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A Comparative Study on Color Removal From Textile Industry Effluent Using Shrimp and Crab Shell Chitosan

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ABSTRACT

Effluent from the textile industry is a major source of water pollution. Textile effluents contain a high amount of color, turbidity, BOD, and COD, which are highly toxic and affect aquatic organisms as well as human beings. Physical and chemical treatments of these effluents are difficult and costly. Adsorption is an effective method to treat textile industry effluent. In the present study, chitosan is selected as an adsorbent, derived from the exoskeleton of marine crustaceans like shrimp and crab by chemical processing. The color was removed using chitosan from shrimp and crab shells separately. On the sixth day of treatment, shrimp shell chitosan removed 100% of color from textile industry effluent and proved to be a better adsorbent.

INTRODUCTION

In India, water pollution is a growing environmental problem because nearly 70 percent of its surface water resources are already polluted and a huge percentage of groundwater reserves are contaminated by biological, toxic, organic, and inorganic pollutants. In many cases, these sources have been rendered unsafe not only for human consumption but also for other organisms in the environment and other activities such as irrigation and industrial needs. In 1995, the Central Pollution Control Board (CPCB) has identified 18 major rivers in India as severely polluted. Every day, over 600 people in India die as a result of water pollution (Lakshmi 2014).

One of the largest water pollution-causing industries in the world is the textile industry. The textile industry is one of the oldest as well as most important industries in the Indian economy. Factors that make India shine in the textile industry are low cost, skilled manpower, availability of cheap raw materials, availability of numerous varieties in cotton fiber, a big and potential national and international market, and independent textile industry (Dey & Islam 2015).

More than 80,000 tons of reactive dyes are produced and consumed each year, this conforms to the estimate of total pollution caused by their use. The dye bath has a very high salt concentration and is heavily colored. Textile effluent is characterized by a high value of suspended solids, dissolved organic matters, chemical oxygen demand, biological oxygen demand, color, and turbidity. Normally color is noticeable at a dye concentration higher than 1 $mg.L^{-1}$, and an average concentration of 300 $mg.L^{-1}$ has been reported in effluents from the textile manufacturing process (O'Neill et al. 1999, Goncalves et al. 2000).

The most widely adopted methods for removing color from wastewater include physico-chemical and biological methods, such as flocculation, coagulation, precipitation, adsorption, membrane filtration, electrochemical techniques, ozonation, and microbial decolorization (Sotelo et al. 2002, Chen et al. 2003). Among the various techniques of pollutant removal, adsorption is an effective and useful process because of its low cost, easy availability to treat dyes in a more concentrated form (Saad et al. 2007). Adsorption techniques for wastewater treatment are becoming more popular in recent years due to their efficiency in the removal of pollutants.

Chitosan is derived from chitin, which is naturally found in the exoskeleton of crustaceans, insects, and some fungi. Class Crustacea is the largest group of marine arthropods comprising shrimps, crabs, lobsters, etc. Chitin is the second most abundant natural polymer in the world, functions as a natural structural polysaccharide, and is composed of glucosamine and N-acetyl glucosamine units linked by β (1-4) glycosidic bonds. Worldwide over 39000 tons of chitin are obtained from shellfish annually. Chitosan has been suggested as a good adsorbent to remove impurities from wastewater, used as a natural seed treatment and plant growth enhancer, as well as bio-pesticide. Shrimp and crab shell chitosan categorized as solid animal waste can be used as a bio adsorbent for the removal of color from textile industrial effluent. In the present work, the ability of chitosan prepared from shrimp and crab shell exoskeleton to decolorize an actual textile industrial effluent is demonstrated.

MATERIALS AND METHODS

Collection of shrimp and crab shell: The shrimp and crab exoskeleton were collected from a seafood processing unit in Colachel, Kanyakumari district, Tamil Nadu, India.

Pre-conditioning: The collected shrimp and crab shells were washed separately 5-6 times by distilled water, and the samples were dried under sunlight for 24- 48 hours and stored in an airtight container until future use.

Preparation of chitosan

***Processing of chitin*:** The shrimp and crab shells were separately allowed to soak in 0.05 M acetic acid solution for 24 h. Then the shells were washed thoroughly with water and dried to remove excess water.

***Demineralization*:** The dried shells were demineralized using 0.68 M HCL (1:10 w/v) at ambient temperature (approximately 30°C) for 6 h. The residue was washed with distilled water until pH in the range of 6.5 -7.5 was obtained and then the residue was dried.

***Deproteinization*:** The demineralized shrimp and crab shells were deproteinized using 0.62 M NaOH solution (1:10 $w.v^{-1}$) at ambient temperature (approximately 30°C) for 16 h. The residue was washed thoroughly with water followed by distilled water until pH in the range of 6.5 -7.5 was obtained. The chitin was dried, ground, and screened with a 150 µm sieve.

***Deacetylation*:** The chitin obtained from the above process was deacetylated in 25 M and NaOH (1:10 $w.v^{-1}$) for 20 h at 65°C. After deacetylation, the chitosan was washed thoroughly with water followed by distilled water until pH in the range of 6.5 -7.5 was obtained.

Collection of textile effluent: The textile effluent was collected from the textile industry in Tirupur. The textile effluent was collected from the discharge tank of the textile industry in a sterile plastic can and transported immediately to the laboratory stored in a dark environment until further analysis.

Adsorption experiment: In the first set of experiments, 0.5 g of shrimp shell chitosan was added to varying concentrations (30%, 35%, 40%, 45%, and 50%.) of the textile industrial effluent in 250 mL conical flasks. In the second set, 0.5 g of crab shell chitosan was added to each of the concentrations respectively.

Determination of OD: Samples were withdrawn on the zero-day and at 24 h interval and the OD of the culture supernatant was estimated. The percentage decolorization was calculated using the following formula,

$$\% \text{ Dye Decolorization (DD)} = \frac{OD\ (Zero\ day) - OD\ (Sample)}{OD\ (Zero\ day)} \times 100$$

Toxicity bio-assay: Adult guppy fish (*Poecilia reticulata*) were collected from a private fish farm in Thickanamcode. They were kept in aerated tanks for 2 weeks and fed with standard pelleted fish feed to acclimatize them to laboratory conditions.10 healthy fishes of the same size were selected for the toxicological studies. These fishes were introduced into 250 mL cups which had the simulated effluent at varying concentrations ranging from 10% to 100%. The mortality was recorded for 96 h after exposure. The percentage mortality was calculated using Abbot's formula.

$$\text{Corrected mortality } \% = \frac{\%\ living\ in\ control - \%\ living\ in\ treatment}{\%\ living\ in\ control} \times 100$$

Toxicity bioassay was carried out for the textile industrial effluent both before and after treatment using crab and shrimp shell chitosan.

HPLC analysis: The HPLC analysis was carried out in Shimadzu LC-10AT VP with a C18 column (250 mm ×4.6 mm). Samples were prepared by dissolving the effluent with 1:1 dilution with distilled water as untreated and effluent with chitosan from shrimp and crab as treated samples. It was sonicated for complete dissolution and equilibrated at room temperature. The run time was 65 min, mobile phase used was methanol: water at the flow rate of 1 $mL.min^{-1}$ with a 0.02 µl injection. The detection was performed with a D2 lamp at 224 nm wavelength. SPINCHROM software was used for integration to find peak area and percentage of the injected samples.

RESULTS

Considering decolorization of textile effluent using shrimp shell, 100% decolorization was attained for 45% and 50% diluted effluents on the twelfth day of treatment (Table 1).

Crab shell chitosan at 40% concentration showed maximum decolorization (68.30%) on the 6^{th}day (Table 2). Probit analysis of toxicity response of the guppy fish, *P. reticulate,* exposed to the textile effluent was used to find out the LC_{50}

Table 1: Percentage decolorization in textile effluent treated with shrimp shell chitosan.

Days of treatment	Concentration of textile effluent				
	50%	45%	40%	35%	30%
0	0	0	0	0	0
2	33.57	36.57	70.84	100	100
4	46.85	56.72	84.11	_	_
6	80.42	80.88	100	_	_
8	90.57	90.51	_	_	_
10	92.85	97.70	_	_	_
12	100	100	_	_	_
14	_	_	_	_	_
16	_	_	_	_	_
18	_	_	_	_	_
20	_	_	_	_	_

Table 2: Percentage decolorization in textile effluent treated with crab shell chitosan.

Days of treatment	Concentration of textile effluent				
	50%	45%	40%	35%	30%
0	0	0	0	0	0
2	37.88	35.43	50.15	55.86	61.13
4	55.24	50.44	62.00	67.39	100
6	56.48	55.19	68.30	100	_
8	58.28	59.05	73.66	_	_
10	61.89	60.46	79.31	_	_
12	66.17	66.75	84.82	_	_
14	68.43	69.31	100	_	_
16	72.94	73.04	_	_	_
18	74.85	80.35	_	_	_
20	79.25	83.05	_	_	_

Table 3: Mortality response of *Poeicilia reticulata* exposed to textile effluent.

Concentration (mL)	Hours of exposure			
	24	48	72	96
30%	-	-	-	-
35%	-	-	10	-
40%	-	20	30	50
45%	30	40	60	-
50%	40	60	90	100

N= 10

values (Tables 3 and 4). After 72 h of exposure, 90% mortality of the fish was recorded at 50% dye concentration. The LC_{50} value was 42.730, LCL was 0.204, and UCL was 0.219. The X and Y values were 1.629 and 4.981 respectively with a *b* value of 16.278. After 96 h of exposure, 100% mortality of the fish was recorded at 50% dye concentration. The $LC_{50,}$ LCL, and UCL values were 39.984, 0.195, and 0.213 respectively. The X and Y values were 1.612 and 5.253 respectively with a *b* value of 24.049.

Probit analysis of toxicity response of the guppy fish *P. reticulata* exposed to textile effluent treated with shrimp shell chitosan was used to find out the LC_{50} values. The toxicity response of the fish under different concentrations of the effluent for different hours of exposure was studied. After 72 h of exposure, 20% mortality of the fish was recorded at 50% dye concentration. The X and Y values were 1.680 and 3.985 respectively with a *b* value of 9.623. After 96 h of exposure, 20% mortality of the fish was recorded at 50% dye concentration. The X and Y values were 1.680 and 3.985 and respectively with a *b* value of 9.623 (Tables 5 and 6).

Probit analysis of toxicity response of the guppy fish *P. reticulata* exposed to textile effluent treated with crab shell chitosan was used to find out the LC_{50} values. The toxicity response of the fish under different concentrations of the dye for different hours of exposure was analyzed and given in Tables 7 and 8. After 72 h of exposure, 20% mortality of the fish was recorded at 50% dye concentration. The X and Y were 1.680 and 3.985 respectively with a *b* value of

Table 4: nhr LC_{50} and confidence intervals for *Poecilia reticulata* exposed to textile effluent (untreated).

Sl. No	Hours	LCL	LC_{50}	UCL
1	24	0.162	55.269	0.307
2	48	0.211	47.454	0.236
3	72	0.204	42.730	0.219
4	96	0.195	39.984	0.213

Table 5: Mortality response of *Poeicilia reticulata* exposed to textile effluent treated with shrimp shell chitosan.

Concentration (mL)	Hours of exposure			
	24	48	72	96
30%	-	-	-	-
35%	-	-	-	-
40%	-	-	-	-
45%	-	-	-	10
50%	-	-	10	20

Table 6: nhr LC_{50} and confidence intervals for *Poecilia reticulata* exposed to textile effluent treated with shrimp shell chitosan.

Sl. No	Hours	LCL	LC_{50}	UCL
1	96	0.159	61.109	0.327

Table 7: Mortality response of *Poecilia reticulata* exposed to textile effluent treated with crab shell chitosan.

Concentration (mL)	Hours of exposure			
	24	48	72	96
30%	-	-	-	-
35%	-	-	-	-
40%	-	-	-	-
45%	-	-	10	20
50%	-	10	20	30

N= 10

Table 8: nhr LC_{50} and confidence intervals for *Poecilia reticulata* exposed to textile effluent treated with crab shell chitosan.

Sl. No	Hours	LCL	LC_{50}	UCL
1	72	0.159	61.109	0.327
2	96	0.144	59.411	0.332

9.623. After 96 h of exposure, 30% mortality of the fish was recorded at 50% dye concentration. The X and Y values were 1.677 and 4.327 respectively with a *b* value of 7.000.

HPLC analysis was carried out on 50% of untreated textile effluent (control), 50% textile effluent treated with shrimp shell chitosan, and 50% of textile effluent treated with crab shell chitosan. The HPLC elution profile of untreated textile effluent (control) showed 7 peaks (Fig. 1) with retention time (RT) 0.847, 1.897, 2.140, 2.333, 2.983, 3.937 and 4.420 (Table 9). The HPLC profile of textile effluent treated with shrimp shell showed 4 peaks (Fig. 2) with RT 0.843, 2.103, 2.300, 4.390, 21.900, 27.077, 42.213, 58.320, and 64.010 (Table 10). RT 1.897, 2.983, and 3.937 did not appear in the effluent treated with shrimp shell chitosan. Textile effluent treated with crab shell chitosan showed 7 peaks (Fig. 3) with RT of 0.863, 1.210, 1.873, 2.140, 2.300, 2.623, 4.313, 20.200, 22.337, 29.610, and 55.120 (Table 11).

The retention times vary from one to another. The textile effluent treated with crab shell chitosan and control have the same number of peaks but the RT was different. The same RT 2.983 and 5.937 did not appear in the effluent treated with crab shell chitosan. But the appearance of 2 new peaks with RT 1.210 and 2.623 in control, treated with crab shell chitosan can be assumed that there is a degradation of dye from one form to another. The elution profile obtained from the shrimp shell chitosan treated samples significantly differed from the control in terms of numbers, the height of peaks obtained and RT.

DISCUSSION

Chitosan is a natural, biodegradable bio-adsorbent polymer obtained from shrimp and crab shells that have many properties such as non-toxic, high porosity, biodegradability, biocompatibility, and adsorption. Effective utilization of these wastes significantly improves color adsorption from textile wastewater. Chitosan is used as a traditional coagulant, in wastewater treatment (Bina et al. 2014, Ariffin et al. 2009). Sobahi et al. (2014) investigated the different studies of chitosan derivatives for the separation of metal solution from textile wastewater.

In the present study, chitosan from shrimp and crab shells was used for biodegradation and detoxification of textile effluent. The shrimp shell chitosan treated dye effluent showed 100% decolorization. Similar studies were published by Kos (2016), who found that chitosan was the best decolorizer for wastewater, removing color up to 83-99% of the time.

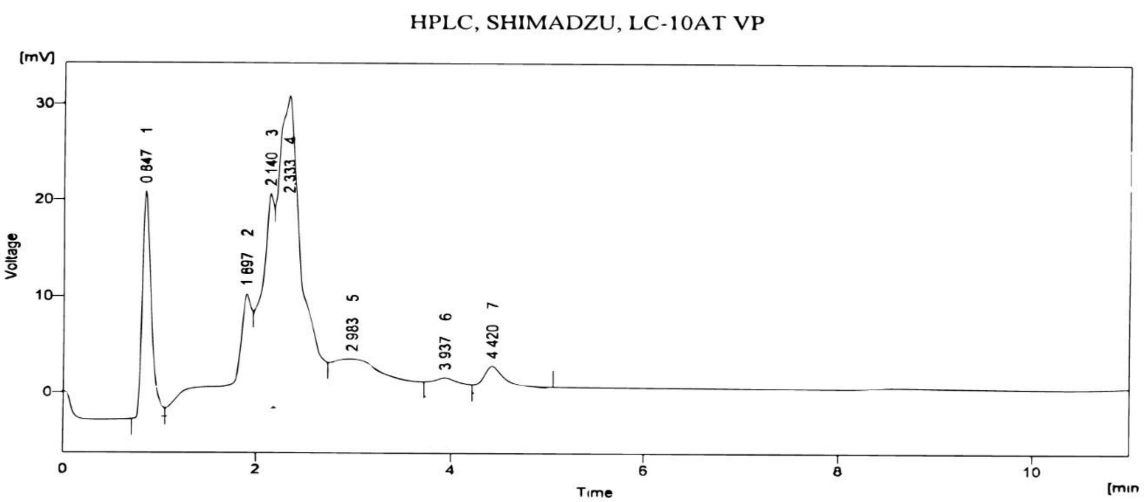

Fig. 1: HPLC chromotogram of Textile Effluent (control).

Table 9: Analytical HPLC chromatogram of untreated textile effluent (Control).

Sl.No	Retent. Time [min]	Area [mV.s^{-1}]	Height [mV]	Area [%]	Height [%]	Wo5 [min]
1	0.847	152.946	23.529	11.0	23.7	0.10
2	1.897	198.450	12.050	14.3	12.2	0.13
3	2.140	200.334	22.280	14.4	22.5	0.16
4	2.333	559.865	32.363	40.3	32.6	0.26
5	2.983	188.957	4.450	13.6	4.5	0.85
6	3.937	43.067	1.813	3.1	1.8	0.49
7	4.420	47.140	2.642	3.4	2.7	0.25
	Total	1390.760	99.127	100.0	100.0	

Similarly, Mary et al. (2019) used shrimp shell chitosan membrane for the decolorization of simulated textile effluent and reported maximum decolorization of 80.70% on the 20th day at 80% effluent concentration.

The effluent treated with crab shell chitosan gave a maximum decolorization of 68.30% as reported by Devi et al. (2012) for the effective decolorization of textile effluent. Similar observations were made by Shirsath & Shrivastava (2012) who reported that crab shell is a good adsorbent for the removal of Ponceau dyes. Similarly, Kair et al. (2019) observed that chitosan was a successful and cost-effective approach for removing color and turbidity from wastewater containing printing ink, with 15 mg chitosan in 100 mL wastewater removing up to 93% of color.

Probit analysis indicated the reduction of LC_{50} values of textile effluent treated with shrimp and crab shell chitosan. The 96 h of LC_{50} value recorded for the untreated textile effluent was 39.984 mL.dL^{-1}. The present observation is in close agreement with the findings of Raj et al. (2015) who observed that toxicity of *Poecilia reticulata* exposed to simulated effluent for 96 h and reported 100% mortality at a concentration of 60%. Similarly, Shengkani & Dhas (2014) reported LC_{50} values as 19.77 and 18.99 mL.dL^{-1} during the toxicity study of *P. reticulata,* exposed for 24 h and 96 h respectively. Likewise, Sharma et al. (2006) reported a 96-h LC50 value of 27.2 ppm for methyl red.

The 96 h LC_{50} value recorded for the shrimp shell chitosan treated textile effluent was 61.109 mL.dL^{-1} whereas the corresponding value recorded for the treated dye with the crab shell chitosan was 59.411 mL.dL^{-1}. In a related study, 96 h LC_{50} of azo dye methyl red for *P. reticulata* was recorded as 24 ppm (Kirandeep et al. 2015). The percentage mortality of fishes was found to be increased with an increase in the concentration of the effluent (Ikpi &

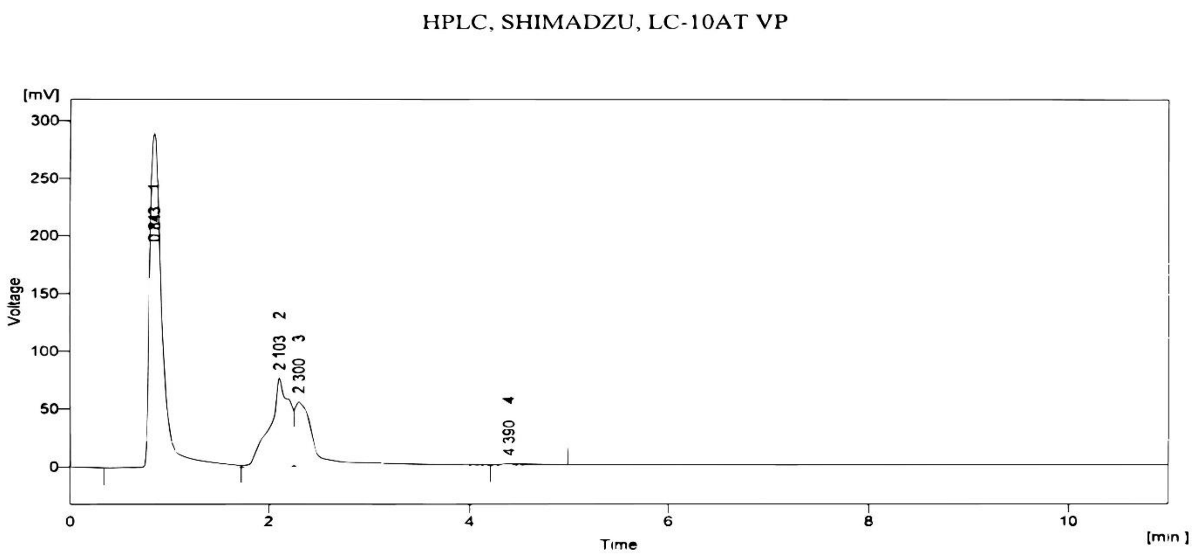

Fig. 2: HPLC chromatogram of textile effluent treated with shrimp shell chitosan.

Table 10: Analytical HPLC chromatogram of textile effluent treated with shrimp shell chitosan.

Sl.No	Retent. Time [min]	Area [mV.s^{-1}]	Height [mV]	Area [%]	Height [%]	Wo5 [min]
1	0.843	2554.79	289.628	22.4	66.1	0.13
2	2.103	1060.75	76.458	9.3	17.5	0.22
3	2.3	818.283	56.065	7.2	12.8	0.18
4	4.39	25.605	1.288	0.2	0.3	0.32
5	21.9	181.587	0.916	1.6	0.2	1.33
6	27.077	2747.9	3.917	24.1	0.9	10.27
7	42.213	3131.42	3.563	27.5	0.8	15.13
8	58.32	563.514	2.762	4.9	0.6	3.74
9	64.01	318.971	3.247	2.8	0.7	1.57
	Total	11402.8	437.845	100	100	

Table 11: Analytical HPLC chromatogram of textile effluent treated with crab shell chitosan.

Sl.No	Retent. Time [min]	Area [mV.s^{-1}]	Height [mV]	Area [%]	Height [%]	Wo5 [min]
1	0.863	75.185	11.223	1.5	16.8	0.11
2	1.21	11.938	0.807	0.2	1.2	0.28
3	1.873	73.808	9.489	1.5	14.2	0.14
4	2.14	145.215	13.999	2.9	20.9	0.19
5	2.3	232.996	17.762	4.6	26.5	0.22
6	2.623	32.961	4.322	0.7	6.5	0.12
7	4.313	10.5	0.717	0.2	1.1	0.25
8	20.2	17.24	2.482	0.3	3.7	0.22
9	22.337	174.628	0.746	3.5	1.1	1.24
10	29.61	4207.48	3.082	83.9	4.6	26.67
11	55.12	34.401	2.278	0.7	3.4	0.44
	Total	5016.35	66.907	100	100	

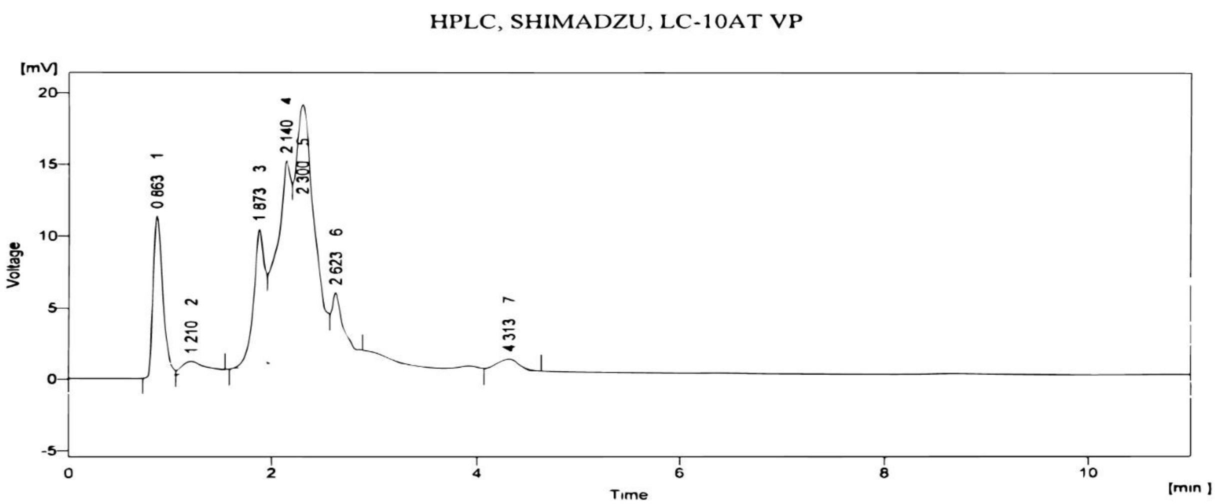

Fig. 3: HPLC chromatogram of textile effluent treated with crab shell chitosan.

Offem 2013). Moreover, the mortality was also directly proportional to the increase in time of exposure to the effluent. Similar observations were made by Angelin et al. (2015) when *P. reticulata* was exposed to chrome plating industry effluent. This toxicity study indicates that chitosan from shrimp and crab shells were good adsorbents and detoxification agents for the removal of the toxins and pollutants present in water.

The HPLC analysis also confirmed the biodegradation of textile effluent. The HPLC elution profile of untreated textile effluent showed 7 peaks, while the shrimp and crab shell chitosan treated sample showed 4 and 7 peaks respectively. The variance in the number of peaks, the height of peaks, and retention time may be assumed as there is biodegradation. In a related study, Raj et al. (2012) obtained 3 peaks with RT 1.98, 2.18, and 2.39 in the HPLC profile of Congo red dye treated with bacteria *Hafnia alvei.* The retention times vary from one to another. The results agree with the findings of Ilavarasi et al. (2019) who attained 5 peaks with RT 2.800, 2.953, 3.990, 4.790, and 5.437 in the HPLC profile of textile dye treated with chitosan. The disintegration of peaks and the different retention times confirmed the degradation of the effluent.

CONCLUSION

When comparing the shrimp and crab shell chitosan for the textile wastewater treatment, shrimp shell chitosan seems to be an efficient and better bio adsorbent for the decolorization and detoxification of textile wastewater. It could be used in water purification plants all over the world and the water purification filters can be modified based on the chitin materials as it is a good alternative for activated carbon.

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