}±3.78	0.284^{a}±0.003	0.225^{a}±0.002	0.022^{a}±0.0025	90.06^{a}±2.473
TW	4.80^{a}±0.458	17.87^{a}±1.921	0.269^{b}±0.009	0.190^{b}±0.002	0.019^{ab}±0.0031	90.00^{a}±2.051
UTW	1.73^{b}±0.152	7.80^{b}±0.986	0.221^{c}±0.006	0.150^{c}±0.002	0.016^{b}±0.0026	89.33^{a}±2.535

**=Mean±SD (n=3); TW=treated wastewater; UTW=untreated wastewater*
**Within each column, values not followed by same superscript are significantly different at p≤0.05.*

Table 4. Effect of treated and untreated tannery wastewater on pigment content (mg g^{-1} fresh weight) in *Phaseolus mungo* L. var. PU-19*

Treatments	*Chlorophyll a*	*Chlorophyll b*	*Chlorophyll a/b ratio*	*Total Chlorophyll*	*Carotenoid*
Control	$1.10^{a} \pm 0.0984$	$0.700^{a} \pm 0.0100$	$1.58^{a} \pm 0.023$	$1.811^{a} \pm 0.093$	$0.681^{a} \pm 0.008$
TW	$0.884^{b} \pm 0.009$	$0.683^{a} \pm 0.0133$	$1.29^{b} \pm 0.080$	$1.582^{b} \pm 0.023$	$0.756^{a} \pm 0.011$
UTW	$0.550^{c} \pm 0.0133$	$0.475^{b} \pm 0.009$	$1.16^{c} \pm 0.040$	$1.027^{c} \pm 0.047$	$0.954^{a} \pm 0.455$

**= Mean±SD (n=3); TW=treated wastewater; UTW=untreated wastewater; *Within each column, values not followed by same superscript are significantly different at p≤0.05.*

Moreover, fresh weight, dry weight and moisture content (biomass production in seedling) has also shown decrease in the untreated wastewater which might be ascribed to the lowered photosynthesis and chlorophyll 'a' production in the seedlings

4. Conclusions

Trichoderma viride SPFT1 isolated from untreated tannery wastewater exhibited detoxification and decolorization of tannery wastewater.

The seed toxicity assessment using *Phaseolus mungo* L. var. PU-19 showed increase in germination, seedling growth and pigment content in treated tannery wastewater compared to untreated wastewater.

In future, there is a need to conduct process parameter optimization studies to improve the bioremediation efficiency of the isolate.

Acknowledgements

The corresponding author (PM) gratefully acknowledges the financial support provided by Department of Biotechnology, Government of India in the form of research project.

References

Ahsan N., Lee S.H., Lee D.G., Lee H., Lee S.W., Bahk J.D., Lee B.H., (2007), Physiological and protein profiles alternation of germinating rice seedlings exposed to acute cadmium toxicity, *Comptes Rendus Biologies*, **330**, 735-746.

Alam M.Z., Fakhrul-Razi A., (2001), Enhanced settleability and dewaterability of fungal treated domestic wastewater sludge by liquid state bioconversion process, *Water Research*, **37**, 1118-1124.

Arnon D.I., (1949), Copper enzymes in violated chloroplast polyphenol oxidase in Beta vulgaris, *Plant Physiology*, **24**, 1-15.

Aziz H.A., Alias S., Adlan M.N., Faridah, Asaari A.H., Zahari M.S., (2007), Colour removal from landfill leachate by coagulation and Flocculation, *Bioresource Technology*, **98**, 218-220.

Bajpai P., Mehna A., Bajpai P.K., (1993), Decolorization of Kraft bleach plant effluent with white-rot fungus *Tramates versicolor*, *Process Biochemistry*, **28**, 377-384.

Begum S.S., Miranda L.R., Srinivasan S.V., (2006), Adsorption of remazol brilliant red (5B) by activated carbon produced from rubber wood, *Indian Journal of Environmental Protection*, **6**, 769-775.

Chandra R., Bharagava R.N., Kapley A., Purohit H.J., (2011), Bacterial diversity, organic pollutants and their metabolites in two aeration lagoons of common effluent treatment plant (CETP) during the degradation and detoxification of tannery wastewater, *Bioresource Technology*, **102**, 2333–2341.

Czabator F.J., (1962), Germination value, an index combining speed and completeness of pine seed germination, *Forest Science*, **8**, 386-396.

De Gisi S., Galasso M., De Feo G., (2009), Treatment of tannery wastewater through the combination of a conventional activated sludge process and reverse osmosis with a plane membrane, *Desalination*, **249**, 337-342.

Duxbury A.C., Yentsch C.S., (1956), Plankton pigment monographs, *Journal of Marine Research*, **15**, 92-101.

Fakhrul-Razi A., Molla A.H., (2007), Enhancement bioseparation and dewaterability of domestic wastewater sludge by fungal treated dewatered sludge, *Journal of Hazardous Material*, **147**, 350-356.

Greenberg A.E., Connors J.J., Jenkins D., Franson M.A., (1995), Standard Methods for the Examination of Water and Wastewater, 15th Edition,. American Public Health Association, Washington DC.

Huffer S., Taeger T., (2004), Sustainable leather manufacturing a topic with growing importance, *Journal of the American Leather Chemists Association*, **99,** 423-428.

Ingole S.P., Kakde A.U., Maggirwar R.C., (2012), Biodegradation of tannery effluent by using tannery effluent isolate, *International Multidisciplinary Research Journal*, **2**, 43-44.

Kankaria S., Andukuri A., Hemamailini C.G., Krishnaveni M., (2011), *Impact of Tannery effluent on Groundwater and Agriculture with a Remedial measure – A case study*, Int. Conf. on Chemical, Biological and Environment Sciences, Bangkok, 383-388.

Kannan A.R., Upreti R.K., (2008), Influence of distillery effluent on germination and growth of mungbean (*Vigna radiata*) seeds, *Journal of Hazardous Material*, **153**, 609-615.

Kenneth E., Pallet K.E., Young J., (2000), *Carotenoids*, In *Antioxidants in Higher Plants*, Alscher R.G., Hess J.L., (Eds.), CRC Press, 60-81.

Kohli R., Malaviya P., (2013), Impact of tannery effluent on germination of various varieties of wheat (*Triticum aestivum* L.), *Journal of Applied and Natural Science*, **5**, 302-305.

Lonigro A., Montemurro N., Rubino P., Vergine P., Pollice A., (2015), Reuse of treated municipal wastewater for irrigation in Apulia Region: the "IN.TERRA" project, *Environmental Engineering and Management Journal*, **14**, 1665-1674.

Malaviya P., Hali R., Sharma N., (2012), Impact of dyeing industry effluent on germination and growth of Pea (*Pisum sativum*), *Journal of Environmental Biology*, **33**, 1075-1078.

Malaviya P., Rathore V.S., (2001), A correlation study on some physicochemical quality parameters of pulp and paper mill effluents, *Pollution Research*, **203,** 465-470.

Malaviya P., Sharma A., (2011), Impact of distillery effluent on germination behaviour of *Brassica napus* L., *Journal of Environmental Biology*, **32**, 91-94.

Malaviya P., Singh A., (2011), Physicochemical technologies for remediation of chromium-containing waters and wastewaters, *Critical Reviews in Environment Science and Technology*, **41**, 1111–1172.

Mert H.H., Dizbay M., (1977), The effect of osmotic pressure and salinity of the medium on the growth and sporulation of *Aspergillus niger* and *Paecilomyces lilacinum* species, *Mycopathologia*, **61**, 125-127.

Mohamed A.M., Sekar P., John G., (2011), Efficacy of microbes in bioremediation of tannery effluent, *International Journal of Current Research*, **3**, 324-326.

Mohorcic M., Teodorovic S., Golob V., Friedrich J., (2006), Fungal and enzymatic decolorization of artificial textile dye baths, *Chemosphere*, **63**, 1709-1717.

Pant D., Adholeya A., (2010), Development of a novel fungal consortium for the treatment of molasses distillery wastewater, *The Environmentalist*, **30**, 178-182.

Rao J.V.S., Rama M.R.K., Samba M.S., (1979), Allelopathic effect of some weed of vegetable crops on the germination and early seedling growth of bajra, *Tropical Ecology*, **20**, 5-8.

Rodger J.B.B., Williams G.G., Davis R.L., (1957), A rapid method for determining winter hardiness of alfalfa, *Agronomy Journal*, **49**, 88-92.

Sharma S., Malaviya P., (2013), Bioremediation of Tannery Wastewater by *Aspergillus niger* SPFSL 2 - a Isolated from Tannery Sludge, *International Journal of Basic and Applied Sciences*, **2**, 88-93.

Singh A., Malaviya P., (2013), Phytotoxic effect of chrome liquor on growth and chlorophyll content of *Spirodela polyrrhiza* L. Schleid, *Journal of Applied and Natural Science*, **5**, 165-170.

Singh R., Bishnoi N.R., Kirrolia A., Kumar A., (2013), Synergism of *Pseudomonas aeruginosa* and Fe for treatment of heavy water contaminated effluents using small scale laboratory reactor, *Bioresource Technology*, **127**, 49-58.

Sinha S., Gupta A.K., Bhatt K., Pandey K., Rai U.N., Singh K.P., (2006), Distribution of metals in the edible plants grown at Jajmau, Kanpur (India) receiving treated tannery wastewater: relation with physico-chemical properties of the soil, *Environment Monitoring and Assessment*, **115**, 1-22.

Srivastava S., Thakur I.S., (2006), Evaluation of bioremediation and detoxification potentiality of *Aspergillus niger* for removal of hexavalent chromium in soil microcosm, *Soil Biology and Biochemistry*, **38,** 1904–1911.

Vajpayee P., Tripathi R.D., Rai U.N., Ali M.B., Singh S.N., (2000), Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L., *Chemosphere*, **41**, 1075-1082.

Wang X.J., Tao S., (1998), Spatial structures and relations of heavy metal content in wastewater irrigated agricultural soil of Beijing's Eastern farming regions, *Bulletin of Environmental Contamination and Toxicology*, **61**, 261-268.

Zucconi F., Pera A., Forte M., De Bertoldi M., (1981), Evaluating toxicity of immature compost, *Biocycle*, **22**, 54-57.