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BIOREMEDIATION OF COOUM WATER USING ALGAE

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ABSTRACT

The name cooum appears to be derived from Tamil literature. The name may have been derived from the Tamil term coopam meaning 'well' or 'deep pit'. The word *coovalan* denotes a person who is well versed in the science of ground water, well water and stagnant water. Cooum has been an integral part of the socio-economic and cultural life of the city. During floods, both the watercourses inundate the whole area. Polluted part of the Cooum is presently spoiled by filth and pollution, and the water quality is considered to be highly toxic and completely non-potable. The 2004 tsunami cleaned the mouth of the river; however, the river returned to its usual polluted self within a short period and the unpolluted part is still being used for drinking water needs of many villages in the banks of the cooum. As the Nation's growing urban major problem is the removal of wastes, humans dump these waste directly into rivers. When this contaminated water is directly consumed by Aquatic life and indirectly by humans, it affects their health and leads to a lot of diseases such as Chlorella, Typhoid etc. Thus the availability of good quality water is necessary for preventing such disease and improving the quality of life. Newer technologies are being now proposed to assess the treatment of waste water. Heavy metals such as Lead, cadmium, mercury, nickel, zinc, aluminum, arsenic, copper and iron are considered to be the major environmental pollutant which causes severe poisoning conditions. Microalgae Mass Culture for Wastewater Treatment exist throughout natural water systems in various forms and concentrations, which makes it viable for wastewater treatment. The mass culture of microalgae in wastewater can significantly contribute to the management of water ecosystems by providing an inexpensive, environmentally sound addition or alternative to conventional energy intensive wastewater treatment systems.

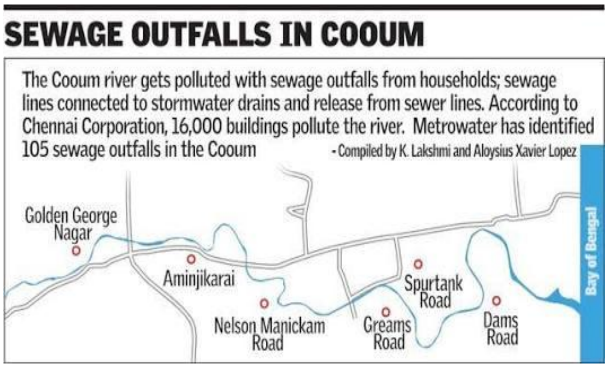
KEYWORDS: Cooum, Coovalan, Contamination, Heavy Metals and Wastewater Treatment Systems.

INTRODUCTION

The ***Cooum River*** is the shortest river draining into the ***Bay of Bengal***. This river is about 74 km in length, flowing 32 km in the urban part and the rural part (**Ayyappan, 2012**). The river is highly polluted due to the waste produced by the city. The river trifurcates the city and separates Northern and Central Chennai (**Figure 1**).

Figure 1. Cooum River.

In Chennai district, the river flows through ***three corporation zones*** - ***Kilpauk, Nungambakkam*** and ***Triplicane***. The total length is 16 km (10 miles) (**Aishwarya, S, 2009*)***. Owing to intensive use of surface water upstream for agriculture, indiscriminate pumping of groundwater leading to reduced base flow in the river, discharge of untreated sewage and industrial effluents and industrial effluents and encroachment along the banks has been highly polluted (**Ahmad F *et. al.*, 2013).** The cooum was earlier known as the ***Triplicane River*** **(Lakshmi, K, 2011).** The 2004 tsunami cleaned the mouth of the river; however, the river returned to its usual polluted self within a short period (**Map 1**) (**Asaolu *et.al.*, 1997**). However the unpolluted Part is still being used for drinking water needs of many villages in the banks of the unpolluted part of the cooum (**Table 1**).



Map1: **Sewage outfalls in Cooum.**

Table 1: Sources of heavy metals from various industries.

Metals	Sources
Chromium	Leather, pulp, petroleum and Electroplating
Cadmium	Paint, plastics, pesticides, copper refineries.
Lead	Fertilizers, vehicle, aircraft plating
Copper	Steel works, paper and pulp
Zinc	Rubber industries, alkalis, detergents
Mercury	Light bulb, leather, ointment, adhesive
Arsenic	Automobile, dyes
Iron	Metal refining

Bioremediation is a pollution control technology that uses biological system to catalyze the degradation or transformation of various chemicals to less harmful forms. Developing biological based treatment system is considered to be economically cheaper and more environment friendly (**Aziz, M. A.** and **Ng, W. J, 1992**). Microalgae culture offers an interesting step for wastewater treatments, because they provide a tertiary bio-treatment coupled with the production of potentially valuable biomass, which can be used for several purposes. Microalgae cultures offer an elegant solution to tertiary and quartinary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth and also, for their capacity to remove heavy metals, as well as some toxic organic compounds and thus does not lead to secondary pollution. Thus Algal bioremediation is being used in waste water treatment as it exhibits high potential in waste water remediation in an economically cheaper way (**Benemann, J. R, 1979).**

Chlorella vulgaris

The microalgae ***Chlorella vulgaris*** is a genus of single-cell algae belonging to the phylum Chlorophyta. It is spherical in shape, about 2- 10µm in diameter and it is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll a and b in its chloroplast. Through photosynthesis, it multiplies rapidly, requiring on CO_2, H_2O, Sunlight and a small amount of minerals to reproduce ***(*Fukami M *et. al.*, 1988**). This green microalgae is known for their high hydrocarbon accumulation in their biomass. The hydrocarbons are very good source of Biofuel, which can be biologically produced (**Figure 2**). *Chlorella* is one of the most extensively used microalgae for waste removal. It is widely distributed around the world. Most species of this genus are spherical and under 10 µm in diameter. *Chlorella* has become a good candidate for biofuel production due to its rapid growth rate and high lipid content (**Li *et. al.*, 2007**). The use of *Chlorella* for wastewater treatment is not a new idea, and many researchers have developed techniques for exploiting the fast-growing isolates and increasing their nutrient removal capacity (**Chen H**. and **Pan Shan Shan, 2005)**. This raises the possibility of the dual-use of microalgae cultivation for wastewater treatment coupled with biofuel production.

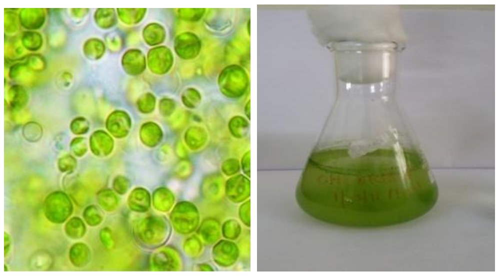


Figure 2: *Chlorella vulgaris.*

Microalgae have been always used for wastewater treatment. The oxygen produced by algae in wastewater is utilized by heterotrophic bacteria for the conversion of wastewater nutrients to biomass. Algal treatment methods are used for the removal of nutrients, pathogens and other type of contaminants (**Griffiths M** and **Harrison S, 2009)**. Other mechanisms for the reduction of pollutants in algal based system can be nutrient competition, toxins produced by algae and adhesion or attachment to the algal cells. Microalgae grown in wastewater can accumulate valuable lipids and fatty acids which can be extracted from the dried biomass of algae and can be used for biodiesel production. The residue obtained from this process can be used as aquaculture feed, animal food supplement or a source of pharmaceuticals (**Hoffmann, J. P, 1998)**.

MATERIALS AND METHODOLOGY

Water samples were collected from 5 different places of Chennai in bottles which was previously washed with 10% HNO_3. It was labeled and few drops of HNO_3 was added to prevent from metal loss.

Physico-chemical Analysis

The physico-chemical parameters such as Colour, pH, Acidity, Alkalinity, Hardness, Total Suspended Solids, Total Dissolved Solids, Total Solids, Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand and Ammonical N_2 of the collected waste water samples were determined before and after treatment by following the Standard Method Examination of water. Waste water as given in "Environment Pollution" of **Ambast (1990)** and **APHA (1998).** The data were statistically analysed by taking the value of standard error.

Collection and isolation of specific algae culture (*Chlorella vulgaris)*

Different types of algae can be used for the bioremediation of waste water. *Chlorella vulgaris* which was previously identified were collected from **Psycospectrum Laboratory**, Anna Nagar, Chennai and were cultured in BBM media (**Table 2**).

BBM MEDIA COMPOSITION

Table 2: **Composition of BBM media.**

S. NO.	CHEMICALS	per 100ml
1	$NaNO_3$	2.5g
2	$MgSO_4.7H_20$	0.75g
3	NaCl	0.25g
4	K_2HPO_4	0.75g
5	KH_2PO_4	1.75g
6	$CaCl_2.H_2O$	0.25g
	TRACE ELEMENTS	**per litre**
7	$ZnSO_4.7H_2O$	8.82g
8	$MnCl_2.4H_2O$	1.44g
9	MoO_3	0.71g
10	$CuSO_4.5H_2O$	1.57g
11	$Co(NO_3).6H_20$	0.49g
12	H_3BO_3	11.42g
13	EDTA	50.0g
14	KOH	31.0g
15	$FeSO_4.7H_2O$	4.98g
16	H_2SO_4 (conc)	1.0ml

Qualitative analysis of algae

The following tests such as Carbohydrates, Saponins, Glycosides, Proteins and Amino acids, Phenols, Lignin and Terpenoids were carried out qualitatively to analyse the Algae.

Assessment of the algal isolates in the bioremediation of the Waste Water samples

30ml of different water samples were inoculated with 30ml algal isolates of particular density in a flask and was kept under UV illumination at 30^0C. For the first 48 hours of incubation the flask was kept in a shaker at 100rpm for the purpose of uniform mixing of algae and effluents. Then periodically monitoring of the samples was done for investigating the physiochemical characteristics and biodegradability of the effluents. On the basis of Physico-chemical analysis and the efficiency of algal isolate it was screened on the basis of the reduction efficiency for the above tests done.

Water Analysis at Scientific Food Testing Laboratory

The water analysis was done at **Scientific Food Testing Laboratory** as per **IS 14543: 2004 and IS 15410: 2003** to check the water quality and to compare the degradation of metals and waste before and after treating with the algae.

Extraction of Oil from Algae

The extraction of oil from algae was done by solvent extraction method. Here Hexane was a solvent that was used to separate the oil from the algae. By this method we can extract nearly 95% of algae's oil content. Hexane can also be mixed with the pulp that remains after expression to extract additional oil. It is the most popular and easy method for oil extraction. On the basis of phytochemical analysis and the reduction efficiency of the above tests done, the most efficient algal isolate was screened.

RESULT AND DISCUSSION

The water samples were collected from five different sites in Chennai and the test was done to check the potential of the algae in the bioremediation of waste water treatment (**Figures 3a - e**).

3a 3b 3c 3d 3e

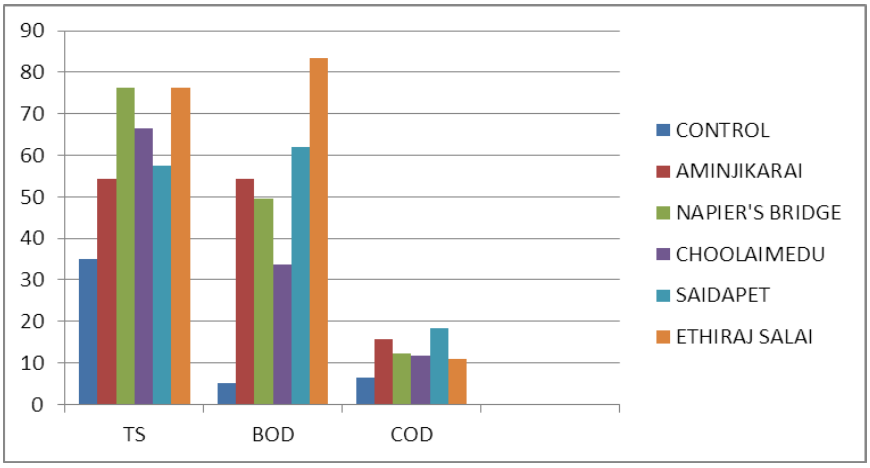
Figure. 3. a) ***Aminjikarai, b) Napier's bridge, c) Choolaimedu, d) Saidapet and, e) Ethiraj bridge.***

Physico - Chemical Analysis of Untreated Water

The waste water was collected from different sites and were subjected for analysis of physicochemical parameters. The waste water (untreated) was tested at the time of collection while the treated water was tested after few days so that treatment can be checked at the particular time. The physico- chemical parameters such as pH, Total Alkalinity, Total Acidity, Hardness, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Solids (TS), Dissolved Oxygen(DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Ammonical N_2 were carried out in the laboratory and the results were tabulated (**Table 3 and Graph1**).

Table 3: **Physico–chemical Analysis of Waste Water.**

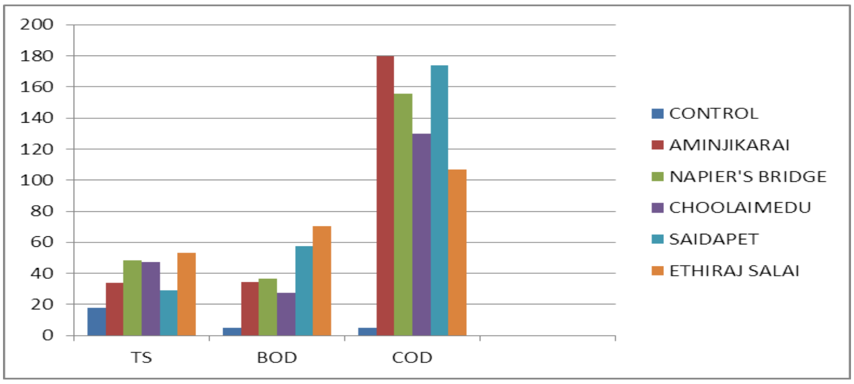
Physical Examination	Control	Aminjikarai	Napier's bridge	Choolaimedu	Saidapet	Ethiraj Salai
Colour	Colourless	Black	Brown	Brown	Black	Brown
Appearance	Clear	Turbid	Turbid	Turbid	Turbid	Turbid
Odour	None	Foul smell	Foul smell	Foul smell	Foul smell	Foul smell
Ph	7.4	7.7	7.8	7.9	8.2	8.8
Acidity	-	-	-	-	-	-
Alkalinity(mg/L)	110	610	429	498	380	540
Hardness(mg/L)	176	288	316	368	348	332
Total suspended Solids(mg/L)	0.13	0.29	0.32	0.39	0.42	0.33
Total Dissolved Solids(mg/L)	35	54	76	66	57	76
Total Solids	35.13	54.29	76.32	66.39	57.42	76.33
Dissolved Oxygen (mg/L)	33.6	57.6	280	83.2	134.4	142.4
Biological Oxygen Demand(mg/L)	5.2	54.4	49.5	33.6	62.1	83.5
Chemical Oxygen Demand(mg/L)	6.4	198.4	183.2	133.6	183.3	122
Ammonical N_2 (mg/L)	0.34	15.6	12.2	11.7	18.3	10.9



Graph 1: **TS, COD, BOD of Untreated Water.**

Physico - Chemical Analysis of Treated Water

Among all the physico-chemical parameters, pH, Total alkalinity, Total acidity, Hardness, Total dissolved solids(TDS, Total suspended Solids(TSS), Total Solids(TS), Dissolved Oxygen(DO), Biological Oxygen Demand(BOD), Chemical Oxygen Demand(COD), Ammonical N_2 were found to be reduced quantitatively when compared to untreated and treated waste water. The reduction percentage were noticed in different samples and in different test **(Graph 2 & Table 4).**



Graph 2: TS, BOD, COD of Treated Water.

Table 4: Physico - Chemical Analysis Treated Water.

Physical Examination	**Control**	**Aminjikarai**	**Napier's bridge**	**Choolaimedu**	**Saidapet**	**Ethiraj Salai**
Colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Appearance	Clear	Clear	Clear	Clear	Clear	Clear
Odour	None	None	None	None	None	None
pH	7.2	7.4	7.3	7.5	7.9	8.5
Acidity	-	-	-	-	-	-
Alkalinity(mg/L)	97	560	359	442	324	486
Hardness(mg/L)	129	288	254	287	319	448
Total suspended Solids(mg/L)	0.06	0.14	0.17	0.23	0.29	0.21
Total Dissolved Solids(mg/L)	18	34	48	47	29	53
Total Solids	18.06	34.04	48.17	47.23	29.29	53.21
Dissolved Oxygen (mg/L)	25.7	42.3	18.4	77.5	104.6	127.6
Biological Oxygen Demand(mg/L)	5.2	34.5	36.7	27.7	57.3	70.5
Chemical Oxygen Demand(mg/L)	4.9	179.5	155.7	129.8	173.8	107
Ammonical N_2 (mg/L)	2.2	12.5	10.02	9.8	12.3	8.8

Collection and cultivation of specific algae culture (*Chlorella vulgaris)*

Current study involves the cultivation of Algae from the master culture. The master culture was taken from the Research Institute Psycospectrum in Anna Nagar West. The cultivation of algae was done in BBM media. One month time is required for the cultivation. The different water samples were inoculated for treatment and were kept in a shaker for 48 hours for continuous shaking of the effluents (**Figure** 4). **The major biochemical constituents including Carbohydrate, Protein, Saponins, Glycosides, Flavanoids, Phenols, Lignin, Terpenoids were estimated (Table 5).**

Figure 4: Cultivation of *Chlorella vulgaris.*

Qualitative Analysis of Algae

Table 5: Phyto-chemical Analysis of Algae.

PHYTOCHEMICAL TEST	***C. vulgaris***
Carbohydrate (***Fehling's Test)***	+
Saponin (***Froth Test)***	+
Glycosides (***Keller-killiani Test)***	+
Protein and Amino acid (***Ninhydrin Test)***	+
Flavanoids (***Lead acetate Test)***	+
Phenols (***Ferric Chloride Test)***	+

Lignin (***Wiesner Test)***	-
Terpenoids (***Libermann-Burchard Test)***	-

Phyto-chemical analysis

The **Lead Acetate Test method** was done for the detection of flavanoids and the test was positive **(Figures 5 – 11).**

Figure 5: Flavanoids test.

The **Ferric Chloride Test** was done for the detection of phenols and the test was positive.

Figure 6: **Phenol test.**

The **Fehling's Test** was done for the detection of Carbohydrate and the test was positive.

Figure 7: **Carbohydrate test.**

The **Keller-Killiani Test** was done to check the presence of Glycosides and the result was found to be positive.

Figure 8: **Glycosides test.**

The **Wiesner *Test*** was done to check the presence of lignin which revealed negative result.

Figure 9: Lignin test.

The **Froth Test** was done to check the presence of saponin. It was present for the algae.

Figure 10: **Saponins test.**

The **Ninhydrin Test** was done to check the presence of amino acids and protein and it was confirmed positive.

Figure 11: **Protein test.**

Assessment of the algal isolates in the bioremediation of the waste water samples and screening the efficiency of Algal isolate

Waste water was inoculated in algal culture to check the potential of the algae in bioremediation of waste water**.** The physico - chemical analysis of water was checked after 15 days of inoculation (**Figure 12**).

Figure 12: Inoculation of waste water samples in algal isolates.

Water Analysis

Untreated (Heavy Metals in Waste Water Sample)

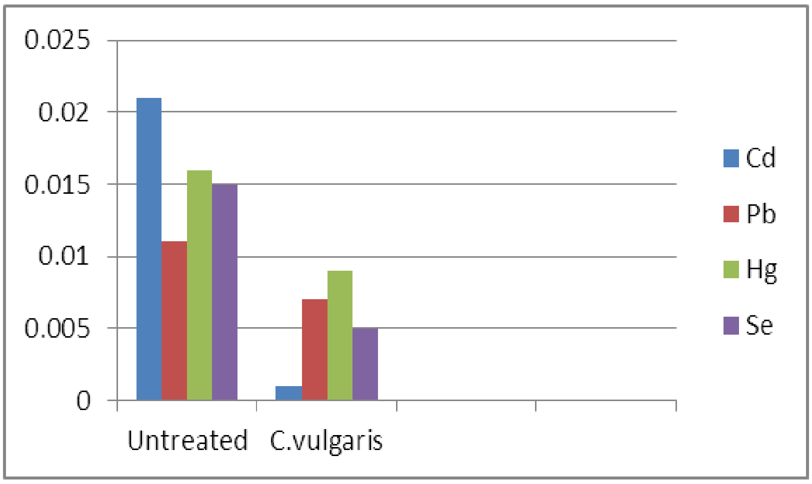
From the overall study, it can be confirmed that the microalgae was beneficial for the bioremediation of waste water treatment. It has shown the reduction in the BOD, COD, TDS, TSS and Alkalinity in the waste water. The biochemicals which accumulated can be utilized for effective biofuel production in large scale in the upcoming future. From this study, it has been proved that *Chlorella vulgaris* is efficient in reducing heavy metals (**Tables 6 - 8**) (**Graph 2**).

Table 6: Heavy metals in Untreated Water Sample.

Element	Blank concentration µg/L	Sample concentration µg/L	Result in mg/L	LOQ In mg/L
Cadmium as Cd	2.588	13.03	0.0104	0.001
Lead as Pb	35.65	19.21	0.0549	0.007
Mercury as Hg	0.0101	0.0369	0.00003	0.0009
Selenium as Se	0.1437	0.0744	-0.0001	0.005

Table 7: Heavy metals degradation after treatment with *Chlorella vulgaris*.

Element	Blank concentration µg/L	Sample concentration µg/L	Result in mg/L	LOQ In mg/L
Cadmium as Cd	2.588	14.35	0.0118	0.021
Lead as Pb	35.65	31.47	0.0042	0.0011
Mercury as Hg	0.0101	0.0268	0.00002	0.0016
Selenium as Se	0.1437	0.1839	0.0000	0.015



Graph 2: Comparison between untreated and treated in terms of heavy metal degradation.

Table 8: **Heavy metal degradation in Waste Water.**

HEAVY METAL DEGRADATION	*C. vulgaris*	
	RESULT	LOQ(mg/L)
Cadmium as Cd	0.0118	0.021
Lead as Pb	0.0042	0.0011
Mercury as Hg	0.00002	0.0016
Selenium as Se	0.0000	0.015

LOQ*=Limit of Quantification.

Extraction of Oil from Algae

The extraction of oil from algae is done by Solvent Extraction Method. Here Hexane is a solvent that is used to separate the oil from the algae. By this method we can extract nearly 95% of algae's oil content. But hexane doesn't stop there; it can also be mixed with the pulp that remains after expression to extract additional oil. Thus this is a more popular and easy method for oil extraction (**Figure 13**).

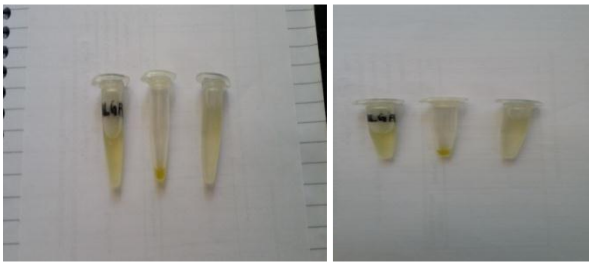


Figure 13: Extraction of oil from Algae.

SUMMARY AND CONCLUSION

Summarizing the work, *Chlorella vulgaris* algae was cultivated from the master culture. The Physico-chemical parameters were done before and after the treatment. The water analysis was done to check the heavy metal degradation and Phytochemical Analysis was also done to check the presence of phytochemicals in algae which was positive in all the tests. The extraction of oil was done by Solvent Extraction Method. Here Hexane was used as a solvent to separate the oil from the algae. Nearly 95% of algae's oil content was extracted by this method.

In conclusion, *Chlorella vulgaris* showed the best bioremediation in almost all the tests which was done in the laboratory and as well as in all the analysis which was done outside of the lab. The oil which was extracted from both the algae was due to the presence of phytochemicals in it and maximum oil has been extracted by Hexane Solvent Method, This can be useful in biofuel production in future by implementing it for Large Scale Production. This production can also be used as feed for aquatic animals. Thus this promising study can be regarded as an initiatory step towards Future Bioremediation in Large Scale.

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