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BIOREMEDIATION OF INDUSTRIAL LEATHER WASTE BY *OSCILLATORIA LUTEA*

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ABSTRACT

Meeting environmental regulations for both liquid and solid wastes are produced during the manufacture of leather items is one of the long-term issues facing the leather industry. Insufficient treatment of these wastes will cause environmental pollution and endanger human health. Trimmings have generally been underutilized among other trashes that are produced. Hair is not utilized, however collagen found in trims and garbage. Many organic and inorganic particles together with the discharge of suspended or gas-solid oil and grease, nitrogen-containing compounds, and heavy metals either by themselves or in their reduced salt form, chlorides, sulphates, chemical oxygen demand (COD) and total dissolved solids (TDS) are all considerably generated and influenced by tanning operations *Oscillatoria lutea*. Formaldehyde used in the production of finished leather that are difficult to biodegrade and can cause the production of free formaldehyde, a recognized carcinogen. Microbial bioremediation is a novel technique that may be used in a variety of soil and water environments due to microorganisms' adaptability to remove hazardous pollutants that could offer a safer and affordable strategy.

KEYWORDS: Environmental pollutants, leather industry *Oscillatoria lutea* waste, human health, re-tanning agent, microbial bioremediation.

INTRODUCTION

Cyanobacteria are aquatic and photosynthetic that is, they live in the water, and can manufacture their own food. Because they are bacteria, they are quite small and usually unicellular, though they often grow in colonies large enough to see. They have the distinction of being the oldest known fossils, more than 3.5 billion years old, in fact. It may surprise you then to know that the cyanobacteria are still around they are one of the largest and most important groups of bacteria on earth. Many Proterozoic oil deposits are attributed to the activity of cyanobacteria. They are also important providers of nitrogen fertilizer in the cultivation of rice and beans. The cyanobacteria have also been tremendously important in shaping the course of evolution and ecological change throughout earth's history. The oxygen atmosphere that we depend on was generated by numerous cyanobacteria during the Archaeanproterozic Eras. Before that time, the atmosphere had a very different chemistry, unsuitable for life as we know it today.

The other great contribution of the cyanobacteria is the origin of plants. The chloroplast with which plants make food for themselves is actually a cyanobacterium living within the plant's cells. Sometime in the late Proterozoic, or in the early Cambrian, cyanobacteria began to take up residence within certain eukaryotes cells, making food for the eukaryote host in return for a home. This event is known as endosymbiosis, and is also the origin of the eukaryotic mitochondrion. Because they are photosynthetic and aquatic, cyanobacteria are often called blue-green algae. This name is convenient for talking about organisms in the water that make their own food, but does not reflect any relationship between the cyanobacteria and other organisms called algae. Cyanobacteria are relatives of the bacteria, not eukaryotes, and it is only the chloroplast in eukaryotic algae to which the cyanobacteria are related.

Though cyanobacteria do not have a great diversity of form, and though they are microscopic, they are rich in chemical diversity. Cyanobacteria get their name from the bluish pigment phycocyanin, which they use to capture light for photosynthesis. They also contain chlorophyll a, the same photosynthetic pigment that plants use. In fact the chloroplast in plants is a symbiotic cyanobacterium, taken up by a green algal ancestor of the plants sometime in the Precambrian. However, not all blue-green bacteria are blue some common forms are red or pink from the pigment phycoerythrin. These bacteria are often found growing on greenhouse glass, or around sinks and drains. The Red Sea gets its name from

occasional blooms of a reddish species of *Oscillatoria*, and African flamingos get their pink color from eating *Spirulina*.

Cyanobacteria are very important organisms for the health and growth of many plants. They are one of very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia. It is these fixed forms of nitrogen which plants need for their growth, and must obtain from the soil. Fertilizers work the way they do in part because they contain additional fixed nitrogen which plants can then absorb through their roots. Nitrification cannot occur in the presence of oxygen, so nitrogen is fixed in specialized cells called heterocysts. These cells have an especially thickened wall that contains an anaerobic environment. A larger cells among the filaments of *Nostoc*.

Many plants, especially legumes, have formed symbiotic relations with nitrifying bacteria, providing specialized tissues in their roots or stems to house the bacteria, in return for organic nitrogen. This has been used to great advantage in the cultivation of rice, where the floating fern *Azolla* is actively distributed among the rice paddies. The fern houses colonies of the cyanobacterium *Anabaena* in its leaves, where it fixes nitrogen. The ferns then provide an inexpensive natural fertilizer and nitrogen source for the rice plants when they die at the end of the season. Cyanobacteria also form symbiotic relationships with many fungi, forming complex symbiotic organisms known as lichens.

The cyanobacterium *Spirulina*, has long been valued as a food source; it is high in protein, and can be cultivated in ponds quite easily. In tropical countries, it may be a very important part of the diet, and was eaten regularly by the Aztecs; it is also served in several Oriental dishes. In the US, the popularity of *Spirulina* is primarily as a "health food", being sold in stores as a dried powder or in tablet form.

Many other species of cyanobacteria produce populations that are toxic to humans and animals. Blue-green pond scums have been linked to the poisoning of cattle and dogs, and occasionally people. It is therefore not recommended that wild populations be gathered and eaten without some knowledge of the organisms involved. *Cyanobacteria* may cause other problems as well; a species of *Lyngbya* is responsible for one of the skin irritations commonly known as swimmer's itch.

Role of Cyanobacteria in Wastewater Treatment

Cyanobacteria have long been recognized as having enormous potential for use in biotechnology, especially in agriculture, and now slowly drift is towards their use in wastewater treatment, because of the following reasons. Cyanobacterial growth does not require energy rich compounds like other non photo synthetic microorganisms. Cyanobacteria have simple growth requirements which use water as a source of reluctant. This character gives them as edge over other photosynthetic bacteria. Many cyanobacteria combine photosynthesis and nitrogen fixation. This is another advantage over other eukaryotic photosynthetic organisms. Cyanobacterial biomass production is in abundance and this can be used as food for animals (Mosbach 1987) an important source for extraction of high value substances like vitamins and drug intermediates (Venkararamanan 1994) Nitrogen fixation (Stewart *et al*., 1987) hydrogen production light energy photo conversion and amino acid production. They are environmental friendly and do not cause toxicity to other biotic components. Separation of cyanobacterial biomass is much easier than other microbial biomass due to their size.

MATERIALS AND METHODS

Chemicals and regents

BG-11Cyanobacterial medium was pursued from SIGMA–ALORICH company in USA, sodium chloride (Nacl) was purchased from SRL Company in India, silver nitrate (AgNo3) was purchased from fisher scientific in USA, Sulphuric acid (H_2So_4)was purchased from Merck in USA, methyl red was purchased from SRL Company in India. Digital pH meter, digital water tester, Burette, Burette stand, centrifuge tube, Test tube stand.

Collection of samples

For the present study, water sample were collected from Periyar River, Kerala, India. Collection of Industrial leather waste from Kerala Leather Corporation in jews-street, ernakulam, India.

Morphological identification of Cyanobacteria

Morphological identification of Cyanobacteria was done by spreading isolated culture on glass slide using forceps. Culture were covered with glass cover slips and observed their size, shape, colour and other features under low (10X) and high power (45X) objective lens of Trinocular microscope.

Culture and maintaince of *Oscillatorialutea*

Isolated *Oscillatoria* was inoculated in Erlenmeyer flask having BG11 medium and incubated at room temperature under continuous dark and sunlight period for 15-20 days, for their growth.

Treatment of Industrial leather waste by *Oscillatoria lutea*

After incubation period, *Oscillatoria lutea* were harvested and the harversed *Oscillatoria* is treated with collected industrial leather waste sample by following way. Here four centrifuge tubes were taken for the treatment of industrial leather liquid waste by harvested *Oscillatoria lutea* in different ratios. (Tube1 with ratio 1:5, Tube 2 with ratio 2:4, Tube 3 with ratio 3:3, Tube 4 with ratio 4:2). D Then treatment of industrial leather solid waste by harvested *Oscillatoria lutea* with same ratio 2:4 in triplicates.

Effect of Cyanobacterial biodegradation on Physiochemical properties of leather waste

pH

Leather waste water sample 5ml was dissolved in 10 ml of distilled water. The mixture was kept at room temperature for 20 min. The pH was measured for the clear solution using digital pH meter.

Estimation of NPK

Estimation of NPK was done by the kit procedure (Agrinex soil doctor). Breifly, the water sample was mixed with double distilled water in 1:2 ratios and mixed thoroughly. The sample was kept for 30 min for clear water separation.

Estimation of Nitrogen

Leather waste water sample 5 ml was taken from the clean test tube and mixed with Soil Doctor-N Capsule. The solution was mixed thoroughly, until the chemical was dissolved. The tube was kept at room temperature for 20 minutes for colour development. The colour formation was referred with the given reference chart to find out the concentration of the sample.

Estimation of Phosphorous

Leather waste water sample5 ml was taken from the clean test tube and mixed with Soil Doctor-P capsule. The solution was mixed thoroughly, until the chemical was dissolved. Then, four drops of TCA reagent was added carefully and mixed well. The tube was kept at

room temperature for 20 minutes for colour development. The colour formation was referred with the given reference chart to find out the concentration of the sample.

Estimation of Potassium

Leather waste water sample 5ml was taken from the clean test tube and mixed with Soil Doctor-K capsule. The solution was mixed thoroughly, until the chemical was dissolved. The tube was kept at room temperature for 20 minutes for colour development. The colour formation was referred with the given reference chart to find out the concentration of the sample.

Temperature

Leather waste water sample 5ml was taken and dissolved in 10ml of distilled water .The mixture was kept without any disturbance for few minutes. And the temperature was measured for the clear solution by using digital water tester.

Colour

The term colour means true colour that is the colour of water sample from which turbidity has been removed. True colour of the water is due to dissolved material. Colour of the sample is determined by visual comparison of Industrial leather waste before and after treated with *Oscillatoria lutea.*

Decolourization study

The decolouration study was carried out in treated and non-treated Industrial leather waste by *Oscillatoria lutea.* Here the decolourization study was carried out by using calorimeter maintained at 620nm. Now, all the *Oscillatoria* treated liquid waste water and soil waste water were treated by calorimeter and marks the reading for decolourization study. Same procedure was carried out for few more days in order know the decolourization percentage in Industrial leather waste before and after treatment with *Oscillatoria lutea*.

Estimation of Carbonate

For the estimation of carbonate take 15ml of leather wastewater sample was taken in a conical flask and first phenolphthalein is added as indicator. Add the standard acid on burette and allow it to drop into conical flask containing sample drop-wise. The pink colour disappeared, at this stage methyl red is added as indicator and noted the colour changes and the point reached.

Estimation of Sulphate

Sulphate ions are precipitated as BaSo4 (Barium sulfate) in acidic media (HCL) with Barium chloride. The absorption of light by this precipated suspension is measured by spectrophotometer at 420 nm.

Estimation of Chloride

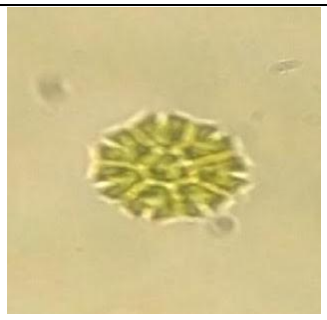
Chloride is determined in a natural or slightly alkaline solution by titration with standard silver nitrate. Take 5ml of leather waste water sample and dissolved in 10ml of distilled water in a conical flask and Slowly add standard silver nitrate solution from burette and shake the solution well. At the end point, white colour is formed in sample containing conical flask. And finally mark the volume for the calculation of chloride estimation.

Thin layer chromatography of *Oscillatoria lutea* extract

Thin layer chromatography is a technique useful in analyzing the purity of component. Take a beaker and required quantity of solvents are taken into the beaker. Take a thin layer plate and cut it to required length and witdh. Now, mark a line about 1cm from the bottom and placed a dots at center. Then 1% of sample solution is spotted using a micropipette. Put the TLC plate carefully into the beaker. The solvent should not touch the marked line. Close the beaker with glass. Allow the plate to run for 30minutes.

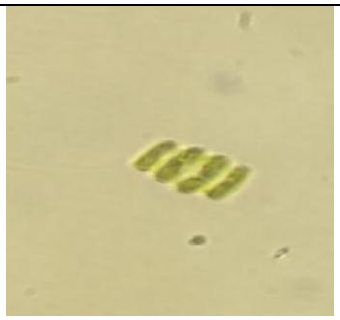
RESULTS AND DISCUSSION

Plate -1: Isolation and identification of Cyanobacteria from Periyar River Water.



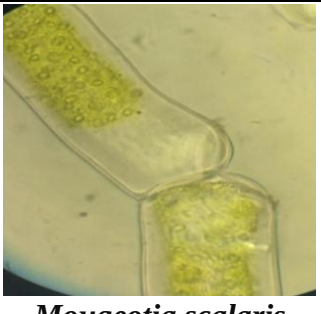
Scenedesmus quadricauda

Pediastrum sp.



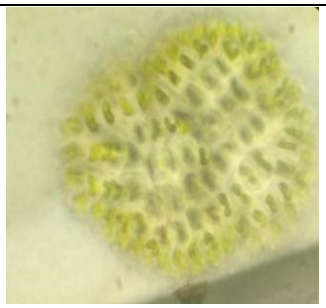
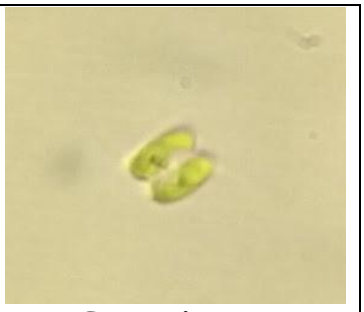
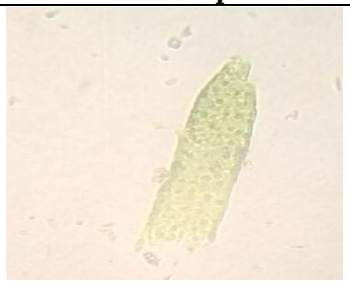
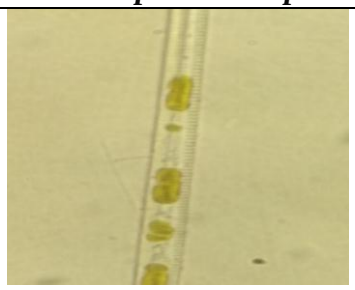
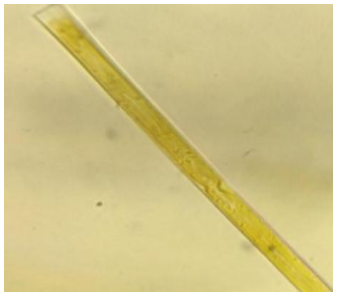
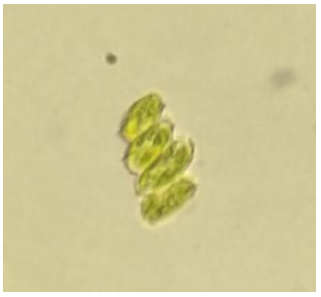
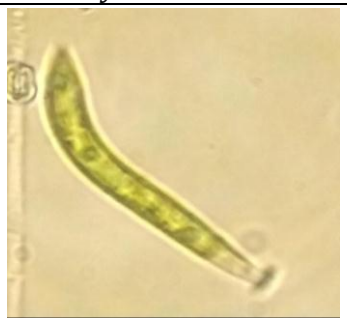
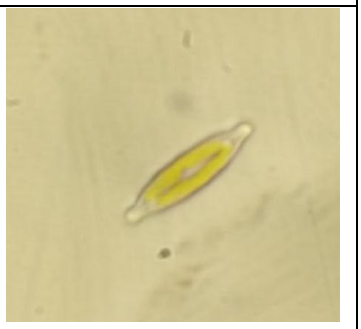
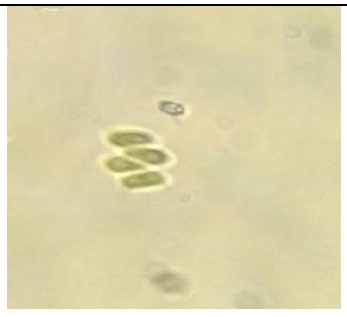
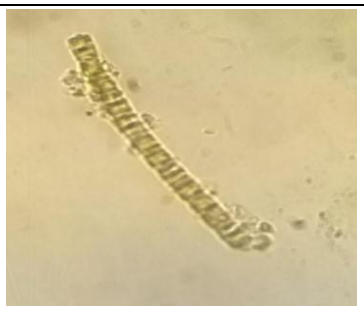
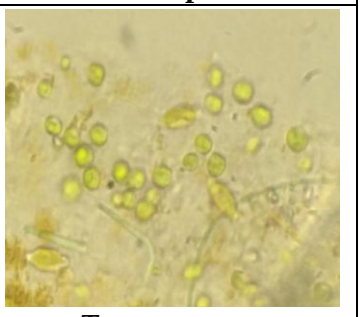
Desmodesmus serratus

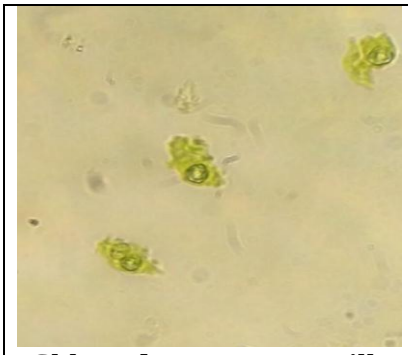
Chlorochytrium lemnae



Mougeotia scalaris

Cyclotella meneghiniana

 *Nostoc sp.*	 *Coelosphaerium sp.*	 *Cosmarium sp.*
 *Hydrodictyon sp.*	 *Tribonema sp.*	 *Fragilaria sp.*
 *Synedra ulna*	 *Westella botryoides*	 *Scenedesmus arcuatus*
 *Closterium sp.*	 *Pinnularia diatom*	 *Caloneis amphisbaena*
 *Dimorphococcu slunatus*	 *Ulothrix speciosa*	 *Tetraspora sp.*

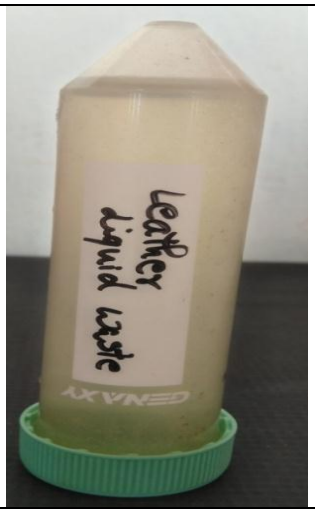


Chlamydomonas perpusillu

Oscillatoria lutea.

Plate – 2 Collection samples in Periyar river and leather industry.

Kollida river water



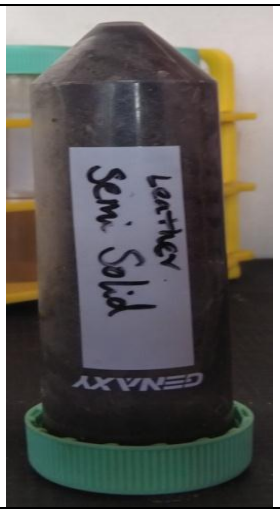


Plate 3: Culture and maintance of isolated *oscillatoria.*

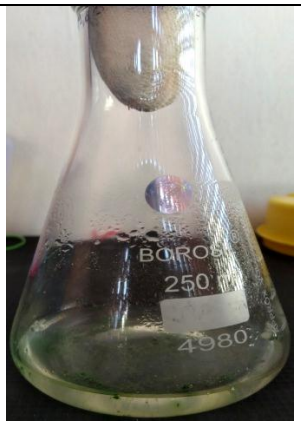


Plate 4: Colour of leather waste before and after treated with *oscillatoiralutea.*

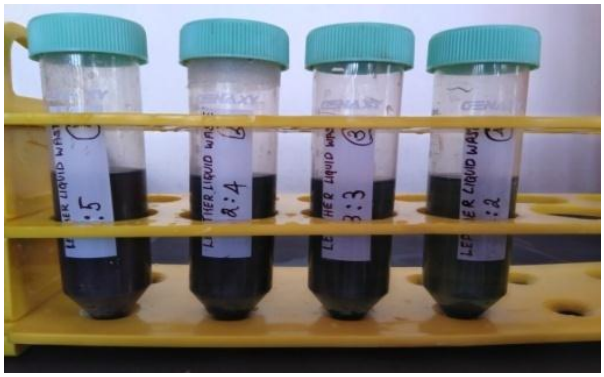
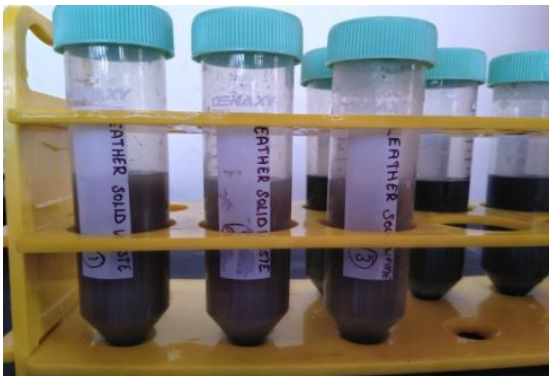
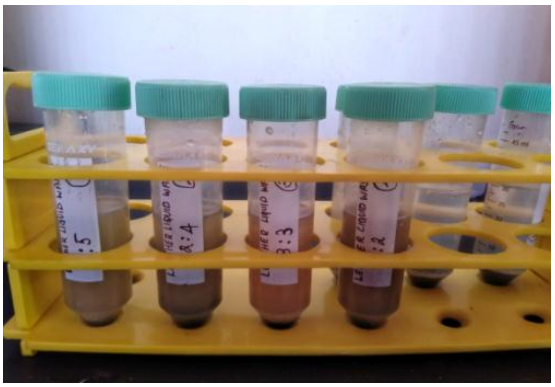
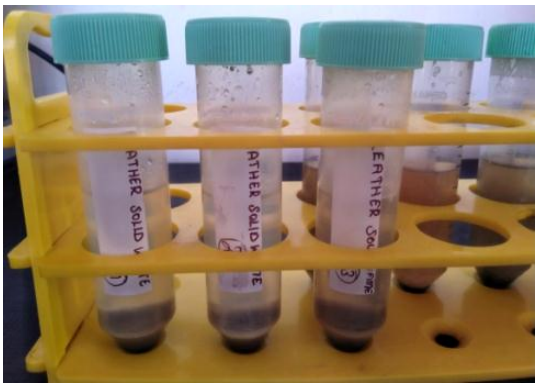
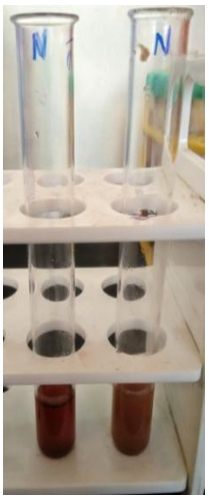
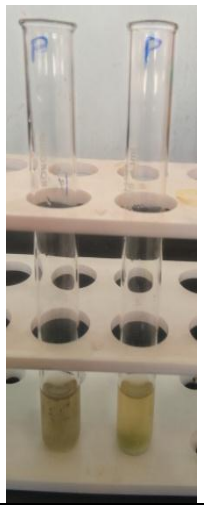
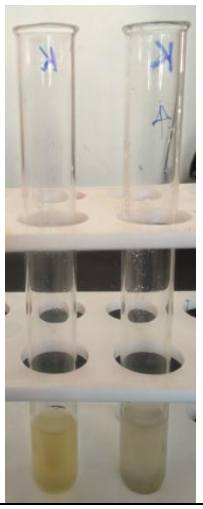
Before treatment  **Leather liquid waste**	 **Leather solid waste**
After treatment  **Leather liquid waste**	 **Leather solid waste**

Plate 5: Effect of *Oscillatoria sp.*, on macro nutrient level (NPK).

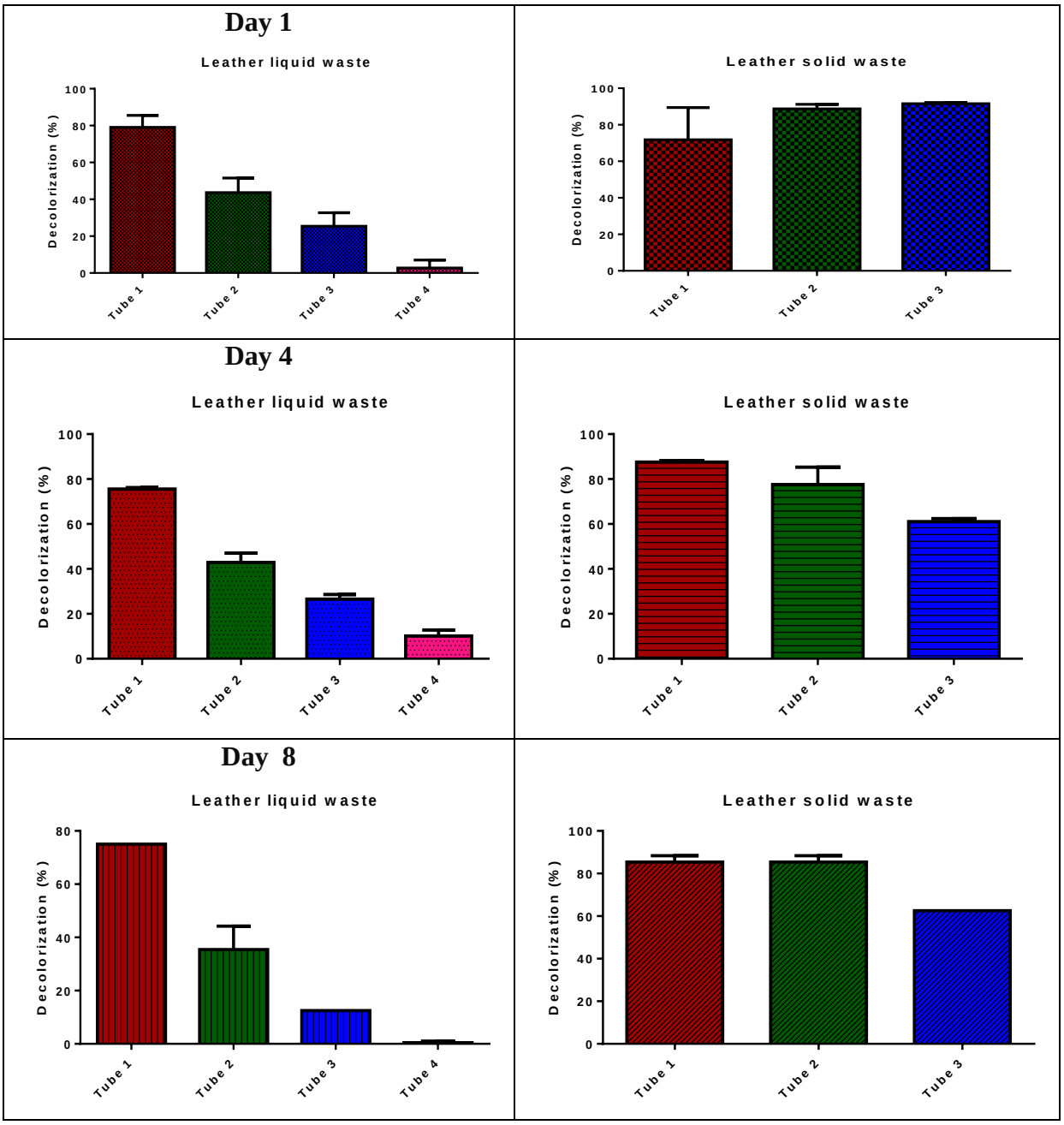
Nitrogen test	Phosphorous test	Potassium test
		

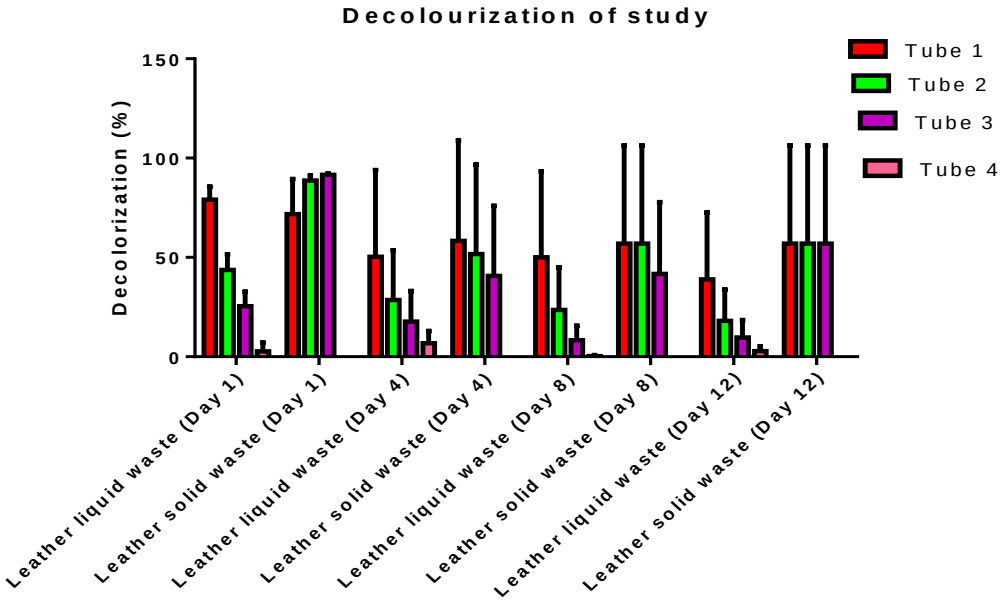
TLC of *Oscillatoria sp.*, extract

Table 1: Morphological characteristics of identified Cyanobacteria.

S.no	Name of the organisms	Family	Size	Shape
1	*Scenedesmus quadricauda*	Scenedesmaceae	10-17µm	Spherical
2	*Pediastrum sp.,*	Hydrodictyaceae	8-32µm	Flat and circular
3	*Desmodesmus sceratus*	Scenedesmaceae	9-20µm	Ovoid
4	*Chlorochytrium lemnae*	Chlorochytriaceae	6-9µm	Cocci
5	*Mougeotia scalaris*	Zygnemataceae	19µm	Rod
6	*Cyclotella meneghiniana*	Stephanodiscaceae	6-18µm	Flat and circular
7	*Nostoc sp.,*	Nostocaceae	4-12µm	Spherical
8	*Coelosphaerium sp.,*	Coelosphaeriaceae	3-9µm	Colonial
9	*Cosmarium sp.,*	Desmidiaceae	57µm	Spherical
10	*Hydrodictyon sp.,*	Hydrodictyaceae	20-30µm	Flat and circular
11	*Tribonema sp.,*	Tribonemataceae	20cm	Filament
12	*Fragilaria sp.,*	Fragilariaceae	45µm	Filament
13	*Synedra ulna*	Fragilariaceae	10µm	Restrate-wedge
14	*Westella botryoides*	Scenedesmaceae	31µm	Circular and coccus
15	*Scenedesmus arcuatus*	Scenedesmaceae	15µm	Linear
16	*Closterium sp.,*	Closteriaceae	25µm	Lanceolate
17	*Pinnularia diatom*	Pinnulariaceae	300µm	Boat shape
18	*Caloneis amphisbaena*	Amphisbaenidae	71µm	Oval
19	*Dimorphococcus lunatus*	Scenedesmaceae	50µm	Irregular in shape
20	*Ulothrix speciosa*	Ulotrichaceae	10µm	Barrel shape
21	*Tetrasporasp*	Tetrasporaceae	0.5-10µm	Coccus
22	*Chlamydomonasperpusillu*	Chlamydomonadaceae	10µm	Cup shaped
23	*Oscillatoria lutea*	Oscillatoriaceae	12µm	Filament

Graph 1: Effect of *Osillatoria* Decolorization in leather liquid and solid waste.





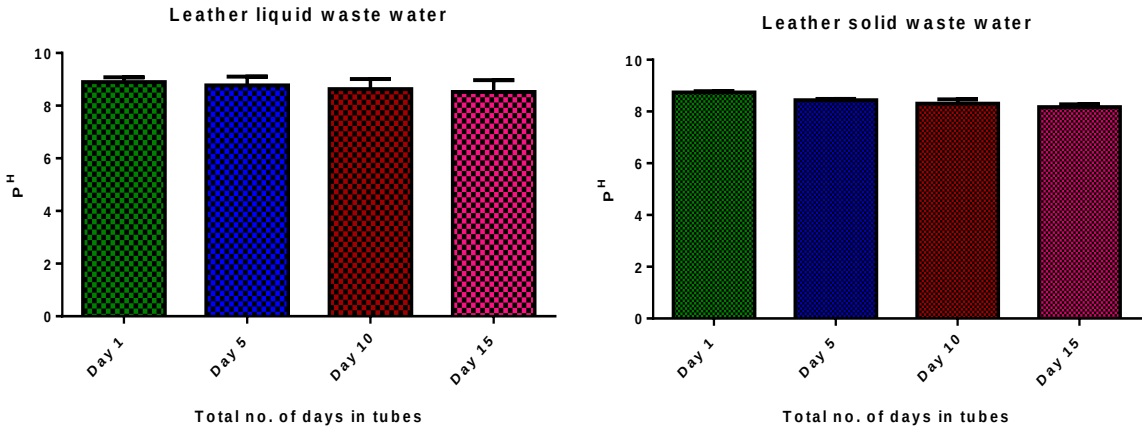
Graph 2: Over all *Osillatoria* Decolorization studies on leather waste.

Table 2: Effect of *Osillatoria sp.*, Decolorization in leather in liquid waste.

S.No	Name of the study	Total No. of working day	Name of the test sample OD Value at 620 nm				Percentage of decolorization (%)			
			Tube 1	Tube 2	Tube 3	Tube 4	Tube 1	Tube 2	Tube 3	Tube 4
1	Leather liquid waste	Day 1	86.36	50	31.1818	0.09	79.06	43.64	25.37	2.71
			76.92	40.16	26.92	7.69				
			73.91	34.78	17.39	0.34				
2		Day 4	76.9	40	28	12	75.5	42.91	26.5	10.16
			75	45.83	25	8.33				
3		Day 8	75	29.16	12.5	0.83	75	35.41	12.5	0.41
			75	41.66	12.5	0				
4		Day 12	58.33	35	16.66	4.16	43.33	27.08	14.58	4.16
			58.33	29.16	12.5	4.16				

Table 3: Effect of *Osillatoria sp.*, Decolorization in leather in solid waste.

S.no	Name of the study	Total No. of working day	Name of the test sample			Percentage of decolorization (%)		
			Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3
1	Leather solid waste	Day 1	86.3636	86.3636	90.9090	71.82	88.70	91.50
			76.9230	88.4615	92.3076			
			52.1739	91.3043	91.3043			
2		Day 4	87	83	62	87.5	77.5	61
			88	72	60			
3		Day 8	83.333	87.5	62.5	85.41	85.41	62.5
			87.5	83.33	62.5			
4		Day 12	83.333	83.333	87.5	85.41	85.41	85.41
			87.5	87.5	83.333			



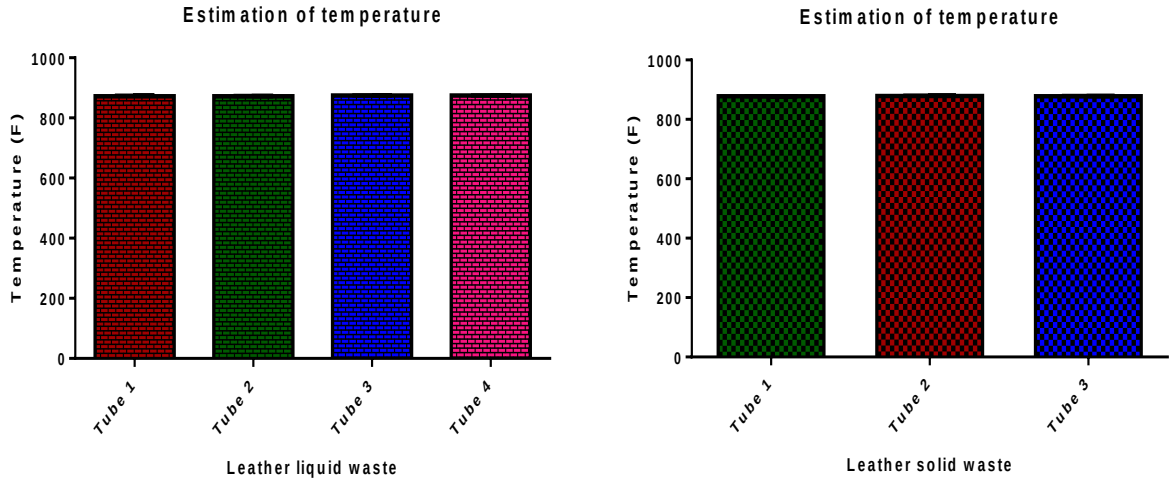
Graph 3: Effect of *Osillatoria sp.*, on pH.

Table 4: Effect of *Osillatoria sp.*, on pH in Leather liquid waste.

S.No	Name of the study	Total no tubes	Name of the test sample			
			Day 1	Day 5	Day 10	Day 15
1	Leather liquid waste	Tube 1	8.7	8.4	8.2	8.1
2	Leather liquid waste	Tube 2	8.8	8.6	8.4	8.2
3	Leather liquid waste	Tube 3	9.0	9.0	8.9	8.8
4	Leather liquid waste	Tube 4	9.1	9.1	9.0	9.0

Table 5: Effect of *Osillatoria sp.*, on pH in Leather solid waste.

S.No	Name of the study	Total no tubes	Name of the test sample			
			Day 1	Day 5	Day 10	Day 15
1.	Leather solid waste	Tube 1	8.8	8.5	8.5	8.3
2.	Leather solid waste	Tube 2	8.7	8.4	8.2	8.1
3.	Leather solid waste	Tube 3	8.7	8.4	8.2	8.1



Graph 5: Effect of *Oscilatoria sp.*, on Temperature.

Table 6: Effect of *Oscilatoria sp.*, on Temperature in leather liquid waste.

Time	Tube 1	Tube 2	Tube 3	Tube 4
1	874 °F	872°F	874°F	874°F
2	872°F	872°F	874°F	874°F
3	871°F	873°F	875°F	875°F

Table 7: Effect of *Oscilatoria sp.*, on Temperature in leather solid waste.

Time	Tube 1	Tube 2	Tube 3
1	878 °F	879°F	877°F
2	878°F	879°F	877°F
3	877°F	878°F	879°F

Graph 6: Effect of *Oscilatoria* on macronutrients level.

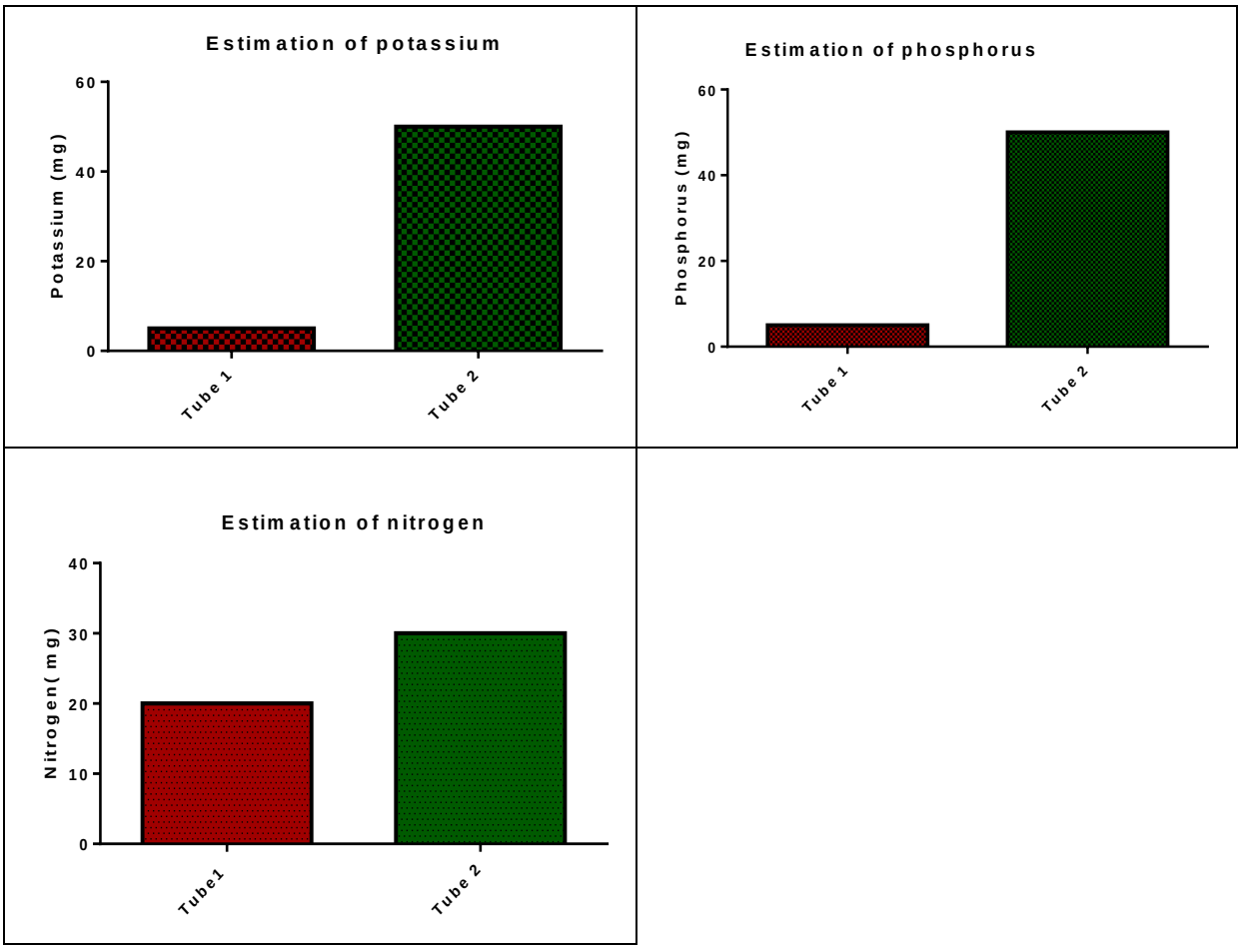


Table 8: Effect of *Oscilatoria* on macronutrients level.

S.NO	Total No. of tubes	Estimation of Macronutrients		
		Potassium	Phosphorus	Nitrogen
1	Tube 1	5 mg	5 mg	20 mg
2	Tube 2	50 mg	50 mg	30 mg

Graph 7: Effect of *Oscilatoria* on micronutrients level.

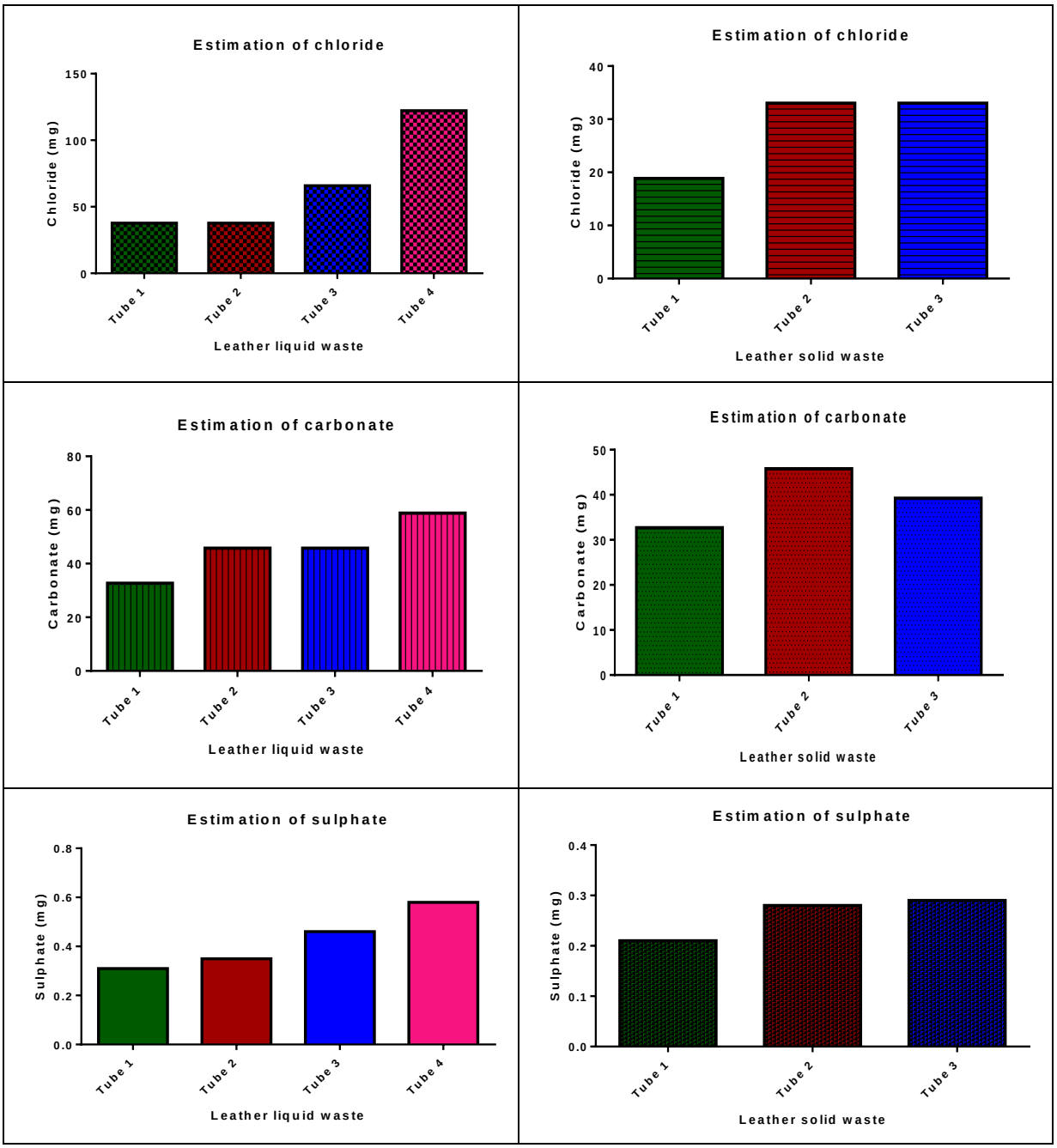


Table 9: Effect of *Oscilatoria* on micronutrients level.

S.NO	Name of the test sample	Total no. of tubes	Estimation of Micronutrients		
			Chloride mg/l	Carbonate mg/l	Sulphate(450nm)
1	Leather liquid waste	Tube 1	37.72 mg/l	32.693 mg/l	0.31
2		Tube 2	37.71 mg/l	45.7702 mg/l	0.35
3		Tube 3	65.8 mg/l	45.7702 mg/ml	0.46
4		Tube 4	122.2 mg/l	58.8474 mg/l	0.58
	Leather solid waste	Tube 1	18.85 mg/l	32.693 mg/ml	0.21
6		Tube 2	33 mg/l	45.7702 mg/l	0.28
7		Tube 3	33 mg/l	39.2316 mg/l	0.29

TLC of *Oscilatoria lutea* extract

Rf = distance moved by the solute / distance moved by a solvent

Here, the distance moved by solute = 1.9

The distance moved by solvent = 5.2

Therefore, Retention factor (Rf value) = 0.3653

CONCLUSION

This is shown through a careful analysis of the traditional leather processes and the underlying principles of each stage. The pre-tanning and tanning procedures account for the majority of the pollution, however post-tanning and finishing activities are equally harmful to the environment. The waste water from the tannery and the sludge produced by leather industries has drastic impact on entire ecology. The necessity for green technology has been highlighted by the detrimental effects of the leather industry. Additionally, the physio-chemical treatment procedures use a lot of chemicals and environmentally friendly. In order to degrade and detoxify the solid wastes and tannery wastewater produced by the leather industries in a way that is safe for the environment, bioremediation technologies may be a good choice which is eco-friendly, cost effective and proposes a promising method to enhance environmental quality. This study shows a number microbes which are reported to show their ability to remove or remediate toxic hazards present in the wastes generated from leather industries. In spite of having so many microbes, successful eco-friendly and cost-effective methods, leather industry wastes and wastewater continue to cause environmental pollution and toxicity issues that are frequently encountered. On that note this study will raise a social massage for the shake of good will of the human population. So that, proper steps can be taken, proper treatment of solid wastes and the tannery waste water which will be eco-friendly and uses of toxic chemicals can be controlled and also draw the sincere attention of the leather industries and the governments about this crucial scenario.

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