Efficacy of treatment of Various Solvent Extracts of *Enteromorpha intestinalis* on Tannery Industry Effluent

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Abstract – The untreated liquid waste generated from tannery industries have managed to take away the calmness and serenity from our hectic and tensed lives by tampering our greatest wealth i.e. health. It has also degraded our environment at a fanatic pace. Moreover, with rapid industrialisation under the guise of automation, modernization and progress have also contaminated our water bodies as the untreated effluent are release directly into them, therefore leading to a catastrophic impact on the quality of water and aquatic life.The present study being first of its kind investigate the impact of different solvent extract of marine algae *Enteromorpha intestinalis* on the various physicochemical parameters like TDS, Hardness, Chloride, Nitarate, Sulphate and Chromium (VI) of tannery industry effluent. Phytochemical analysis of various solvent extract namely EIET, EIMT, EICT, EIBT and ETAT were performed and the retentate was used as biofertilizer for growth of *Vigna mungo*. The length and protein content of the plant showed a significant increase in comparison to the control. With the help of Imvic test, the organism isolated from tannery industry effluent was identified as *Bacillus* (MIC 1200 mg/L for Cr VI). Methanolic extract of *Entromorpha intestinalis* was found to be most effective in improving the quality of water and removal of toxic metals like Cr VI.

Keywords- Phytochemicals, Imvic test, Chromium, Nitrate, Chloride, Sulphate, Cr VI

1.INTRODUCTION

Water is the part and parcel of our existence without which existence of life cannot be even imagined. Worldwide civilization had originated on the banks of large water bodies as water is the most 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 [1,2]. A large number of enteric bacterial and viral pathogens may be excreted by infected individuals and may therefore be present in untreated domestic wastewater [3].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 been receiving 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. The untreated liquid wastes generated from tannery are characterized as high-coloured, foul-smelling, acidic, and alkaline [4] with high BOD (Biological oxygen demand) and COD (Chemical oxygen demand) [5]. The waste product of electroplating and leather industries are contain huge amount of chromium and it is also major cause for the high influx of chromium to the Biosphere [6].The huge quantity of chromium salts discharge into tannery waste has raised several ecological concerns. The slug generated by chromium based industries is usually damped on the ground which pollutes in surface and subsoil water in the vicinity of industrial units. Hexavalent chromium is formed due to oxidation of Cr (III) compounds which percolates down into the soil during rainy season and polluting underground water [7]. In general, industrial waste contains both hexavalent and trivalent forms of chromium which are most stable and exist in aqueous system. [8].The hexavalent chromium is of particular concern due to its great toxicity. It is known to be carcinogenic and mutagenic to living organisms Thus it is necessary to remove or recover

the chromium before disposal of industrial waste. *Enteromorpha intestinalis* is an invasive brown seaweed that has recently found its way near the coast of Ireland. Numerous species are distributed throughout the temperate and tropical oceans of the world, where they inhabit shallow water and coral reefs. The species of *Enteromorpha intestinalis* containing wide range of bioactive metabolites which has various application like medicinal importance, biofuel and cosmetic industries [10]. Due to physiochemical and biological activities of *Enteromorpha intestinalis* it is used to enhanced the soil quality by nutrients supply and toxicity removal [11,12]. However the genus may be best known for its planktonic species. *Enteromorpha intestinalis* is also cultivated and cleaned for use as an herbal remedy. Many Chinese herbalists prescribe powdered *Enteromorpha intestinalis* in paper packets, to be dissolve in warm water and drunk as tea. Batch experiment using Sargassum biomass indicated that it was possible to attain high removal efficiencies of various parameters including TDS, conductivity and salinity.Hardness and TDS are the major criteria which can be significantly reduced by by leaf extract of Moringa oleifera and Murraya koengii [13,14]. A similar study using leaf extracts of Prosopisjuli flora, Nymphae ampla , Annona squamosa , Manilkara zapota and Moringa oleifera were performed to treat the paint industry effluent. [14,15,16]. The present study uniquely utilizes the various solvent extracts of *Enteromorpha intestinalis* to improve the physicochemical characteristics and reduce Cr VI concentration of tannery industry effluent collected from Nagalkeni village, Pallavaram Chennai (12°57'51.6"N 80°07'53.8"E). The phytochemical analysis of the solvent extract was performed and the retentate obtained after extraction was used as biofertilizer to grow Vigna mungo. Lastly, Cr (VI) resistant bacteria was isolated and was identified using IMVIC test.

2. MATERIALS AND METHODS

2.1 Collection of sample

Leather and paint industry effluents were collected from the surrounding areas of Nagalkeni village, Pallavaram, Chennai (12°57'51.6"N 80°07'53.8"E) and stored in refrigerator for avoiding further contamination in the effluent. *Enteromorpha intestinalis* was collected from the coast of Tuticorin (8° 45' 50.9976" N and 78° 8' 5.4024" E.). Leaves were separated manually and dried under shade for 10 days. After complete drying, it was made as a fine powder and was stored.

2.2 Preparation of plant extract

The powdered samples were soaked in different solvents such as methanol, ethanol, chloroform benzene and water for 48 hrs and the extracts were filtered out using whatmanNo.1 filter paper and were tested by following protocol (Sharmila et al 2013).

2.3 Phytochemical analysis of leaf extract

Various qualitative phytochemical test were performed. Briefly for testing Terpenoids and Triterpenoids Salkowski Test was done in which Few drops of conc sulphuric acid was 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. For Phenol, 2ml of extracts was taken and few drops of ferric chloride were added. Presence of phenol was confirmed by the appearance of green/blue/ bluish green/ brown/ brownish red color. To 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. Neutral Ferric chloride test was done for tannins in which Few drops of 0.1% ferric chloride was added to 2ml plant extracts. Appearance of blue/ black/ bluishgreen precipitate indicates the presence of tannins. To check the presence of amino acids Ninhydrin test was done by adding few drops of ninhydrin into two ml of extract. Sodium bicarbonate test was done for checking the presence of carboxylic acid. Presence of glycoside was done by Molisch's Test in which Two ml of sample was treated with 2-3 drops of α-napthol and few drops of concentrated sulfuric acid. Keller killani test for Cardiac glycoside by adding Few drops of glacial acetic acid and 2-3 drops of ferric chloride into 2 ml of extract along with 1 ml of concentrated Sulfuric acid. For testing Anthraquinone Borntrager's test was performed by adding 2 ml of extract was mixed with 10% of 5ml ammonia. Carbonyl group was tested by treating the 2 ml of plant extract with 2-3 drops of 2,4 diphenyl hydrazine. Saponin was tested by adding 2 ml of plant

extract 5 ml distilled water and boiled with vigorous mixing. Coumarin was tested by reacting the plant extract with 1N NaOH or KOH. For testing Phlobatanin, distilled water was added to the extract and then filtered. Filtrate was boiled with 2% HCl.

2.4 Treatment of effluent

Tannery effluent was treated with various plant extracts and some important parameters were checked. In which estimation of TDS was done. Briefly the sample was filtered and the sediment leftover on the filter was scrapped off and dried in oven. Then the dry weight of the sediment was measured. Determination of Hardness was done by dissolving an aliquot containing 25ml of extract 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 colour changes from reddish to blue tinge. Sulphate concentration was checked by nephlometry method. About 100ml of 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 standard graph, the concentration of sulphate was measured. Aliquot containing 50ml of sample was added to 1ml of HCl and OD was measured using calorimeter by using Phenol disulphonic acid method. The nitrate concentration was measured for the given sample using standard graph. 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. Chloride was estimated by adding Ten milliliter of effluent samples in a conical flask 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.

2.5 Effect of retentate as Bio fertilizer on growth of Vigna mungo

The soil was tested by soil testing kit (HIMEDIA K054). After the extraction of the phytochemical the plant biomass was mixed with soil in two proportions i.e. 5g in 50 g soil and 10 g in 50 g soil and the growth and protein content of Vigna mungo was checked using Lowry method. It was watered with nutrient solution for almost a week.

2. 6.Microbiological Assay

2.6.1 Catalase test:

Pure growth of the organism from the agar to a clean slide with a loop or glass rod was transferred. Immediately 3% hydrogen peroxide was added to the growth and the release of bubbles was observed. 2.6.2 Oxidase test

With the help of glass rod, a colony from 24hrs growth of the test organism was picked up and rubbed on oxidase disc. A change in color from blue or purple within 10sec was observed.

2.6.3 MRVP test:

Organism was inoculated into MR/VP both and incubated at 370 c for atleast48hrs. Then the broth was divided into two equal halves and to one tube 0.5ml of MR reagent was added and other tube 0.2ml VP reagent A and 0.2mlVP reagent B was added and was allowed to stand for 15min.

2.6.4 Citrate utilization test:

Citrate agar slant was prepared and the organisms were streaked on it. It was then incubated for 18-24 hrs and the result was read.

2.6.5 Indole test:

Tube of tryptone broth was inoculated with organism and was incubated for 24-48 hrs at 370 c.Then0.2ml of kovac's reagent was added and it was allowed to stand for few min.

2.6.6 TSI test:

A butt and a slant was prepared in the same tube. Organism from top of a single colony was picked and was stabbed at the centre if the agar butt carefully. The needle was withdrawn carefully and then the surface of slant was streaked carefully and was incubated at 370 c for nearly 18-24 hrs.

3. RESULT AND DISCUSSION

3.1 Phytochemical analysis of various solvent extracts of *Enteromorpha intestinalis*

Phytochemical are non-nutritive plant chemicals that have protective or disease preventive properties. It is well known that plant produce these chemicals to protect them but recent research demonstrate that they can also protect humans against diseases. The methanolic extract of *Enteromorpha intestinalis* contained steroids, terpenoids, amino acid and

carbonyl in medium amount whereas phenol, coumarin, glycoside, cardiac glycoside and saponins were weakly present. In the ethanolic extract, triterpenoids, coumarin, cardiac glycoside and carbonyl were present in medium amount whereas steroid, terpenoids, phenol, falavanoid, tannin, phlobatanin and carboxylic acid were weakly present. The chloroform extract had steroid, triterpenoids and coumarin in medium amount whereas terpenoids, phenol, flavonoid, tannin, phlobatanin, carboxylic acid and glycoside were weakly present. However benzene and aqueous extract showed ony triterpenoids in medium amount and aqueous extract showed weak presence of steroid.

Table 1. Phytochemical analysis of *Enteromorpha intestinalis* in various solvent extract

S.No	Phytochemical	EIMT	EIET	EICT	EIBT	EIAT
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	Saponins	+	+	+	+	+
14	Anthraquinone	-	++	-	-	-
15	Carbonyl	++	++	++	++	++

(Highly prominent= +++, Medium amount= ++ fewer amount= + Absent= -).

3.2 Effect of extracts on physicochemical properties of tannery effluent.

Methanolic extract of *Enteromorpha intestinalis* was most effective in reducing almost all parameters including TDS, Hardness, sulphate, chromium, nitrate except chloride followed by benzene , ethanol , chloroform and aqueous extracts. From the phytochemical analysis, it can be revealed that the methanolic extract has terpenoids and amino acid in maxm amount which may be responsible for bringing maximum reduction in all parameters. Chloride was reduced maximum in chloroform extract followed by benzene, ethanol, aqueous and methanolic extract. The minimum reduction in chloride in the methanolic extract of Enteromorpha intestinalis suggested a probability of amino acid hindering with chloride reduction. (Fig 1)

Methanolic extract of *Enteromorpha intestinalis* was able to remove 93.75% Cr (VI) from the tannery effluent which may be attributed to the presence of Phytochemical like steroid, Terpenoids, amino acids and carboxyl group.

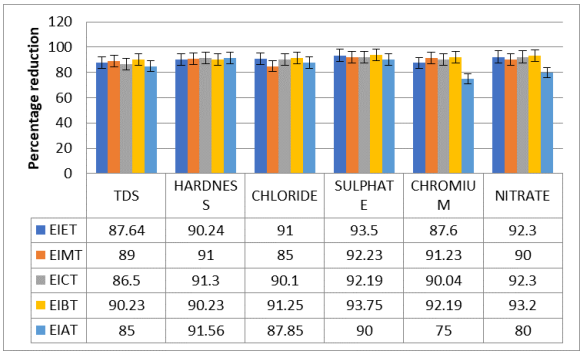

Fig 1- Percentage reduction of various parameters by solvent extract of *Enteromorpha intestinalis*

3.3 Effect of retentate on growth of *Vigna mungo*

A biofertilizer is a substance which contains biological or natural agents which when applied to seed or plant surfaces promotes growth without any ill effect. The retentate after extraction of phytochemical were used as biofertilizer in 2 different amounts and it showed a positive effect on growth of plant in terms of height and protein content. The seedling without retentate (control) was 13 cm long with 0.38 µg / ml of protein whereas the seedling with 5g and 10 g of retentate showed a considerable increase in height and protein content. (Table 2)

Table 2.Effect of biofertilizer on growth of green grams

Amount of biomass	Height of plant(cm)	Protein content(µg/ml)
0(control)	13	0.38
5g	18	0.42
10g	19	0.46

3.3.Identification of isolate

A total of 10 different colonies were isolated and purified. Out of 10 isolates, one colony with MIC 1800 mg/l Cr (VI) was identified using primary and secondary tests and the organism was identified as Bacillus sp. based on Bergey's Manual of Systematic Bacteriology.(Table 3 and 4)

Table 3.Morphlogical test result of bacterial strain

Test	Result(Organism)
Colony morphology	
configuration	circular
texture	moist
pigment	Blue green
opacity	opaque
Grams reaction	
Cell shape	rods
spore	-
motility	+

Negative(-),Positive(+).

Table 4.Biochemical test result of bacterial strain.

test	organism
Methyl red test	-
Vogesprausker test	-
citrate	-
indole	+
catalase	+
oxidase	+
TSI	
Acid production	+
H_2S production	+
Gas production	+

Negative (-),Positive(+).

4.CONCLUSIONS

Methanolic extract of *Enteromorpha intestinalis* has many bioactive compounds like steroids, terpenoids, amino acids and carbonyl which are medicinally important as well as they can can be used in improving the physicochemical properties of contaminated water and they are also important in remediating many toxic metals like Cr (VI). In future quantitative determination of these phytochemicals can be done and their impact on large scale bioremediation of tannery effluent can be studied. Moreover, the isolated strain can be used for bioreduction of Cr (VI) to Cr (III).

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