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A STUDY ON BIODEGRADATION OF LEATHER INDUSTRY EFFLUENT USING *SARGASSUM SP.*

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ABSTRACT

The Increase in the population since the 19th century had initiated a demand for more food products and housing. Thus, the production of wastewater and the subsequent deterioration of environment due to the wastewater being discharged into watercourses have been increasing since the 1800's. Many methods have been adopted for treating the effluent to reduce its harmful chemicals. But they are not an economic one. In this study, a new approach to treat effluent was focused on finding an economic way of treating effluent. The present study investigated the efficacy of various extracts of *Sargassum sp.* collected from Tuticorin (Tamil Nadu) in treating leather effluent. Five different solvent systems such as ethanol, methanol, water, benzene and chloroform were used for extracting phytochemical constituents from seaweeds. Then these extracts were used for treating leather effluent which was collected from Nagalkeni, Chennai, and Tamil Nadu. The results showed that reduction of various chemicals present in the treated effluent was more than 75% by all the algal extracts.

KEYWORDS: **Effluent, industrialization, modernization, *Sargassum sp.***

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INTRODUCTION

Worldwide civilization had originated on the banks of large water bodies as water is an essential commodity for survival. Of late water is polluted by lots of factors among which industrial and domestic effluent play vital role, as they are discharged in the untreated form to the water bodies, canals, and drainage ditches, land and water resources. This method of waste disposal has greatly reduced the amount of potable water. The main constituent in domestic wastewater is human excreta with smaller contributions from food preparations, washings, laundry and surface drainage[1]. A large number of enteric bacterial and viral pathogens may be excreted by infected individuals and may, therefore, be present in untreated domestic wastewater[2]. The limited availability of fresh water is a global crisis. The growing consumption of fresh water by anthropogenic activities has taken its toll on available water resources. Unfortunately, water bodies are still used as sinks for wastewater from domestic and industrial sources. However, in recent times, the need to replenish our water resources has received increasing attention. This has led to the development of strategies to return water to its source in the least toxic form possible, to enable reutilization of water. These strategies and processes may be collectively termed as 'wastewater treatment'. Precipitation technique is the first stage of treatment which usually involves the removal of solids, which are separated as sludge[3]. Untreated or allegedly treated effluents have increased the level of surface water pollution up to 20 times the safe level in 22 critically polluted areas of the country. It is found that almost all rivers are polluted in most of the stretches by some industry or the other[4]. Many researchers had found that the rapid swiftness of urbanization has contributed to the pollution of water in the country[4-9]. As populations in towns and cities grew, the rivers could not absorb the pollution. They began to smell and became unable to support life. Hence the safe treatment of wastewater and its return to the natural environment is a key part of the water cycle. It protects the life of rivers and ensures that all water sources are clean and may be easily used for the public supply. The Hindu reported about the seriousness of the leather effluent at Pallavaram on 19th November 2007 (Fig.1).

Figure 1

Stagnation of effluent from tanneries at Pammal and Tiruneermalai (The Hindu, 2007)

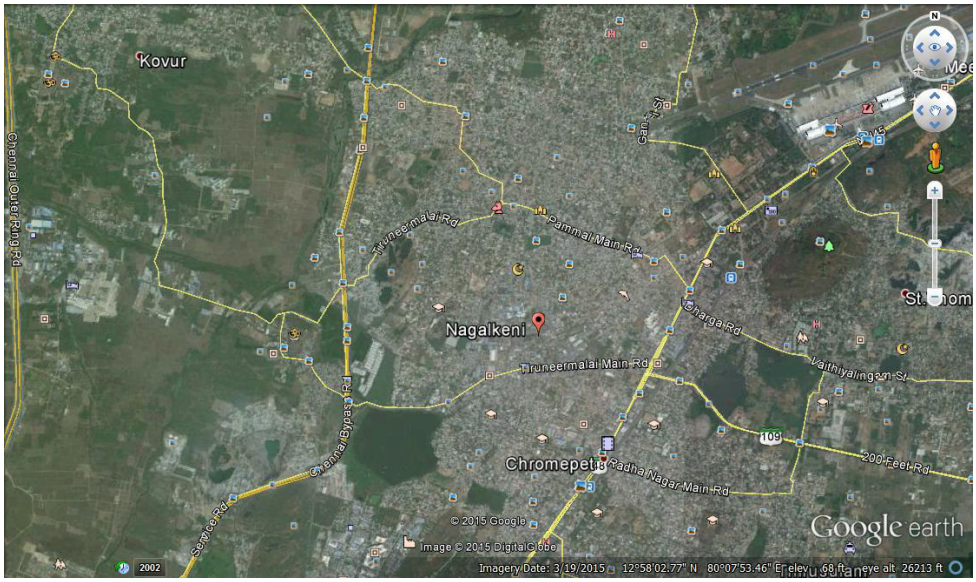
Many methods have been adopted for treating the industrial waste water and were found to be very costly. The coast of Tamil Nadu bears luxuriant growth of seaweeds. More than two hundred species of seaweeds have been found in this area and most of them are treated as waste. Plant extracts were effectively used for treating paint industry effluent[10]. Hence in this study, seaweeds were used for treating leather effluent.

MATERIALS AND METHODS

Collection of sample

Marine algae Sargassum sp., was collected from Tuticorin, Tamil Nadu and was identified by Dr.Balusamy Head, department of Plant Biology, Madras Christian College, Chennai, Tamil Nadu. Then it was washed thoroughly with water to remove sand and epiphytes. Then were dried under sunlight and were made into powder using mixer. Then it was stored. The algal sample was identified by Dr.M.Baluswami, Head, Dept. of plant biology and Plant Biotechnology, Madras Christian College, Tambaram, Chennai. Leather industry effluents were collected from the surrounding areas of Nagalkeni village, Pallavaram (Fig.2). Since the underground water and the nearby river is highly polluted by the leather effluent. The collected effluent was stored in the refrigerator for avoiding further contamination.

Figure 2
GPS location of effluent collection place (Nagalkeni, Pallavaram, Chennai)



Preparation of plant extract
Five gram of powdered samples was soaked in 50ml of different solvents such as methanol, ethanol, chloroform benzene and water and the extracts were filtered out using Whatman No.1 filter paper.

Salkowski Test for Terpenoids and Triterpenoids
Few drops of concentrated sulphuric acid were added to 2 ml of extract. Then it was shaken well and left it for some time. Appearance of red color indicates the presence of steroids and yellow color indicates the presence of triterpenoid. Appearance of Brown color indicates the presence of terpenoids[11].

Test for phenol
To the 2ml of extracts, few drops of 10% ferric chloride were added. The presence of phenol was confirmed by the appearance of green/blue/ bluish green/ brown/ brownish red color[12].

Test for flavanoids
Three ml of distilled water was added to two ml of sample and filtered. Then 10% ferric chloride is added to this filtrate. Appearance of greenish blue/ violet color confirms the flavanoids[13].

Neutral Ferric chloride test for Tannins
Few drops of 0.1% ferric chloride were added to 2ml plant extracts. Appearance of blue/ black/ bluish green precipitate indicates the presence of tannins[14].

Ninhydrin test for aminoacid
Few drops of 10% ninhydrin were added to the extracts. Color changed from blue to pink confirms the amino acid[15].Sodium bicarbonate test for carboxylic acid 2% Sodium bicarbonate was added to two ml of plant extract. Formation of brisk effervescence confirms the presence of carboxylic aciD[16].

Molisch's Test for glycoside
Two ml of sample was treated with 2-3 drops of 2% α-napthol and few drops of concentrated sulfuric acid. Appearance of red brown/ violet ring confirms the presence of glycoside[17].

Keller killiani test for Cardiac glycoside
Few drops of glacial acetic acid and 2-3 drops of ferric chloride were added to two ml of extract along with 1ml of concentrated sulfuric acid. Appearance of brown ring at the interface confirms the cardiac glycoside[16,18].

Borntrager's test for anthraquinone
Two ml of extract was mixed with 10% of 5ml ammonia. Appearance of pink red/ violet color at the lower phase indicates the presence of anthraquinone[19].

Test for Carbonyl group
2 ml Plant extracts were treated with 2-3 drops of 2% 2,4 diphenyl hydrazine. Then it was shaken well. Appearance of yellow crystals confirms the presence of carbonyl groups[20].

Test for Saponin
To the 2ml of a plant extract, 5 ml distilled water was added and boiled with vigorous mixing. Saponin was confirmed by the froth formation[21].

Test for Coumarin
Plant extracts were reacted with 1N NaOH or KOH. Appearance of dark yellow color confirms the presence of coumarin[22].

Test for Phlobatanin
Distilled water was added to the extract and then filtered. The filtrate was boiled with 2% HCl. Presence of red precipitate confirms the phlobatanin[23].

Treatment of effluent

Twenty-five ml of algal extracts (100mg/l) were added to the 250ml of effluent and was left for five days. After 5 days, the changes in TDS, hardness, sulfate, nitrate, chloride and Cr(VI) were analyzed.

Estimation of TDS

The sample was filtered and the sediment leftover on the filter was scrapped off and dried in an oven. Then the dry weight of the sediment was measured[24].

Determination of Hardness

An aliquot containing 25ml of extract was dissolved in 50ml of distilled water and 1 or 2 drops of EBT indicator was added to it. The solution was titrated with EDTA solution till the color changes from reddish to a blue tinge[24].

Analysis of Sulphate concentration

Sulfate concentration was checked by nephlometry method. About 100ml of the sample was treated with 20ml of buffer solution A(30 g of MgCl2 was dissolved in 5g of sodium acetate, 1g of KNO3 and 20ml of CH3COOH in 500ml distilled water). A spoonful of BaCl2 was added to it. The turbidity was measured. Using a standard graph, the concentration of sulfate was measured[25].

Determination of Nitrate level

Aliquot containing 50ml of the sample was added to 1ml of HCl and OD was measured using calorimeter. The nitrate concentration was measured for the given sample using standard graph[25].

Determination of chromium

Hexavalent chromium was measured spectrophotometrically by diphenyl carbizide method which is nearly specific for Cr(VI) Adding diphenyl carbized solution to samples develops a pink color which can be measured with a UV-spectrophotometer at 540nm.

Estimation of chloride[26]

Ten milliliters of effluent samples in a conical flask was taken and 1ml potassium chromate was added to get light yellow color. It was then titrated with standard silver nitrate solution till color change from yellow to brick red[24].

RESULTS AND DISCUSSION

Industrial effluents which are released into the environment in the untreated form are a major threat in developing countries. In this work, different solvent extracts of *Sargassum sp.* were used for treating the leather and paint industry effluent and the results were correlated with phytochemical constituents.

Table.1
Phytochemical Analysis of Plant Extracts

S.No	Plant constituents	Methanol	Ethanol	Chloroform	Benzene	Water
1	Steroid	++	+	++	-	+
2	Triterpenoids	-	++	++	++	++
3	Terpenoids	++	+	+	-	-
4	Phenol	+	-	+	-	-
5	Flavanoid	-	-	+	-	-
6	Coumarin	+	++	++	-	-
7	Tannin	-	+	+	-	-
8	Phlobatanin	-	+	+	-	-
9	Amino acid	++	-	-	-	-
10	Carboxylic acid	-	+	+	-	-
11	Glycoside	+	-	++	+	+
12	Cardiac glycoside	+	-	++	+	+
13	Carbonyl	++	+++	+++	++	++
14	Saponins	+	+	-	+	+
15	Anthraquinone	_	++	-	-	-

Table.2
National Environment Standard of effluent

S.No	Parameters	Permissible limit (mg/l)
1	TDS	1200
2	Chloride	500
3	Sulphate	500
4	Chromium	0.05
5	Nitrate	10

Table.3
EPA Classification of water hardness

S.No	Hardness	mg/l as CaCO3
1.	Soft	0 to 50
2.	Moderately hard	50 to 150
3.	Hard	150 to 300
4.	Very Hard	Above 300

Table.4
Effect of plant extract on tannery effluent

S.No	Parameters	Untreated effluent (mg/l)	Effluent treated with algal extracts (mg/l)				
			Methanol	Ethanol	Chloroform	Benzene	Water
1	TDS	2200	200	250	275	210	300
2	Hardness	3220	210	250	250	300	275
3	Chloride	1604.67	209.95	159.96	124.97	154.96	194.96
4	Sulphate	4000	250	300	300	250	400
5	Chromium	1600	100	175	125	125	375
6	Nitrate	540	25	25	30	25	100

Many technologies have been adopted for treating effluent to remove its harmful contents. Chemical contents of untreated effluent were found to be very much higher than the permissible limit of The National Environment (Standards for Discharge of Effluent into Water or on Land) Regulations, S.I. No 5/1999 (Under section 26 and 107 of the National Environment Act, Cap 153) (Table.2&3). All the algal extracts showed good reduction (Table.4) of harmful chemicals present in the effluent. Water extracts showed less reduction of Cr(VI) compared to other extracts. Methanolic extract showed highest reduction of 94% which was higher than the Cr(VI) reduction by *Vogococcus sp*[27].. Since the hardness of effluent was too high (3220 mg/l), it was unable to bring down to soft water but almost all the extracts reduced the maximum of 90% reduction in hardness. It is comparatively higher than the electrolysis process (80%)[28]. All the extracts were able to reduce minimum of 90% nitrate content in the effluent, except water extract. All the extracts had a good effect on nitrate reduction. In previous studies of reducing TDS with extracts of Murraya koenigii showed a maximum of 69% from domestic effluent[28]. But in this work, nearly 90% of TDS content was removed from the effluent (Fig.3). Nearly 90% of chloride content was removed by chloroform extract which was almost equal to the reduction of chloride by benzene extract of Prosopis juliflora[30]. Carbonyl compounds were identified in all extracts and these may the reason for the reduction all compounds present in the effluent.

CONCLUSION

Algal extracts were found to be the best source for reducing harmful chemicals present in the effluent. In future, further analysis may be carried out to optimize its concentration.

REFERENCES

1. Gray NF. Biology of water treatment. Oxford Scientific Publications, United State 1989; 124-828.
2. Samuel RF, Bulton G. 1983, *J.* Applied Environ. Microbiol. 2013; 45:174
3. Fair GM. Element of Water Supply and Waste Disposal, 2nd ed., John Wiley and Sons
4. Modak DM, Singh KP, Ahmed S and Ray PK. Trace metal ion in Ganga water system, Chemosphere 1990;21(1-2): 275-287.
5. Rajaram T, Das A. Water pollution by industrial effluents in India: discharge scenarios and case for participatory ecosystem specific local regulation. Futures 2008; 40(1):56–69.
6. Khurshid S, Abdul B, Zaheeruddin A, Usman SM. Effect of waste disposal on water quality in parts of Cochin, Kerala. Indian J Environ Health 1998; 40(1): 45–50.
7. Pachpande BG, Ingle ST. Recovery of the chromium by chemical precipitation from tannery effluent. Orient J Chem 2004; 20(1): 117–23.
8. Prabha S, Selvapathy P. Heavy metal pollution in Indian Rivers. Indian J Environ Prot 1997; 17(6): 641–49.
9. Singare PU, Lokhande RS, Pathak PP. Study on Physico-Chemical properties and Heavy Metal Content of the Soil Samples from Thane Creek of Maharashtra, India. Interdisciplinary Environmental Review 2010; 11(01): 38-56.
10. Sharmila S, Jeyanthi Rebecca L and Md Saduzzaman, Effect of plant extracts on the treatment of paint industry effluent, Int.J.Pharma and Biosci., 2013; 4(3): (B) 678 – 686.
11. Salkowski, Ernst Leopold. A Laboratory Manual of Physiological and Pathological Chemistry. 1904; New York: John Wiley & Sons.

12. Mace ME, Histochemical Localization of phenols in healthy and diseased tomato roots. Phytopathology, 1963; 16: 915-925.
13. Sofowora A. Medicinal Plant and Traditional Medicine in Africa; Spectrum Books Limited, Ibadan. 1993; 1(12): 101-108.
14. Segelman AB, Fransworth NR and Quimbi MD. False negative saponins test results induced by the presence of tannins. Lloydia, 1969; 32: 52-58.
15. Yasuma A, Ichikawa, Ninhydrin-schiff, Alloxan-Schiff staining. A new histochemical staining method for proteins J.Lab clin Med. 1953; 41:296-299.
16. Harborne JB. Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis. 1stedn: Chapman and Hall; London, UK. 1998.
17. Ramakrishnan SK, Prasannan G and Rajan R. Textbook of medical biochemistry.Orient Longman, New Delhi.India., 1994; 582.
18. Kokate CK, Purohit AP and Gokhale SB, Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy,: 2001; 7, edition:133 -166.
19. Trease GE, Evans WC. Pharmacognosy, 4th edition, WB Saunders Company: UK, 1997; 50: 150.
20. Venkatesan D, Karrunakarn CM, Selva Kumar S and Palani Swamy PT. Identification of Phytochemical Constituents of *Aegle marmelos* Responsible for Antimicrobial A ctivity against Selected Pathogenic Organisms, *Ethnobotanical Leaflets* 2009; 13: 1362- 1372.
21. Kokate CK, Practical pharmacognosy.4th Edition.Vallaph prakashan publication, New Delhi, India. 1999; 115.
22. Kiran Kumari SP, Sridevi V and Chandana Lakshmi MVV, Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants, Journal of Chemical, Biological and Physical Sciences, 2012, 2(3): 1326-1332.
23. Pravin S. Jogi1 and Akkewar DM, Phytochemical screening and antimicrobial activity of medicinal plant Pergularia daemia From Chandrapur Forest Region, International Journal of Natural Products Research, 2012; 1(3): 61-63.
24. Kotaiah, B and Swamy, KN. Environmental Engineering Laboratory Manual, Charotar Publishing House, Gujarat, India, 1994; 25-26.
25. SAP (Standard Analytical procedure), Hydrology Project, Govt.of India & Govt. of Netherlands, 1999.
26. APHA. Standard Methods for the Examination of Water and Waste Water, 1998; 20th Edition, AWA, WEF.
27. Kinnari Mistry, Chirayu Desai, Krishna Patel, Chromate Reduction by *Vogococcus sp.* Isolated from Cr (VI) Contaminated Industrial Effluent, Electronic Journal of Biology, 2010; 6(1): 6-12.
28. Agostinho LCL, Nascimento L and Cavalcanti BF, Water Hardness Removal for Industrial Use: Application of the Electrolysis Process, Open Access Scientific Reports, 2012; 1(9): 1-5.
29. Sharmila S, Jeyanthi Rebecca L and Md Saduzzaman. Biodegradation of domestic effluent using different solvent extracts of *Murraya koenigii.* Journal of Chemical and Pharmaceutical Research 2013; 5(2): 279-282.
30. Sharmila S, Jeyanthi Rebecca L and Md Saduzzaman. Biodegradation of Tannery effluent using Prosopis juliflora, Int.J.Chem Tech Res. 2013; 5(5): 2186-2192