

Characterization of tannery effluents of Hazaribagh area, Dhaka, Bangladesh

Kabir, M.M.^{1*}, Fakhruddin, A.N.M.¹, Chowdhury, M.A.Z.², Fardous, Z.² and Islam, R.³

1. Department of Environmental Sciences, Jahangirnagar University, Dhaka-1342, Bangladesh
2. Agrochemical and Environmental Research Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka-1349, Bangladesh
3. Department of Microbiology, Jahangirnagar University, Dhaka-1342, Bangladesh

Received: 16 Dec. 2016

Accepted: 6 Mar. 2017

ABSTRACT: The present investigation characterizes tannery effluents by determining water quality parameters and some selected trace metallic constituents in order to measure the magnitude of environmental pollution. Effluents and sediments have also been used to isolate chromium-resistant bacteria, collected from Hazaribagh area, Dhaka, claimed to be increasing Cr (VI)-enriched environments, and utilized to reduce Cr (VI). The pH, TDS, DO, and EC values of all investigated samples have been within the range of 4.35 to 9.74, 290 to 9,200 mg/L, 1.5 to 4.90 mg/L, and 587 to 19,000 $\mu\text{s}/\text{cm}$, respectively. The concentrations of trace metallic constituents have been determined by means of Atomic Absorption Spectrophotometer (AAS), giving the following ranges: Cr: 14.282 to 6,769.554 mg/L; Cd: 1.546 to 2.214 mg/L; Pb: 18.808 to 32.026 mg/L; Cu: 1.522 to 2.578 mg/L; Zn: 0.682 to 8.688 mg/L; Fe: 1.37 to 108.556 mg/L; and Mn: 3.494 to 17.17 mg/L. The order of trace metallic constituents in the effluent samples was $\text{Cr} > \text{Fe} > \text{Pb} > \text{Mn} > \text{Zn} > \text{Cd} > \text{Cu}$. The water quality parameters and metallic constituents' concentration were higher than that of standard permissible limits in all the examined samples. The average number of Cr (VI)-resistant bacteria in the sediment samples with glucose (0.5%, w/v) supplementation was 2.35×10^3 CFU/gm at 24h. One of the representative isolate was able to tolerate up to 400 mg/L of $\text{K}_2\text{Cr}_2\text{O}_7$ as Cr (VI). The bacteria, isolated in the present study, can be used as eco-friendly biological agents for the remediation and/or detoxification of chromium (Cr) pollution from the chromium contaminated environments.

Keywords: Cr (VI) resistant bacteria, metal, pollution, tannery wastewater, water.

INTRODUCTION

Leather tanning industry is recognized as one of the major sector to produce industrial pollution throughout the world and the processes of leather tanning are completely-wet processes, in need of enough water, turning almost 90% of the used water to

wastewater. Due to the existence of excessive extremely-colourful compounds, NaCl and $\text{Na}_2(\text{SO}_4)_3$, different types of organic and inorganic compounds, hazardous metallic compounds with various biologically oxidizable tanning materials, and a huge quantity of decomposing suspended materials, tannery effluents hold severe

* Corresponding author Email: mahbubkabir556@gmail.com

pollution loads (Akan et al., 2007). As tanning processes are completed, a tiny proportion of the large amounts of chemicals are immersed by leather with the rest being discharged as wastewater that deteriorates the usual functioning of life of recipient aquatic bodies and ground surfaces (Cooman et al., 2003). At present there are 220 tanneries in Bangladesh in total, 95% of which are established in Hazaribagh, a heavily populated suburban area of Dhaka. This particular area is popularly known for its tannery industries, listed fifth in the list of top 10 polluted places of the world (Bernhardt & Gysi, 2013). During the off season the tanneries, located in Hazaribagh area, usually process 60,000 tons of raw hides and skins on an annual basis, which release nearly 95,000 litres effluents without treatment along with 115 tons of solid wastes daily into the open environment (Rusal et al., 2006).

Various industrial applications of chromium and its compounds, e.g. pigments, dyes, leather tanning, and refractory materials, result in extensive environmental pollution. Trivalent chromium is considered a significant nutritive trace element. On the contrary, soluble hexavalent chromium is severely toxic, with mutagenic and carcinogenic impacts on living organisms (Horitsu et al., 1978). Traditional methods for removing trace metals from wastewater include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies, and evaporation recovery (Ahluwalia & Goyal, 2007). It is very unfortunate that it is not possible for developing countries to achieve economic viability through these methods, a few of which might even produce huge amounts of waste as secondary pollution (Ahluwalia & Goyal, 2007). The microbial reduction of rigorously toxic Cr (VI) to less toxic Cr (III) is an essential step, regarding remediation of Cr (VI)-contaminated environments. Biological removal of hexavalent chromium from contaminated

environments ensures the negligible risks to both living biota and the environment.

A diverse group of micro-organisms have been reported to remove chromium from environments, contaminated with this metal, through biosorption and/or reduction of highly soluble and toxic Cr (VI) to less soluble and less toxic Cr (III). A few examples are Bacteria: *Pseudomonas putida* (Sadeeshkumar et al., 2012), Fungi: *Saccharomyces cerevisiae* (Benazir et al., 2010), Algae: *Oedogonium sp.* (Kidgell et al., 2014), etc. The present study tries to determine the magnitude of environmental pollution from the discharge of tannery wastewater and isolate and evaluate the status of Cr (VI)-resistant bacteria, so that it can assess their bioremediation potentials for removing chromium from Cr-contaminated environments.

MATERIALS AND METHODS

Tannery effluents and sediment samples were collected from different sites of the combined tannery industries at Hazaribagh area in Dhaka city, located in south-western part of Dhaka. Figure 1 offers the map of the studied location of Hazaribagh Thana, Dhaka, Bangladesh. Sterile plastic containers (500 ml) and polyethylene bags were used to collect effluents and sediments samples, respectively. The samples were collected in two sets: one, used for determining water quality parameters and metallic constituents, and another for microbiological analysis. The samples were kept in ice boxes and taken to the laboratory for a short time in order to undergo microbiological analysis. In order to determine trace metallic concentration of the samples, instantly after collection, one ml of concentrated HNO₃ (65%) was poured into each of the samples and mixed properly to bring the pH below 2 and minimize precipitation and adsorption on container's walls (APHA, 1998). To prevent the likelihood of hydrolysis and oxidation, samples were conserved at -20°C in a refrigerator.

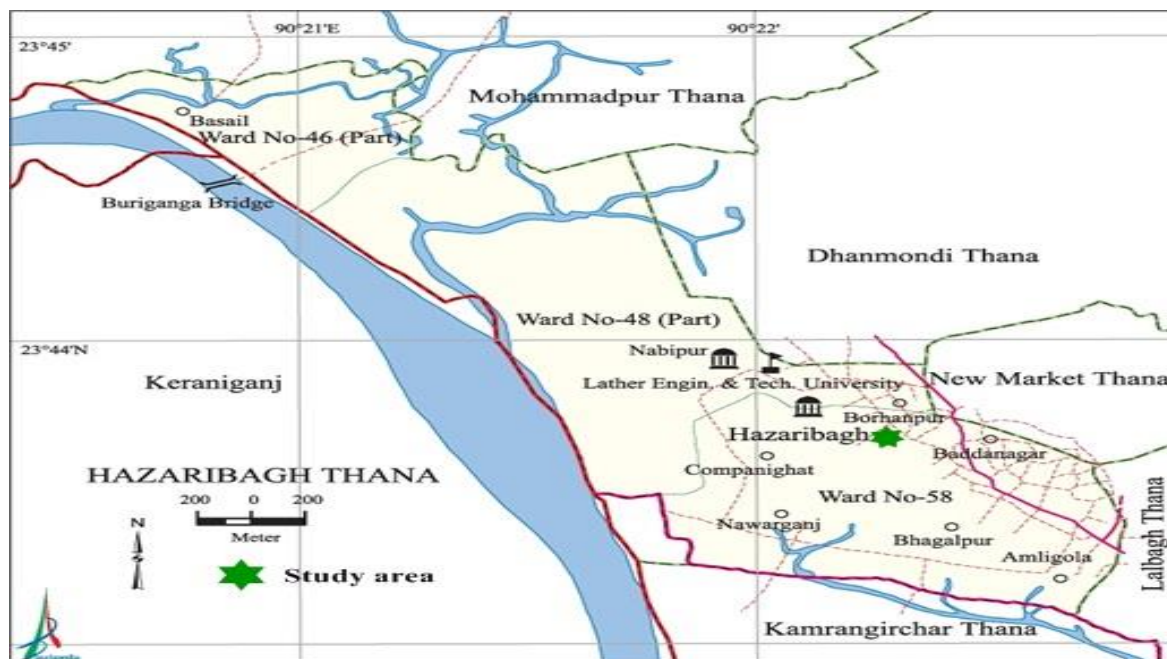


Fig. 1. Map of the study location, Hazaribag Thana, Dhaka, Bangladesh (Banglapedia, 2016)

Measurement of pH, DO, TDS, and EC in tannery effluents

Parameters of water quality, namely pH, Dissolved Oxygen (DO), Total Dissolved Solid (TDS), and Electrical Conductivity (EC), were measured by means of pH-meter (Model- HI 211, HANNA), DO-meter (Model- DO 110, ECOSSEN), TDS-meter (Model-HI 8734, HANNA), and EC-meter (Model-HI 8033, HANNA), respectively. All the instruments were calibrated with standard solution prior to use and the utilized chemicals were of analytical grade.

Determination of trace metallic constituents in tannery effluents

Standards of the heavy metals, i.e., cadmium, copper, lead, chromium, manganese, iron, zinc, and nickel, were obtained from Kanto Chemical Co. Inc., Japan. Acid digestion was performed according to the methods, described by Baker and Amacher (1982) in order to measure the concentration of trace elements in the effluent samples. The samples were filtered with No. 41 Whatman (0.45 μm pore size) filter paper to estimate the dissolved metal content. The filtrates were preserved at room

temperature with 1 ml of nitric acid to preclude the precipitation of metals and 50 ml of the sample was then transferred into a clean glass and 3 ml of concentrated nitric acid was added to it for digestion. The solution was heated at 95°C without boiling until it got dry. The samples were diluted to 50 ml by de-ionized water after cooling and finally the sample solution was aspirated into air acetylene flame in an atomic absorption spectrophotometer (AAS-6300, SHIMADJU, Japan).

Quality Control (QC) and Quality Assurance (QA) of metallic constituent's determination

Quality control and quality assurance were incorporated into the analysis. The analytical method was standardized by processing spiked samples. The accuracy and precision were also validated in accordance with the European Commission guidelines (EC, 2011). The precision was expressed as Relative Standard Deviation (RSD). Accuracy was measured by analysing the samples with known concentrations and comparing the measured values with actual spiked values. For the recovery experiments, free samples

of the metal were spiked, in three replicates, after being homogenized by the addition of appropriate volumes of standards of Cr, Fe, Pb, Mn, Zn, Cd, and Cu at three different levels (0.125, 0.50, 1.0, and 5.0 mg/L). Control samples were processed along with spiked ones. Average percent recoveries ranged between 87.43% and 103.78% for Chromium (Cr), with its precisions being within 1.67-4.50%. For Iron (Fe), this changed to 94.74%-98.61%, with the precisions ranging from 1.98% to 4.52%. As for Lead (Pb), average recoveries were between 88.90% and 98.92% and its precisions ranged from

3.71% to 6.12%, which changed in case of Manganese (Mn) to 89.52%-99.72% for average recovery and to 2.56%-3.87% for the metal's precisions. Average recoveries and precisions of Zinc (Zn) were within 92.79%-107.73% and 2.91%-3.63%, respectively. As for Cadmium (Cd), the average recoveries ranged between 91.45% and 102.29%, while the precisions were from 0.24% to 5.20%. In case of Copper (Cu), the range of these rates changed to 90.34%-107.51% for average recovery and 0.29%-6.03% for its precisions. The QC and QA data are provided in Table 1.

Table 1. Performance characteristics of the analytical methods, employed for metallic residues analysis at three spiking levels

Spiked Levels (mg/L)	% Recovery (n=3) (Repeatability / %RSD) of trace metals						
	Cr	Fe	Pb	Mn	Zn	Cd	Cu
0.5000	87.4326	94.74	92.32	89.52	92.79	102.29	90.34
	(1.67)	(4.52)	(3.71)	(2.56)	(3.63)	(0.24)	(6.03)
1.0000	97.52	98.61	88.90	96.73	97.91	95.30	107.51
	(2.82)	(1.98)	(4.56)	(3.87)	(2.91)	(5.20)	(2.73)
5.0000	103.78	95.51	98.92	99.72	107.73	91.45	98.67
	(4.50)	(5.62)	(6.12)	(2.91)	(3.54)	(0.41)	(0.29)

n* = number of replicates, values are average of triplicate analysis

Isolation of Cr (VI)-resistant bacteria from tannery effluents and sediments

To isolate Cr (VI)-resistant bacteria from sediment samples, 1gm of air-dried sediment sample was taken in a test tube, into which 9 ml of sterile water was added to make a suspension of cells by using vortex mixture. Subsequently, 0.1 ml of the suspension was added aseptically to the Lauria-Bertani (LB) agar plate, containing 100 mg/L $K_2Cr_2O_7$ and 0.5% dextrose, and spread plate technique was applied. Plates were incubated at 32°C for 24-48 h. To isolate Cr (VI)-resistant bacteria from tannery effluents, the sample bottles were mixed properly and 0.1 ml of the samples got spread on LB agar plate with abovementioned content. The plates were incubated at 32°C for 24-48 h. $K_2Cr_2O_7$ was used in the agar plates as a source of Cr (VI). Morphologically-distinct colonies were then selected for purification and further studies.

Quantification of Cr (VI)-resistant bacteria from tannery effluents and sediment

After 24 h and 48 h of incubation, the colonies were counted in both types of plate, i.e. with and without supplementation. The total number of bacteria per gram of the sediments and per ml of the effluents was then measured by viable plate count method (APHA, 1998).

Purification and preservation of bacterial isolates

The isolated organisms were purified through repeated subculture method. Luria Bertani agar was used as media, containing $K_2Cr_2O_7$ and glucose. When a plate yielded only one type of colony, the organisms were considered pure. In order to preserve the organisms, the Luria Bertani agar plate, containing single and purified colonies, was kept in refrigerator at 4°C, and/ or in 10% glycerol stock for further studies.

Characterization of Cr (VI)-resistant bacterial isolates

Colony and morphological characteristics of the isolates

The purified colonies were sub-cultured in Luria Bertani agar media. The isolates from stock culture were grown on Luria- Bertani agar plate, which contained the same concentration of $K_2Cr_2O_7$ and the dextrose was supplemented separately and incubated at 37°C for 24 h. Well-isolated colonies were selected and evaluated based on their colony and morphological characteristics (Buchanan & Gibsons, 1994).

Influence of various concentrations of Cr (VI) on the growth of bacterial isolates

For this purpose, one of the purified isolates, grown on the LB agar plates, was taken by sterile inoculating loop and

transferred in sterile water in a test tube, which was then put in a vortex and a suspension of cell was prepared. The equal amount of suspension (one ml of each) was then taken aseptically into five conical flasks, containing ten times diluted Lauryl tryptose broth with different concentrations of $K_2Cr_2O_7$ (0-400 mg/L). All the conical flasks were then kept in a shaking incubator and got incubated at room temperature under 120 rpm for 24-48 h. The effects of varying concentrations of Cr (VI) on the tolerance of microbial growth was then measured by monitoring optical density (OD) at 600 nm.

RESULTS AND DISCUSSION

Water quality parameters of tannery wastewater, collected from Hazaribagh Area

Table 2 presents the values of water quality parameters in tannery wastewater, collected from Hazaribagh Area, Bangladesh.

Table 2. TDS, pH, DO, and EC of the tannery wastewater, collected from Haazaribagh area, Dhaka, Bangladesh

Sample ID	Water quality parameters				
	TDS(mg/L)	pH	DO(mg/L)	EC(μscm^{-1})	
H.T.E-1	7,000	4.35	3.75	13,500	
H.T.E-2	9,200	4.77	3.55	19,000	
H.T.E-3	1,030	4.85	3.50	2,500	
H.T.E-4	5,000	6.38	4.67	9,500	
H.T.E-5	3,300	6.20	3.60	6,586	
H.T.E-6	3,960	6.45	3.8	7,912	
H.T.E-7	300	6.64	3.40	1,000	
H.T.E-8	3,000	9.74	1.50	6,200	
H.T.E-9	290	7.86	4.90	587	
Standard permissible limits for discharging into inland surface water	NEQS (2000) ISW-BDS- ECR (1997)	3500 2100	6-9 6-9	4-6 4.5-8	288 1200

NEQS*= National Environmental Quality Standards, ISW-BDS-ECR*= Inland Surface Water in Bangladesh.

In the present study, the TDS concentration was within the range of 290 to 9200 mg/L, and almost all the samples exceeded the standard permissible limits (2100 mg/L). The average concentration of TDS was 3675.555 mg/L, which is approximately double, when compared to the standard value, set by ISW-BDS-ECR (1997). Rouf et al. (2013) found TDS concentration of the tannery wastewater 3455 mg/L in the samples sources from different points of Hazaribagh tannery

industrial area, which were nearly similar to the TDS concentration of the present study (3675.555 mg/L).

The pH values ranged from 4.35 to 9.75. Majority of the tested samples were within the range of standard permissible limits, set by NEQS (2000) and ISW-BDS-ECR (1997) (Table 2). Three wastewater samples show relatively lower pH values, i.e. in acidic state (4.35, 4.77, and 4.85), compared to standard permissible limits. Rouf et al. (2013) observed similar results, while characterizing

the tannery wastewater in Hazaribagh, Bangladesh. The broad scale variations in pH concentration imply different pressures on aquatic environment and detrimentally influence the fragile aquatic life, like the fish, phytoplankton, and zooplankton (Chowdhury et al., 2015).

The highest and lowest value of Dissolved Oxygen (DO) of the analysed effluent samples were 4.5 and 1.5 mg/L, respectively, with the mean concentration of DO being 3.29 mg/L, which is far below the standard prescribed limits, set by NEQS (2000) and ISW-BDS-ECR (1997). Chowdhury et al. (2015) reported that the DO concentration of the tannery wastewater in Hazaribagh area was 2.6 mg/L in the monsoon, dropping to nearly zero in pre-monsoon and post-monsoon seasons. The excessive level of organic pollution in the wastewaters results in the low DO value, also indicative of an extreme level of BOD₅ in the effluents (Verma et al., 2008).

The EC values of the tested samples ranged from 587 to 19000 $\mu\text{s}/\text{cm}$ and almost all of them showed higher conductivity, compared to the prescribed standards limits (ISW-BDS-ECR, 1997; 1200 $\mu\text{s}/\text{cm}$ and NEQS, 2000; 288 $\mu\text{s}/\text{cm}$). The high concentration of EC in the wastewater samples exhibits the presence of sufficient amount of organic and inorganic compounds and salts, particularly sodium and chromium salts used in the pickling and tanning processes may have enhanced the electrical conductivity of the effluent samples (Priya, 2010).

Concentrations of trace metallic constituents in tannery wastewater samples

Table 3 gives the concentration of trace metallic constituents in tannery wastewater, collected from Hazaribagh area. The results show that the concentrations of metallic constituent's ranges were: Cr: 14.282 \pm 1.17 to 6769.554 \pm 1.59 mg/L; Cd: 1.546 \pm 4.14 to 2.214 \pm 1.98 mg/L; Pb: 18.808 \pm 4.09 to 32.026 \pm 6.92 mg/L; Cu: 1.522 \pm 5.83 to

2.578 \pm 4.43 mg/L; Zn: 0.682 \pm 3.48 to 8.688 \pm 2.07 mg/L; Fe: 1.37 \pm 5.44 to 108.556 \pm 2.97 mg/L; and Mn: 3.494 \pm 2.97 to 17.170 \pm 5.60 mg/L. The concentration of nickel was below detection limit of all tested samples.

The concentration of trace metallic constituents in the tannery wastewater were in the following order: Cr > Fe > Pb > Mn > Zn > Cd > Cu. The average concentration of chromium in the effluents was 1943.646 mg/L, approximately close to chromium concentration (1950 mg/L), measured by Chowdhury et al. (2015). Two effluent samples contain extremely high concentration of chromium (5866.942 \pm 1.02 and 6769.554 \pm 1.59 mg/L), which may be due to the fact that they were collected directly from the equalizer tank of two tanneries. The other samples were collected from different drain water of different tanneries, with their chromium concentration ranging between 14.282 \pm 1.17 and 2857.182 \pm 3.23 mg/L, which was relatively low compared to the first two samples. This may be due to the dilution of the samples with drain water, sedimentation, coagulation, and precipitation of metals in the effluent samples (Chowdhury et al., 2015; Sass & Rai, 1987). However, the concentration of other metals was far above the standard permissible limits, set by NEQS (2000) and ISW-BDS-ECR (1997). The average concentrations of chromium, cadmium, lead, copper, zinc, iron, and manganese in the effluent samples were 3887.292, 4, 236, 4, 2, 23, and 2 times higher respectively, compared to ISW-BDS-ECR (1997) values of 0.5, 0.5, 0.1, 0.5, 5.0, 2.0, and 5 mg/L.

Table 4 shows the Pearson's correlation matrix for the analyzed parameters of the effluents dataset. Correlation analysis showed strong positive correlation between Cd-Cr ($r= 0.701$), Fe-Zn ($r= 0.707$), and Mn-Cd (0.704) at $P < 0.05$ level as well as Pb-Cr ($r= 0.885$) and Mn-Cr ($r= 0.839$) at $P < 0.01$ level. Mn-Pb ($r= 0.674$) at $P < 0.05$ level showed moderate correlation.

Table 3. Concentrations of trace metallic constituents in tannery wastewater

Sample ID	Concentration of trace metallic constituents								
	Cr	Cd	Pb	Cu	Zn	Fe	Ni	Mn	
H.T.E-1	5866.942±1.02	2.134±1.88	28.976±6.68	1.774±2.65	0.682±3.48	1.37±5.44	BDL	13.008±1.69	
H.T.E-2	6769.554±1.59	2.214±1.98	32.026±6.92	1.924±2.36	7.326±2.47	108.556±2.97	BDL	17.17±5.60	
H.T.E-3	288.454±1.87	1.546±4.14	20.842±4.88	1.724±5.53	8.688±2.07	98.292±1.42	BDL	9.782±6.89	
H.T.E-4	1474.02±4.99	2.08±4.00	23.384±2.53	1.622±2.37	4.18±7.32	14.26±1.41	BDL	12.806±1.82	
H.T.E-5	2857.182±3.23	2.186±6.60	21.858±2.33	2.026±6.66	3.69±3.01	41.952±7.56	BDL	13.802±2.96	
H.T.E-6	144.604±2.21	2.106±1.54	20.334±2.50	1.824±3.32	6.724±0.48	64.392±4.00	BDL	6.764±5.19	
H.T.E-7	50.792±1.22	1.786±6.79	18.808±4.09	2.578±4.43	7.876±2.52	23.57±6.22	BDL	4.494±2.97	
H.T.E-8	14.282±1.17	1.654±1.34	24.40±1.21	1.522±5.83	6.41±2.39	30.732±5.47	BDL	3.89±2.88	
H.T.E-9	26.984±6.57	1.76±6.66	22.368±5.68	1.974±1.37	2.512±3.76	19.512±4.83	BDL	5.674±3.46	
Standard permissible limits for discharging into inland surface water	NEQS (200)	1.00	0.10	0.50	1.00	5.00	8.00	1.00	1.50
	ISW-BDS-ECR (1997)	0.50	0.10	0.50	0.50	0.50	2.00	1.00	5.00

BDL* = Below detection limit

Table 4. Pearson's correlation matrix among different heavy metals in the wastewater samples

	Cr	Cd	Pb	Cu	Zn	Fe	Mn
Cr	1						
Cd	0.701*	1					
Pb	0.885**	0.481	1				
Cu	-0.061	0.034	-0.337	1			
Zn	-0.332	-0.403	-0.289	0.175	1		
Fe	0.175	-0.025	0.174	-0.073	0.707*	1	
Mn	0.839**	0.704*	0.674*	-0.226	-0.240	0.331	1

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

A positive significant correlation suggests that these metals had come from the same pollution sources, possibly from different tanning processes. Nickel is not used in normal preparation of leather, thus the results of nickel concentration below detection limit in this study. Bosnic et al. (2000) reported that post-tanning processes are the main sources of metallic pollution in the tannery wastewater, where different types of azo dyes, insecticides, biosides, heavy metal's derived pigments, and other chemicals are utilized to increase the visual characteristics of leather products.

Isolation of Cr (VI)-resistant bacteria in tannery effluents and sediments

Figure 2 and Figure 3 illustrate the total number of Cr (VI)-resistant bacteria in tannery effluents and sediment, collected from Hazaribagh area. Total number of Cr (VI)-resistant bacteria in the sediment samples with glucose (0.5%) supplementation ranged between 1.0×10^3 CFU/gm and 4.5×10^3 CFU/gm at 24 h, which without glucose (0.5%) supplementation, altered to 1.3×10^3 CFU/gm - 4.0×10^3 CFU/gm at 24 h.

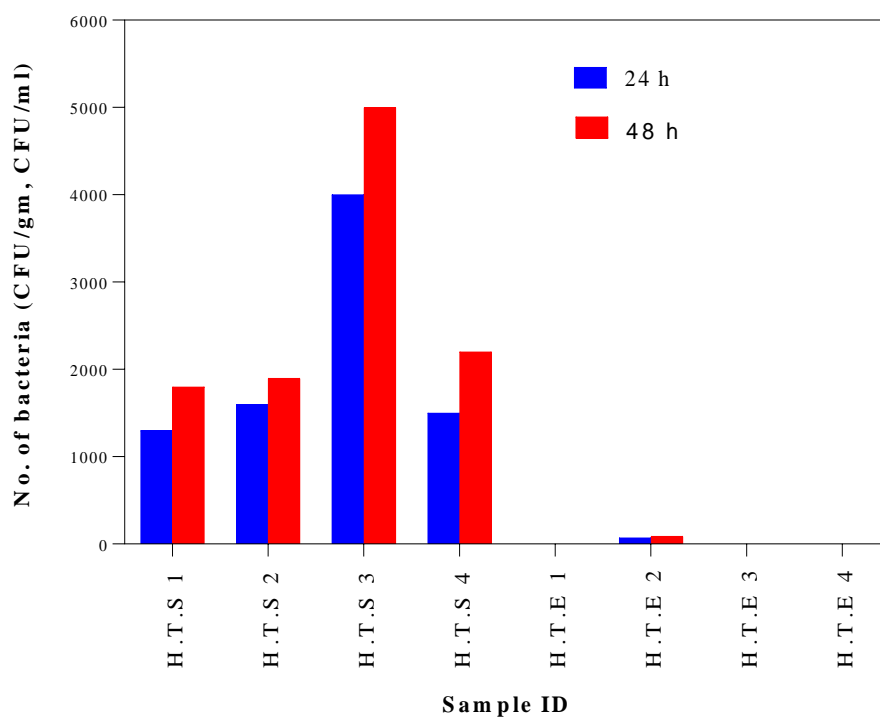


Fig. 2. Total number of Cr (VI)-resistant bacteria with glucose (0.5%, w/v) supplementation at 24 h and 48 h in tannery effluents and sediments (CFU*= Colony Forming Unit)

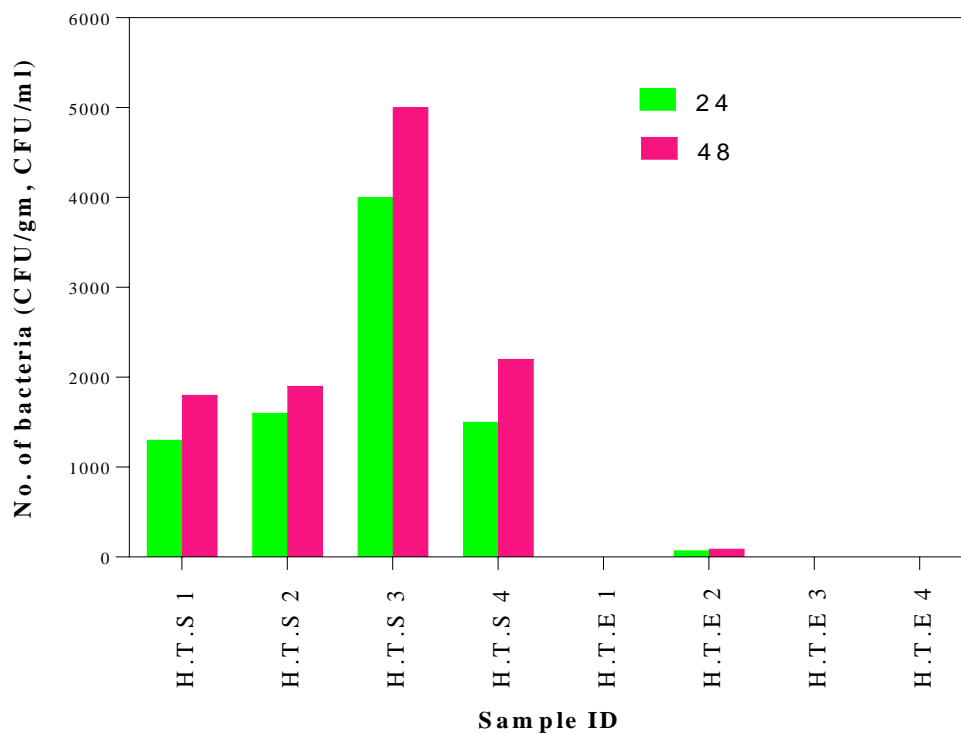


Fig. 3. Total number of Cr (VI)-resistant bacteria without glucose supplementation at 24 h and 48 h in tannery effluents and sediments (CFU*= Colony Forming Unit)

The ranges of total number of Cr (VI) resistant bacteria in the sediment samples with and without glucose supplementation were 1.2×10^3 CFU/gm to 7.5×10^3 CFU/gm and 1.8×10^3 CFU/gm to 5.0×10^3 CFU/gm, respectively at 48 h. The average number of Cr (VI)-resistant bacteria in the sediment samples with and without glucose supplementation was 2.35×10^3 CFU/gm and 2.10×10^3 CFU/gm, respectively at 24 h. The average number of Cr (VI)-resistant bacteria in the sediment samples with and without glucose supplement at 48 h was 3.74×10^3 CFU/gm and 2.7×10^3 CFU/gm, respectively. It is clear from this study that there was no significant difference between the CFU of Cr (VI)-resistant bacteria at 24h and 48h, which can be attributed to the fact that nature of the bacteria, isolated in the present study grew slowly. Basu et al. (1997), isolated Cr (VI)-resistant bacteria from sediment, contaminated with tannery effluents, and counted CFU/g in the order of $2.15-2.77 \times 10^6$, incorporating only 0.0125 to 0.025 mg/mL of Cr (VI) as $K_2Cr_2O_7$. So, it is evident that the isolated bacteria in the present study were more resistant and well-adapted to chromium-contaminated environments.

The number of Cr (VI)-resistant bacteria was relatively higher with glucose supplementation, compared to those, without glucose supplementation. Javor (1984) reported that in the presence of additional carbon sources, salt-tolerant bacterial communities prefer to grow well. The total number of Cr (VI)-resistant bacteria was more in sediment samples, compared to effluent samples (Figs. 2, 3), where only one effluent sample out of four had such bacteria.

The reasons behind greater number of Cr (VI)-resistant bacteria in sediment samples than the effluent ones is the continuous deposition of high concentration of chromium from chromium-rich tannery effluents in the sediment that forms a natural environment for the enrichment of chromium-resistant bacteria. Besides, most

of the effluent samples were collected from drain water that was running and contained excess amount of salts and other toxic chemicals, which did not provide either suitable conditions or enough time for the bacteria for their adaptation and colonization. The sampling period may be another reason, as it took place in the rainy season, diluting the effluent samples to a great extent.

Characterization of Cr (VI)-resistant bacterial isolates

The colony and morphological characteristics of the bacterial isolates were studied. All bacterial isolates were creamy white in colour; isolate 1 and isolate 3 were round, whereas the shape of isolate 2 was irregular. The edges of the bacterial isolates were complete with all the bacterial isolates being mucoid by texture. Two isolates (isolate 1 and isolate 3) were gram- positive, cocci shaped, while isolate 2 was gram-negative, cocci shaped. All the bacterial isolates were almost similar in their size, shape, and colour. To study the effects of varying Cr (VI) concentrations on the growth of bacterial isolates, one of the representative isolates (Isolate-3) was inoculated in conical flasks, containing 10 times diluted Lauryl tryptose broth, amended with different concentration of $K_2Cr_2O_7$ (0-400 mg/L). The effects of varying concentrations of Cr (VI) (as $K_2Cr_2O_7$) on the tolerance of the microbial growth was measured by monitoring Optical Density (OD), using an optical spectrophotometer at 600 nm.

Figure 4 shows the tolerance pattern of isolate 3 at 24 h and 48 h, which can successfully tolerate up to 400 mg/L of $K_2Cr_2O_7$ as Cr (VI). After 24 h and 48 h of incubation, the maximum optical density was found in 100 mg/L of $K_2Cr_2O_7$ and the OD values were 1.509 and 1.335 respectively. The OD values declined as the concentration of $K_2Cr_2O_7$ increased, which might be due to antibacterial activity of $K_2Cr_2O_7$ (Degiam et al., 2011).

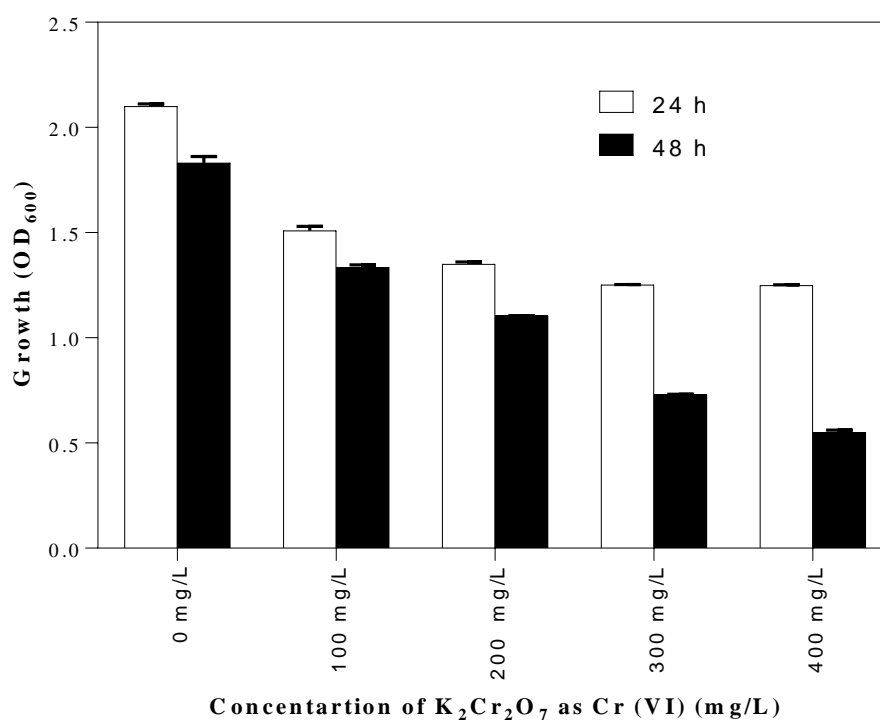


Fig. 4. The effect of Cr (VI) as K₂Cr₂O₇ concentrations on the growth of bacterial Isolate (Bars indicate standard deviation of triplicate samples)

The minimum growth was recorded at 400 mg/L of K₂Cr₂O₇ with the values being 1.250 and 0.550 at 24 h and 48 h, respectively. In 2012, Mullick isolated two Cr (VI)-resistant bacteria, testing their growth rate and Cr removal capacity. Both of the bacteria were resistant from 170 to 270 mg/L of K₂Cr₂O₇ as Cr(VI) and were able to remove up to 98% of chromium from submerged culture. So, it is evident that all the isolated bacteria in this study can be used for the removal of chromium from environments, contaminated with this metal. The predominance of Cr(VI)-resistant bacteria was directly interrelated to the concentration of chromium in the contaminated sites. A continuous exposure to high concentrations of heavy metals provides an extremely selective stresses on the microbial community, which could in turn result in the occurrence of metal tolerant strains (Sundar & Chandrasekaran, 2010). In the present study, the mean concentration of chromium was 1943.646

mg/L and this high concentration may lead to the presence of Cr(VI)-resistant bacteria with high resistant capacity.

CONCLUSIONS

Tannery effluents of Hazaribagh area are extremely polluted with regards to water quality parameters and trace metallic constituents, especially chromium and lead, which were 3887 and 236 times higher than the standard permissible limits, prescribed by ISW-BDS-ECR (1997), respectively. The tannery effluents of Hazaribagh area should not be discharged into inland surface water and its surrounding environment without appropriate treatment. Bioremediation can be a cost-efficient and eco-responsive biotechnological tool for the treatment of composite tannery wastewater in Hazaribagh area, Dhaka, Bangladesh. Particularly, metallic pollution as indigenous bacteria can tolerate a wide range of metal [Cr (VI)] incorporation as well as a continuous exposure to high concentrations of metal-contaminated environments.

REFERENCES

- Ahluwalia, S.S. and Goyal, D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol.*, 98 (12), 2243-2257.
- Akan, J.C., Moses, E.A., Ogugbuaja, V.O. and Abah, J. (2007). Assessment of tannery industrial effluents from Kano metropolis, Kano state, Nigeria. *J. Applied Sci.*, 7(19), 2788-2793.
- APHA (1998). Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association. Washington, D.C.
- Baker, D.E. and Amacher, M.C. (1982). Nickel, copper, zinc and cadmium. In: Page, Miller, A.R, Keeney, D.R. (eds.) *Methods of soil analysis*, American Society of Agronomy; Madison, Wisconsin, USA, 323-336.
- Banglapedia (2016). Hazaribagh thana. Available online at: http://en.banglapedia.org/index.php?title=Hazaribagh_Thana (2016). Accessed on 21 September.
- Basu, M., Bhattacharya, S. and Paul, A.K. (1997). Isolation and characterization of chromium-resistant bacteria from tannery effluents. *Bull. Environ. Contam. Toxicol.*, 58, 535-542.
- Benazir, J.F., Suganthi, R., Rajvel, D., Pooja, M.P. and Mathithumilan, B. (2010). Bioremediation of chromium in tannery effluent by microbial consortia. *Afr. J. biotechnol.*, 9(21), 3140-3143.
- Bernhardt, A. and Gysi, N. (2013). The world's worst 2013: The top ten toxic threats; Clean up, progress and ongoing challenges. New York, Zurich, 1-36.
- Bosnic, M., Buljan J. and Daniels R.P. (2000). Pollutants in tannery effluents: International scenario on environmental regulations and compliance. United Nations Industrial Development Organization, Vienna, 1-51.
- Buchanan, R.F. and Gibbons, N.F. (1994) *Bergey's manual of determinative bacteriology*, 9th ed., Williams and Wilkins Co., Baltimore, USA.
- Chowdhury, M., Mostafa, M.G., Biswas, T.K., Mandal, A. and Saha, A.K. (2015). Characterization of the effluents from leather processing industries. *Environ. Process.*, 2(5), 173-187.
- Cooman, K., Gajardo, M., Nieto, J., Bornhardt, C. and Vidal, G. (2003). Tannery wastewater characterization and toxicity effects on *Daphnia* Spp. *Environ. Toxicol.*, 18, 45-51.
- Degiam, Z.D., Abd-Al adheem, H. and Abbas, F.N. (2011). Influence of some weak acids, weak bases and salts against some pathogenic microorganisms. *Thi-Qar Med. J.*, 5(2), 93-99.
- European Commission (EC) (2011). Method validation and quality control procedures for pesticide residues analysis in food and feed. pp. 1-41. Available online at: http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2011_12495.pdf, Accessed on 6/2/ 2017.
- Horitsu, H., Nishida, H., Kato, H. and Tomoyeda, M. (1978). Isolation of potassium chromate-tolerant bacterium and chromate uptake by the bacterium. *Agr. Boil. Chem.*, 42, 2037-2043.
- ISW-BDS-ECR (1997). Inland surface water in Bangladesh. Ministry of Environment and Forest, Gazette notification 27, Aug.
- Javor, B.J. (1984). Growth potential of halophilic bacteria isolated from solar salt environments: carbon sources and salt requirements. *Appl. Environ. Microbiol.*, 48(2), 352-360.
- Kidgell, J.T., Nys, R.D., Hu, Y., Paul, N.A. and Roberts, D.A. (2014). Bioremediation of a complex industrial effluent by biosorbents derived from freshwater macroalgae. *Plos One*, 9(6), e94706.
- Mullick, U. (2012). Simultaneous removal of sulphate and chromium from tannery waste using microbes. M.Sc. Thesis Dessertations, National Institute of Technology, Rourkela-769008.
- NEQS (2000). National environmental quality standards for municipal and liquid industrial effluents. Available online at: <https://www.elaw.org/system/files/RevisedNEQS.pdf> (2016). Accessed on 16 June.
- Priya, K.E. (2010). Biodegradation of tannery effluent using native fungus *Penicillium* sp. B.Sc Dissertation, University of Madras.
- Rouf, M.A., Islam, M.S., Haq, M.Z., Ahmed, N. and Rabeya, T. (2013). Characterization of effluents of leather industries in Hazaribagh area of Dhaka city. *Bangladesh J. Sci. Ind. Res.*, 48(3), 155-166.
- Rusal, M.G., Faisal, I. and Khan, M.M.K. (2006). Environmental pollution generated from process industries in Bangladesh. *Int. J. Environ. Pollut.*, 28(1-2), 144-161.
- Sadeeshkumar, R., Saranraj, P. and Annadurai, D. (2012). Bioadsorption of the toxic heavy metal chromium by using *Pseudomonas putida*. *Int. J. Pure Appl. Microbiol.*, 2(4), 32-36.
- Sass, B.M. and Rai, D. (1987). Solubility of amorphous chromium (III)- Iron (III) solid solutions. *Inorg. Chem.*, 26, 2228-2232.

Sundar, K. and Chandrasekaran, N. (2010). Seasonal variations in the chromium concentration and chromium resistant bacterial populations in Vellore district. Res. J. Appl. Sci. Eng. Technol., 2(4), 378-380.

Quality assessment of treated tannery wastewater with special emphasis on pathogenic E. coli detection through serotyping. Environ. Monit. Assess., 145, 243-249.

Verma, T., Ramteke, P.W. and Grag, S.K. (2008).

