Use of natural bio-sorbent in removing dye, heavy metal and antibiotic-resistant bacteria from industrial wastewater

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Abstract

In this study, we evaluated the ability of waste shell powder (WSP) and moringa seed powder (MSP) individually or in combination to eliminate dye, heavy metal and resistant bacteria from the industrial wastewater (IWW). The presence of dyes, heavy metals, approximately 7.0 log CFU/ml of aerobic bacteria and 3.0–4.0 log CFU/ml of other pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Serratia liquefaciens* and *Bacillus cereus* was evident in IWW of both tannery and textile industries. In addition, depending on the type of bacterial species each bacterium was resistant to as high as 50 ppm of multi-metal (Cr^{6+} , Cd^{2+} and Pb^{2+} or its combination) and even multi-drug resistant (amoxicillin, ampicillin, cefixime, ceftazidime, and tazobactam). Combined use of MSP (0.8 gm/100 ml IWW) and WSP (0.2 gm/100 ml IWW) treatment was able to discolor the IWW within 4 h and took 24 h to eliminate heavy metals and pathogenic bacteria to non-detectable level from the IWW, simultaneously. On the other hand, individual use of MSP or WSP was not found effective enough to remove or eliminate dye, heavy metal and bacteria simultaneously from the IWW. Similar experimental results were observed in the challenge test with laboratory-prepared effluent water containing 35.0 ppm Remazol Brilliant Blue R dye and maximum 50 ppm Cr^{6+} . Thus, the combination of these two bio-sorbents could be applicable in IWW treatment before being discharged into the environment.

Keywords Industrial effluent water · Multi-drug-resistant bacteria · Discoloration · Heavy metal · And natural bio-sorbents

Introduction

Many industries such as textile, paper and plastics use dye-stuffs in order to color their products and also consume substantial volumes of water (Rodrigues et al. 2013). As a result, they generate a considerable amount of colored wastewater (Kant 2012). It is recognized that public perception of water quality is greatly influenced by the color which is the first contaminant to be recognized with open eyes in wastewater (Alalewi and Jiang 2012). The presence of very small amounts of dyes in water (less than 1.0 ppm for some dyes) is highly visible and undesirable (Dorthy et al. 2012). On the other hand, the presence of heavy metals in industrial effluents is known to have major hazard to

natural water, animal and human health (Ezaka and Anay-anwu 2011; Girma 2015).

High concentrations of all heavy metals have deleterious effect on the environment (Akpore and Muchie 2010; Wang et al. 2014). Toxic heavy metals like mercury (Hg^{2+}), chromium (Cr^{6+}), lead (Pb^{2+}) and cadmium (Cd^{2+}) have no biological role, but they are well known for their toxicities, mutagenicity and carcinogenic impacts on human beings and other aquatic living system especially those metals classified under priority list of pollutants (Akpore and Muchie 2010; Abbas et al. 2014; Girma 2015). Untreated industrial wastewater discharged to the nearby water bodies may cause severe ground water pollution (Pandey et al. 2007). However, wastewater containing dyes is very difficult to treat, since the dyes are recalcitrant organic molecules, resistant to aerobic digestion, and stable to light, heat and oxidizing agents (Islam et al. 2011; Priya and Selvan 2017). Although several new composite materials including polyphenylsulfone or multi-walled carbon nanotubes membrane filters (Nayak et al. 2019), graphene oxide-based nano-materials (Lim et al. 2018) and selective composite

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cation exchanger (Ismail 2010; Nabi et al. 2011) have been developed, however, commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction (Kant 2012; Patel et al. 2013). During the past three decades, several physical, chemical and biological discoloration methods have been reported (Oak et al. 2016); however, few have been accepted by the paper and textile industries. Although some of these techniques have been shown to be effective, they have limitations as well (Hou et al. 2016). Among these, excess amount of chemical usage or accumulation of concentrated sludge with obvious disposal problems; expensive plant requirements or operational costs; lack of effective color reduction; and sensitivity to a variable wastewater input were notably mentioned (Padhi 2012). In view of these disadvantages, bio-sorption or removal of heavy metals, dyes and resistant pathogens by biological materials has gained momentum since naturally occurring biomass/adsorbents or spent biomass can be effectively used (Ali et al. 2009; Aho and Lagasi 2012; Eze and Ananso 2014).

Moringa seed powder (MSP) is a natural coagulant and has antimicrobial properties and absorption capacity that have been tested over the years as an alternative to the use of inorganic and synthetic coagulants, antimicrobials and absorbents (Valverde et al. 2018; Taiwo et al. 2020). *Moringa oleifera* is a sustainable, low-cost, locally available, simple, reliable, acceptable, eco-friendly and household-level point of used water treatment coagulant/technology most suitable for developing countries (Zaman et al. 2017). Advantages of MSP are it produces lesser volume of biodegradable sludge and it does not affect the physicochemical quality of the water. Despite a strong natural coagulant, moringa seed extract also acts as potential adsorbent and antimicrobial agent and has the capability of adsorbing 95% of copper, 93% of lead, 76% of cadmium and 70% of chromium, from water. Metal adsorption occurs due to the high protein content of the seeds, and the flocculation activities of MSP are based on the electrostatic patch charge mechanism. Studies have shown that seeds have the capability to adsorb metal cations and attract highly toxic compounds (Ravikumar and Sheeja 2013).

On the other hand, heated waste shell powder (WSP) has widely been used for killing pathogens and fungus in many environmental samples (Ahmed et al. 2015; Hou et al. 2016; Zaman et al. 2017). Shell powder heated to over 700 °C exhibited a bactericidal activity (Sawai et al. 2003). Likewise, scallop shells are reused in forestry road construction, as absorbent for phosphate (Jeon and Yeom 2009). Thus, heated waste shell possesses adsorbent capabilities to remove heavy metals (Ravikumar and Sheeja 2013), microorganisms or other impurities from the liquid (Sawai et al. 2003; Ahmed et al. 2017). Shell waste is a growing burden

for seafood industries, and shells piled up near the seaside create several problems such as emission of offensive odors and soil pollution of heavy metals (Sawai et al. 2003). Thus, ideal solution was thought to convert the waste shells into a product that is both environmentally beneficial and economically feasible. In addition, combination of these two natural materials was thought to discolor dye, absorb metal and eliminate pathogens from the wastewater effluent. Therefore, the present study was designed to evaluate the effectiveness of two naturally occurring biomass (MSP and WSP) singly or in combination could completely eliminate heavy metals and microorganisms, as well as discolor the effluent water simultaneously.

Methods and materials

The industrial wastewater (IWW)

The effluent wastewater samples of tannery and textile industries were collected from the outlets of the industries at Hazaribagh and Uttara area of Dhaka city, Bangladesh. The sample collection was carried out at about 12:00 h and the peak production time for the industries observed. The effluent wastewater (500 ml) was collected in dark brown plastic bottles from the discharge point of both tannery and textile industries and transported to the laboratory in a cool box within 2 h of collection and used; if not used immediately, the samples were kept at 25 °C in dark until using.

Measurement of physicochemical parameters

Physicochemical parameters including temperature, pH, total dissolved solid (TDS), conductivity, salinity and heavy metals content of the wastewater samples were analyzed and recorded. Both the treated and non-treated effluent wastewater samples were analyzed periodically using pH meter (BT 600, Boeco, Germany), handy TDS meter (HI98301, Hanna, Italy), water conductivity tester (COND502, Taiwan), digital pen salinity meter (AZ8371, China) and atomic absorption spectrometer (AAS, Shimadzu, Japan) according to the manufacturers' instruction.

Preparation of bio-sorbant materials

Heated waste shell powder was obtained from ICHIBAN LIFE CORP., Tokyo, Japan. For the preparation of seed powder, moringa seeds were collected from the mature raw fruits and then dried in an oven at 50–60 °C temperature for 48 h before grinding the dried seeds in mortar–pestle and then sieved with 50-micron mesh to collect the fine powder. These ingredients at different dose rates

were used singly or in combination to evaluate the effectiveness of the ingredients responsible in discoloration and/or reduction of heavy metal(s) in textile and tannery effluent water.

Microbiological analysis

For the determination of resident microorganisms and for isolating the heavy metal-resistant resident and pathogenic bacteria in treated and untreated IWW, various selective and non-selective agar media supplemented with 50 ppm of Cr^{6+} , Pb^{2+} and Cd^{2+} was surface-plated. All the reagents and chemicals were prepared individually and sterilized before being used in the experiment. Tryptic soya agar (Oxoid, England) was used for the isolation of total aerobic bacteria (TAB), where total coliform bacteria (TCB), *Escherichia coli* and *Citrobacter freundii* were isolated on Chromocult agar (Merck, Germany). Bismuth sulfite agar (Oxoid, England) and cetrimide agar (HiMedia, India) were in use for the isolation of *Serratia liquefaciens* and *Pseudomonas aeruginosa*, respectively. After inoculation, all the plates were incubated at 30–35 °C

$$\text{Discoloration ability (\%)} = \frac{\text{Initial absorbance of combinations} - \text{absorbance after combinations}}{\text{Initial absorbance of combinations}} \times 100$$

for 24–72 h before being counted. At least five randomly selected colonies of each bacterium were confirmed through API 20E, 20NE, 50 CHB (BioMurex, USA) and other biochemical tests.

Laboratory-prepared heavy metal solution

Heavy metal solution prepared in the laboratory individually; both of the 2.0 ppm and 50 ppm Cr solution were prepared from the 100 ppm stock solution of $\text{K}_2\text{Cr}_2\text{O}_7$ (Active Fine Chemicals Ltd, Bangladesh).

Antibiotic resistivity

The randomly selected heavy metal-resistant bacterial isolates were subjected to the Kirby–Bauer disc diffusion susceptibility test with 14 broad spectrum antibiotics (chloramphenicol, 30 µg; ciprofloxacin, 5 µg; tetracycline, 30 µg; and nitrofurantoin, 300 µg; amoxicillin, 10 µg; rifampicin, 5 µg; azithromycin, 15 µg; ceftazidime, 30 µg; cefixime, 5 µg; streptomycin, 10 µg; erythromycin, gentamycin, 10 µg; 15 µg; tazobactam, 110 µg; and ampicillin, 10 µg) and six short spectrum antibiotics (nalidixic acid, 30 µg; kanamycin, 30 µg; bacitracin, 10 µg; aztreonam, 30 µg; novobiocin, 30 µg; and polymyxin, 300 µg) to determine the resistivity or sensitivity.

Discoloration assay of IWW

Four 250-ml beakers each containing 150 ml of IWW and various doses WSP and MSP were added separately in three separate combinations (only heated waste shell powder (WSP), only moringa seed powder (MSP) and combination of WSP and MSP) and stirred using glass rod for 20 s and left at room temperature until clear solution was observed. Non-treated (no biocide was added) beaker was served as control. Discoloration activity was expressed in terms of percentage discoloration and was determined by observing the decrease in absorbance at 200–500 nm of wavelength respective dyes. To examine the dye concentration, 3 ml of sample was centrifuged at 13,000 rpm for 15 min and the absorbance values of supernatants were determined at 200–500 nm of wavelength. The degree of discoloration of the tested dye was measured at its respective maximum absorbance wavelength using supernatant by UV–visible spectrophotometer (1800, Shimadzu, Japan). The rate of discoloration of MSP and WSP was calculated using the following formula:

Determination of heavy metal in IWW

For the determination of residual heavy metal (Cr^{6+}) in the treated effluent water, clear water was taken from the upper layer and filtered through 0.45 µm filter paper and the hexavalent Cr^{6+} and Cr^{3+} contents in the filtered water were measured using the diphenylcarbazide-based Cr^{6+} and Cr^{3+} test kits (Kyoritsu, Japan).

Laboratory-prepared dye containing low level of Cr^{6+} content (challenge test)

Each 150 ml of test solution was containing 35.0 ppm dye solutions (prepared from a 100 ppm Remazol Brilliant Blue R stock solution) and 2 and/or 50 ppm Cr^{6+} content (prepared from 100 ppm $\text{K}_2\text{Cr}_2\text{O}_7$ stock solution). WSP and MSP or its combinations were added in separate beaker and stirred for 20 s using glass rod and left at room temperature until discoloration was completed. Non-treated (no biocide was added) beaker was served as control. Discoloration ability was expressed in terms of percentage discoloration and was determined by observing the decrease in absorbance at 200–500 nm of respective dyes as described in the previous section.

The low-level hexavalent Cr^{6+} content of treated and non-treated dye solutions was determined using diphenylcarbazide

color comparison method-based test kits (Kyoritsu, Japan). The clear solutions from the top of each beaker were filtered through Whatman filter paper, and then, total Cr^{3+} and Cr^{6+} (WAK-Cr T, Kyoritsu, Japan) content and hexavalent Cr^{6+} (WAP- Cr^{6+} , Kyoritsu, Japan) contents were checked periodically. The detection limits of total Cr^{6+} kit were 0.5–50 mg/L, and hexavalent Cr^{6+} kit was 0.05–2.0 mg/L, respectively.

Statistical analysis

Each category of effluent water was collected three times in a week from the same industries effluent outlet. Reported data for all parameter tested represent the mean values obtained from three individual trials, with each of these values being obtained from duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington DC, USA). Significant differences in data were established by the least significant difference at the 5% level of significance.

Results and discussion

The presence of dye, heavy metals and resistant microorganisms in industrial effluents is known to have major hazards to natural habitat. High concentrations of dye and heavy metals

have deleterious effect on the environment, and hence, it is necessary to eliminate these from industrial wastewater (IWW) before it is discharged to the environment (Wang et al. 2014). Various physical, chemical and biological technologies for treating these industrial effluents exist (Sajidu et al. 2006). However, physical and chemical technologies got demerits of having membrane logging, short shelf life and periodic replacement of membrane, costly, and create disposal problem (Ravikumar and Sheeja 2013). Adsorption technique for the removal of wide variety of dyes from wastewaters is an ideal alternative to other expensive treatment options (Mangale et al. 2012). In this study, two new natural and eco-friendly ingredients were introduced for effective dye discoloration, heavy metal removal and elimination of pathogens from industrial wastewater. The results revealed that dye discoloration occurs quickly with stirring (150 rpm) within 1 h; however, it takes 4 h to discolor completely without stirring as shown in Fig. 1. In addition, it was also observed that the dyes discoloration capacity depends on the initial concentration of dyes, contact time and stirring time. Only effective treatment concentration was shown in Fig. 1.

Fig. 1 Discoloration effects of MSP and WSP on different EWW (a textile IWW; b tannery IWW; c challenge test with LPEW)

Table 1 Comparison of the physicochemical parameters of textile and tannery EWW with WHO permissible limit

Physicochemical parameters	Sources of wastewater		WHO permissible limit
	Textile	Tannery	
Color	Dark violet	Deep blue	–
pH	6.85	7.4	5.5–9.0
BOD (mg/l)	164.66	4464	30
COD (mg/L)	475.35	1284	250
TSS (mg/L)	1490	1250	600
TDS (mg/L)	912	851	2100
Conductivity ($\mu\text{S}/\text{cm}$)	1370	1430	1200
Salinity (ppt)	0.49	0.80	–
Metals (mg/L)	Fe ³⁺	1.23	14.67
	Cu ²⁺	25.14	0.41
	Zn ²⁺	49.08	1.52
	Cd ²⁺	1.9	1.26
	Ni ²⁺	3.05	0.15
	Pb ²⁺	18.4	0.182
	Cr ⁶⁺	132.5	10.35
	As ³⁺	1.41	3.53

Physicochemical characterization of tannery and textile industries

The physicochemical quality parameters including pH, TDS, conductivity, salinity, BOD (biological oxygen demand) and COD (chemical oxygen demand) and other parameters of IWW were recorded and are presented in Table 1. Deep blue color was observed for both the effluent, and these highly colored compounds may cause significant harm to the environment. A relevant study reported that the deep blue-brown-black color might hinder the penetration of sunlight causing depletion in the rate of oxidation of the pollutants (Verma et al. 2008). The pH of the textile and tannery effluent was recorded as 6.85 and 7.4, respectively, which is within the WHO (World Health Organization) permissible limit (6.0–8.5).

The electric conductivity (EC) of water is a measure of the ability of a solution to conduct an electric current; this ability depends on the dissolved solids, the presence of ions concentration, mobility and temperature of water. The conductivity of the water is one of the important parameters used to determine the suitability of water for irrigation. The conductivity of the textile and tannery effluent was recorded as 1370 and 1430 $\mu\text{S}/\text{cm}$, respectively, which exceeded the upper limit prescribed by WHO (1200 $\mu\text{S}/\text{cm}$). The total suspended solid (TSS) values of textile and tannery effluent ranged from 1250–1490 mg/L, which was far above the standard limits (600 mg/L) for effluent discharge and may cause damage to soil flora and fauna and lead to changes in soil porosity, soil texture and water

holding capacity (Chowdhury and Fatema 2016). The BOD and COD values were found much higher than the WHO maximum permissible level. The TDS values of textile and tannery effluent were recorded as 912 and 851 mg/L, lower than the WHO permissible limit (2100 mg/L). The salinity of the samples was relatively normal than other parameters. In addition, the average concentrations of heavy metals of tannery and textile effluents are also presented in Table 1.

Except cadmium (Cd) and nickel (Ni), all other metal and heavy metals were found higher than the WHO permissible limit. In case of textile effluent, substantially higher presence of copper (25.14 mg/L), Zn (49.08 mg/L) and Cr (132.5 mg/L) observed. On the contrary, 10–12-fold lower values of Cu, Zn and Cr were observed throughout the study. Fe concentration of textile wastewater was recorded as 1.23 mg/L, and this value was 14.57 mg/L in tannery effluent waste. The relative dominance of heavy metals in textile effluent was observed in the following sequence: Cr > Zn > Cu > Pb > Ni > Cd > As > Fe. On the other hand, in tannery effluent, the relative dominance of heavy metals was observed in the sequence: Fe > Cr > As > Zn > Cd > Cu > Pb > Ni. These study results demonstrated that the effluent water of both industries generated huge volume of wastewater containing various harmful chemicals, dyes and heavy metals, which severely impacted the environment.

Microbial characterization of IWW

Irrespective of types of bacteria, higher bacterial population was observed in textile effluent than in tannery wastewater. The textile effluent had high number of coliform bacteria which indicating the possible presence of fecal coliform and other pathogenic bacteria in the IWW (Table 2). In addition, the presence of heavy metal content was evident in effluent water analyzed and the presence of higher number of single and multiple metal-resistant bacteria was observed in the effluent water. The isolated strain was characterized as *Bacillus cereus*, *C. freundii*, *P. aeruginosa* and *E. coli*, and all of them found resistant to multiple heavy metals (Cr⁶⁺ + Pb²⁺ + Cd²⁺) observed by surface plating the wastewater onto tryptic soya agar medium supplemented with heavy metals separately or in combination. *Serratia liquefaciens* was found to be resistant to Cr⁶⁺ but not to Cd²⁺ and Pb²⁺. In recent studies, *Serratia sp.* has been shown to be resistant to a concentration of 1,500 mg Cr⁶⁺/L (Zhang and Li 2011). Although other species of *Serratia* have been found to be resistant to Cd²⁺ and Pb²⁺ (Kono and Cho 2010), there is no recent evidence that shows *S. liquefaciens* is resistant to Cd²⁺ or Pb²⁺. Another isolated heavy metal-resistant strain was *Citrobacter freundii* which is resistant to Cr⁶⁺, Cd²⁺ and Pb²⁺. In other studies, different strains

Table 2 Distribution of bacterial population (log CFU/ml) of treated (MSP and WSP in combination) and control textile and tannery effluent water samples

Name of microorganism analyzed	Textile wastewater				Tannery wastewater			
	Control		Heavy metal ^a -resistant bacteria		Control		Heavy metal ^a -resistant bacteria	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
TAB	7.23 ± 0.14	3.67	3.14 ± 0.09	1.47	4.48 ± 0.12	2.11	2.95 ± 0.07	1.0
TCB	3.38 ± 0.04	2.04	2.81 ± 0.31	1.0	4.04 ± 0.09	1.84	1.49 ± 0.18	< 1.0
<i>E. coli</i>	3.11 ± 0.09	< 1.0	1.17 ± 0.19	< 1.0	2.67 ± 0.21	< 1.0	1.00 ± 0.00	< 1.0
<i>Citrobacter sp.</i>	3.04 ± 0.23	< 1.0	1.22 ± 0.07	< 1.0	2.11 ± 0.23	< 1.0	1.04 ± 0.23	< 1.0
<i>Bacillus sp.</i>	4.34 ± 0.17	1.85	2.56 ± 0.22	1.47	3.15 ± 0.11	1.84	1.69 ± 0.16	< 1.0
<i>Serratia sp.</i>	2.75 ± 0.18	< 1.0	< 1.0	< 1.0	2.15 ± 0.12	< 1.0	< 1.0	< 1.0
<i>Pseudomonas sp.</i>	3.07 ± 0.14	< 1.0	ND		2.30 ± 0.13	< 1.0	ND	

^aResistant to Cr⁶⁺, Pb²⁺ and Cd²⁺ or in combination (50.0 ppm each); ND = not done; < 1.0 = less than detection limit

of *Citrobacter sp.* have been shown to be resistant to Cr⁶⁺, Cd²⁺ and Pb²⁺ including *C. freundii* (Mohseni 2014). The resistance to multiple metal ion indicates that the resistance might have resulted from the presence of single or multiple genes present in their plasmids which may also confer antibiotic resistance to these bacteria (Yamina et al. 2014). The industrial effluents are enriched media to grow and spread microbial population, which are resistant to different metals (Khadivinia et al. 2014). The identification of bacteria resistance against different metals may provide a useful tool for the simultaneous monitoring of several toxic pollutants in the environment (Mohsenzadeh and Shahrokhi 2014). But heavy metal-resistant pathogenic bacteria may also confer health and environmental risk as metal resistance is often associated with antibiotic resistance. However, with proper handling these metal-resistant strains can be used as a bioremediation tool for the treatment of effluent from leather, textile and other industries handling heavy metals.

Thus, the results of this study revealed that the IWW was heavily contaminated with dyes, heavy metal and resistant microorganisms. Hence, discharge of this effluent into nearby river and pond directly can cause acute toxicity to the receiving water bodies. It therefore becomes essential to remove color and eliminate heavy metal, and these resistant bacteria from the tannery and textile effluent wastewater, so as to avoid the hazardous environmental effects. Several treatment methods have been employed to embark upon the problem of dye removal and metal degradation, but degradation becomes further more difficult for effluents containing dye matrix.

Antibiotic resistivity pattern

The microorganisms isolated from the IWW were seen multi-metal resistance at the concentration as high as

50 mg/L. In addition, all these multi-metal resistance bacteria were also found resistant to at least five antibiotics (amoxicillin, ampicillin, cefixime, ceftazidime and tazobactam) in common (Table 3).

Dye removal and Cr⁶⁺ reduction using artificial challenge test

When the LPEW was treated with MSP alone or in combination with WSP, 100% dye removal was observed within 4 h (percentage dye removal was calculated through the equation already mentioned in Sect. 2.7); at the same time, separate application of WSP was not found so effective (Figs. 1c, 2). However, total Cr⁶⁺ content was reduced to less than detection limit (lowest detection limit was 0.5 ppm) at 24 h (Fig. 3). Furthermore, addition of MSP and WSP into IWW resulted in clear solution in the upper layer and sludge layer at the bottom. To see which ingredients are responsible in removing the heavy metal (Cr⁶⁺) from the laboratory-prepared dye solution, each effective concentration of MSP (0.8 gm/100 ml LPEW) and WSP (0.2 gm/100 ml LPEW) was also tested against the LPEW with 2.0–50.0 ppm Cr⁶⁺ and the results were presented in Table 4 and Fig. 3. It was observed that MSP alone was able to remove dye and heavy metal (Cr⁶⁺) to non-detectable level. This finding is an agreement with the report of Ravikumar and Sheeja 2013, which stated that moringa seeds were capable of adsorbing 95% of copper, 93% of lead, 76% of cadmium and 70% of chromium from contaminated water. In addition, some recent research reported that heated shell waste possesses adsorbent capabilities to remove heavy metals from the liquid (Sawai et al. 2003; Rohim et al. 2014; El-Kassas and Mohamed 2014).

Table 3 Resistance pattern of randomly selected bacterial isolates against multiple antibiotics and heavy metals

Types of resistance determinants	Names of determinants	Pattern of sensitivity				
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. freundii</i>	<i>S. liquefaciens</i>	<i>B. cereus</i>
Antibiotics	Broad spectrum	Amoxicillin (10 µg)	R ^a	R	R	R
		Ampicillin (10 µg)	R	R	R	R
		Azithromycin (15 µg)	R	S	R	S
		Cefixime (5 µg)	R	R	R	R
		Ceftazidime (30 µg)	R	R	R	R
		Chloramphenicol (30 µg)	S	I ^b	S ^c	S
		Ciprofloxacin (5 µg)	S	S	S	I
		Erythromycin (15 µg)	R	I	R	S
		Gentamicin (10 µg)	I	S	S	S
		Nitrofurantoin (300 µg)	S	R	I	S
		Rifampicin (5 µg)	R	R	R	R
		Streptomycin (10 µg)	R	I	I	S
		Tazobactam (110 µg)	R	R	R	R
		Tetracycline (30 µg)	I	R	I	R
	Narrow spectrum	Aztreonam (30 µg)	R	R	I	NRU ^d
		Polymyxin (300 µg)	S	S	S	NRU
		Kanamycin (30 µg)	R	R	I	NRU
		Nalidixic acid (30 µg)	R	R	S	NRU
		Novobiocin (30 µg)	NRU	NRU	NRU	S
		Bacitracin (10 µg)	NRU	NRU	NRU	R
Heavy metals (50 ppm each)		Cr ⁶⁺	R	R	R	R
		Cd ²⁺	R	R	R	R
		Pb ²⁺	R	R	S	R
		Cr ⁶⁺ + Cd ²⁺ + Pb ²⁺	R	R	S	R

^aResistant^bIntermediate^cSensitive^dNot recommended to use

Dye discoloration, heavy metal reduction and inactivation of bacteria using combined biological materials

The use of plant materials as natural coagulants to clarify turbidity of wastewaters is of common practice since ancient times. Of all plant material investigated, seeds of *Moringa oleifera* are one of the most effective sources of primary coagulant for water treatment (Oluduro and Ade-riye 2007; Ali et al. 2009). In addition, Hou et al. (2016) reported that use of marine shell waste powder, alkaline in nature and calcium-based materials with high hygroscopicity was proved to remove phosphorous, toxic metallic ions, sulfur dioxide and adsorption of toxic dyes from wastewater which can contribute to preserving water ecosystems and assist with waste discharge problems (Hou et al. 2016). Thus, application of MSP or WSP singly was able to eliminate dyes and heavy metal with MSP; however, WSP was able to reduce toxic metal and eliminate

bacteria. Hence, combined application was able to eliminate dyes, absorb up to 50 mg/L of Cd²⁺, Pb²⁺ and Cr⁶⁺ and eliminate bacteria, when exposed for a 24-h period to effluent wastewater. This result is an agreement with the results of another study, which reported that heated egg shell removes 95% of chromium at an optimum pH 5.0 and 1.45 mg/g of maximum adsorption capacity (Daraei et al. 2015). Thus, combination of effective concentration of MSP and WSP was used to remove color (Figs. 1, 2); heavy metal (Table 4); and eliminate bacteria (Table 2) from effluent water efficiently. However, despite efficient removal of dyes, heavy metal and pathogens from IWW, the sludge volume increased in the waste effluent which contains all the removed heavy metal, dyes and resistant microbial cells along with moringa seed powder and marine shell. Therefore, only water can be reused or discharged into the environment from the industrial wastewater through these techniques, but sludge disposal still remains a challenge, and land disposal or dumping

Fig. 2 Effectiveness of MSP and WSP to remove dye and heavy metal from LPEW

Fig. 3 MSP and WSP treatment in eliminating Cr⁶⁺ from effluent water; color bands mean the presence of Cr⁶⁺, where transparent color indicates the level of Cr⁶⁺ below detection limit (**a** Cr⁶⁺ test strips; **b** Cr⁶⁺ test kits)

in the ocean may contaminate underground water or may destroy sea habitat, respectively. On the other hand, incineration completely evaporates the moisture and converts the organic solids into inert ash, but the heavy metal still remains in the inert ash because heavy metal cannot be decayed, but can convert from one form to another only.

Conclusion

The discharge of dyes in the environment is a matter of concern for both toxicological and esthetical point of view, and these study results demonstrated that combined application of MSP (0.8%) and WSP (0.2%) was able to effectively remove dye (up to 35 ppm), heavy metal (up to 50 ppm Cr⁶⁺) and 3–7 log CFU/ml of microbial contaminants simultaneously from the effluent water within 24 h. Hence, these two bio-sorbents could be applicable in IWW treatment of textile and tannery industries before being discharged into the environment. Therefore, only water can be discharged into the environment from the industrial wastewater through these techniques, but sludge disposal still remains a challenge.

Table 4 Effectiveness of bio-sorbant materials to remove Cr⁺ from LPEW

Treatment time	Cr ⁶⁺ (2.0 ppm)			Cr ⁶⁺ (50.0 ppm)			Cr ⁶⁺ and Cr ³⁺ (2.0 ppm)		
	Treatment with MSP	Treatment with WSP	Treatment combined	Treatment with MSP	Treatment with WSP	Treatment combined	Treatment with MSP	Treatment with WSP	Treatment combined
Immediately after treatment	1.5	2.0	1.5	20	50	20	1.5	2.0	1.5
24 h	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05
48 h	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05
72 h	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05
Fourth day	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05
Fifth day	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05
Sixth day	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05
Seventh day	<0.05	2.0	<0.05	<0.5	50	<0.5	<0.05	2.0	<0.05

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