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Research Article

**Institutional Waste Water
Treatment Using Root –
Zone Method**

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Abstract

Water resources receive pathogens and pollutions from a variety of sources like industrialisation, domestication, cleaning of clothes, washing and bathing of animals. The inadequate water treatment process has resulted in low quality potable water ultimately resulting in health problems. *Eichhornia crassipes*- water hyacinth plant plays a vital role in treating pollution water. The collected waste water, *Eichhornia crassipes* plant treated waste water and filtered wastewater were subjected to different parameters like physico- chemical and biological parameters. In this study after the treatment with Root-Zone method, alkalinity, hardness, chlorides, nitrogen, carbon and sulphates had been reduced in the wastewater. The total solids, dissolved solids and suspended solids are also decreased in the samples. But, the coliform bacteria were remaining same level. So it was concluded that the treatment of wastewater through Root-Zone Method is the best treatment for the recycling of wastewater.

Keywords: *Eichhornia crassipes*- water hyacinth plant, Root-Zone method, waste water, physical, chemical, biological, parameters

Introduction

Wastewater treatment plant treats wastewater from domestic, textile, leather, food, steel industries and a number of other sectors including sewage. Biodegradable organics are the most common water pollutants. The method of using aquatic plants can improve water quality must have come about several centuries ago, with the observation that wastewater flowing out of channels infested with vascular plants, such as water hyacinth, seemed to be cleaner than the wastewater entering such

channels. There were occasional publications on the subject earlier, including a pioneering and significant report from India^[1] which was first over published report on that subject and gave a major spurt to R and D on macrophyte based water treatment systems. They brought about the amazing capability of water hyacinth in treating sewage and industrial effluents^[2]. The tertiary treatment is possible with the same aquatic plant sometimes even in a single unit process^[3]. When cultured over waste water, vascular plants perform several functions including assimilating and storing contaminants, transporting oxygen from the atmosphere to the root- zone facilitating aeration of wastewater and providing a substrate for microbial activity, which helps in the stabilization of wastes.

Materials And Methods

Identification and Collection of sample

The waste water samples were identified and collected in plastic vessels from the open drainage of Ladies Hotel in Gandhigram Rural Institute, Dindigul and Kamaraj College, Tuticorin.

Analysis of Water Samples

The physico – chemical and biological characteristics of the waste water samples were examined by following standard methods.

Determination of Temperature

Temperature of the samples were detected using a thermometer. While collecting the water samples the temperature was recorded and tabulated.

Determination of pH

pH was determined by using digital pH meter. It was calibrated with a buffer solution of pH4/pH7. After calibration, the pH value of the water samples were measured and tabulated.

Determination of Electrical Conductivity

The electrical conductivity was found out by using a conductivity bridge with a cell constant of 0.99. It was measured with help of conductivity meter electrode platinum with black or carbon. The electrical conductivity was calculated by using the formula.

Electrical conductivity = observed conductance x Cell constant x Multiplier factor.

Determination of Percentage of Transmission

Percentage of transmission was determined by Systronics Spectrophotometer. The presence of the colloidal and suspended particles in the water samples varies with the optical density. After setting for 100% transmission using distilled water as blank, the transmission of the waste water was determined and tabulated.

Determination of Alkalinity

The alkalinity can easily be determined by titration with phenolphthalein and methyl orange. 100ml of each sample was taken in two conical flasks. 0.5ml of phenolphthalein indicator was added in one flask. The sample was titrated with 0.02N sulphuric acid until the pink colour disappears. The ml of acid used was noted.

0.5ml of methyl orange was added in the second flask and titrated with 0.2N sulphuric acid until the orange colour was arrived indicating the end point. Again the ml of acid used was noted.

Determination of Hardness

Hardness was generally caused by the Ca⁺ and Mg⁺ ions, present in water samples. 50ml of sample was taken in a conical flask. 1ml of buffer solution and 1ml of sodium sulphide was added. Then 100 to 200mg of Erichrome Black T indicator was added, the solution turns wine red. The contents were titrated against EDTA solution. At the end point colour changes from red to blue. The same procedure was repeated in each step in water treatment and tabulated.

The Hardness amount was estimated by using the formula.

Hardness (mg/L) = ml of EDTA x 1000/ml of sample.

Determination of Total Chloride

The total Chloride of water sample was estimated by silver nitrate method. 50ml of water sample was dried in 250ml conical flask. Later 2ml of 5% Potassium chromate was added. The solution became yellow colour. This was then titrated against 0.02N silver nitrate until a persistent red tinge appears. The same procedure was repeated in each step of wastewater treatment and tabulated.

The total amount of chloride was estimated by us-

ing the following formula,

$$\text{Chloride (mg/L)} = \text{ml of AgNO}_3 \times 1000 \times 35.5 / \text{Volume of sample (ml)}.$$

Determination of total Carbon

100ml of water sample was taken in a 500ml dried conical flask. Later 10ml of 1N, Potassium dichromate solution and 20ml of concentrated sulphuric acid was added. Then the mixture was kept aside to react for about 30mins. After the reaction was over the content was diluted with 200ml distilled water and 10ml of orthophosphoric acid, followed by 1ml of diphenyl amine indicator. It was titrated against 0.4N Ferrous ammonium sulphate. The end point was dark green. Blank was also prepared with same quantity of chemical but without the sample.

The total carbon content was evaluated in all the steps of wastewater treatment and tabulated.

$$\text{Percentage of carbon} = 3.951 / \text{volume of sample} \times (1 - T/S)$$

Where, S = ml of Ferrous ammonium sulphate

T = ml of Ferrous ammonium sulphate solution with sample titration.

Determination of Sulphates

Sulphates are generally found in hard waters and polluted waters. 100ml of sample was taken and a few drops of methyl orange indicator was added and slight excess of Nitric acid. The mixture was boiled to remove dissolved carbon - di -oxide. 10ml of standard barium chloride solution was added in the boiling solution. It was allowed to cool and the volume was made up to 150ml of clear supernatant liquid into a beaker, 1ml of buffer solution was added and some amount of Erichrome black T indicator. It was titrated with EDTA solution until a permanent blue colour was produced indicating end point.

Determination of Calcium

One ml of sample was taken in boiling tube and 1ml of sulphuric acid was added with perchloric acid and 3ml of nitric acid digested in a heating mantle at 45°C. After digestion make up to 25ml with distilled water and filtered using whatmann No.42 filter paper. The amount of calcium was measured in flame photometer.

Determination of Potassium

5ml of sample was taken and 25ml of neutral ammonium acetate was added and put it in a mechanical shaker for 5 minutes. The sample was filtered through whatmann No.42 filter paper. The filtered sample was collected and the amount of potassium was measured in a flame photometer.

Determination of Sodium

The sample was filtered through whatmann No.42 filter paper. The flame photometer was checked with the known solution. 1ml of sample was taken and 5ml of distilled water was added and put in to magnetic stirrer for 5 minutes. The amount of sodium was measured in flame photometer.

Determination of total solids

Total solids of the sample was estimated by evaporating 25ml of water sample in previously weighted china dish at 100°C for half an hour in an oven. The china dish was placed in the oven and the final weight was found out after evaporation of the sample. The total solids present in the water samples was calculated by using the formula and tabulated.

$$\text{Total solids (gm/L)} = (A - B) \times 1000 / V$$

Where,

A = Final weight of the china dish in gram

B = Initial weight of the china dish in gram

V = Volume of sample taken in ml

Determination of Total Dissolved Solids

The total dissolved solids which is the residue left after evaporation of filtered sample was determined by evaporating. The filtered sample was weighted in a previously weighted china dish at 100°C for half an hour in oven. After evaporation of the sample the final weight was found out. The heating, cooling and weighing were repeated until constant weight was obtained.

The total dissolved solids present in the sample was calculated by using the following formula and tabulated.

$$\text{TDS (gm/L)} = (A - B) \times 1000 / V$$

A = Final weight of the china dish in gram

B = Initial weight of the china dish in gram

V = Volume of the filtered sample taken in ml

Determination of suspended solids

The amount of suspended solids in the collected wastewater was found out from the difference between the total solids and total dissolved.

$$\text{TSS}(\text{gm/L}) = \text{TS} - \text{TDS}$$

Determination of Biological Oxygen Demand

Biological Oxygen Demand was estimated by Wrinkler's Iodometric method. The sample was filled in a glass stoppered bottle of known volume without bubbling. 1ml of manganese sulphate and alkaline potassium iodide was poured to fix the oxygen. The precipitate appeared was diluted by pouring 1-2ml of sulphuric acid shaken well to dissolve the precipitate. 50ml of the content was taken in a conical flask and titrated against sodium thiosulphate using starch as an indicator. The end point was colourless.

Determination of Chemical Oxygen Demand

Chemical Oxygen Demand is the measure of O₂ consumption during the oxidation of organic matter. 25ml of sample was taken in 250ml round bottom conical flask with ground joint. If the sample expected of COD is more than 50mg/lit 10ml of 0.25N Potassium dichromate solution was added and a pinch of mercuric silver sulphate. 30ml of concentrated sulphuric acid was added refluxed at least for 2hrs on a water bath or hot plate or heating mantle. After 2hrs the flask was removed, cooled and distilled water was added to make the final volume about 140ml. Two or three drops of ferrion indicator were added and mixed thoroughly. It was titrated against ferrous ammonium sulphate. The end point was disappearance of green colour and appearance of brown colour.

Chemical Oxygen Demand calculated using the following formula.

$$\text{COD}(\text{mg/L}) = (\text{B}-\text{A}) \times \text{Normality of FAS} \times 1000 \times 8/\text{ml of sample.}$$

Estimation of total nitrogen

10ml of water samples were taken in a long neck Kjeldal flask. 2ml of concentrated sulphuric acid was added and a pinch of catalyst mixture was heated in the flask for 4hrs in a heating mantle. This was allowed to cool. Each sample was added

with 5ml of distilled water to the distilling chamber. Later 15ml of 40% sodium hydroxide solution was poured into the chamber. Fifteen ml of boric acid indicator mixture was taken in a conical flask and kept at the receiver end. After distillation, the colour of boric acid indicator mixture turned green. This was titrated against 0.02N sulphuric acid. The end point was formation of dark purple colour. The percentage of nitrogen was calculated using the following formula.

$$\text{Percentage of Nitrogen} = \frac{\text{Molecular weight of Nitrogen} \times \text{Normality of H}_2\text{SO}_4 \times (\text{Titrant value} - \text{Blank value}) \times 100}{\text{Volume of sample (ml)}}$$

Enumeration of *Escherichia coli* Using the MPN Technique: It is also known as presumptive coliform test. Biological examination of water sample was done by the multiple tube fermentation technique. Known volumes of water samples were added to lactose fermentation tubes, production of acid and gas from the fermentation of lactose was a positive test for coliform bacteria. Lactose broth (MacConkey's lactose broth contains peptone, lactose, NaCl, Bile salt, Neutral Red and Distilled water) was prepared in single and double strength. In the double strength broth, 10ml of diluted sample was added and in the single strength broth 1ml and 0.1ml of diluted samples were inoculated and this was later kept in incubator at 37±2°C for 48 hours.

The Durham's tubes were put into the broths which were introduced in tubes before incubation. After incubation the tubes were observed for acid and gas production and colour change of broth from purple to yellow. After fermentation tubes showing production of gas was taken and MPN was found by matching the results in the Most Probable Number table.

Total Plate Count: Nutrient agar medium was prepared and poured into all required Petri plates. 1ml of diluted wastewater was added to each Petri plate and rotated with L-rod. The Petri plates were incubated in room temperature for 48hrs and colonies count was taken and tabulated.

Results

Physical, Chemical and Biological Analysis of Wastewater

The wastewater was collected from the Ladies Hos-

tel in Gandhigram. The wastewater was subjected to various analysis such as Temperature, pH, EC, Percentage Transmission, Alkalinity, Hardness, Chloride, Total Nitrogen, Total carbon, Sulphates, Biological Oxygen Demand, Chemical Oxygen Demand, Total solids, Total Dissolved Solids, Total Suspended Solids, Total Plate Count and MPN of coliforms.

Temperature

The temperature fluctuation was minimum between treatments and maximum difference of 2°C was observed between the two stages of treatment. Among the treatment the filtered sample recorded a minimum of 23.2°C and treatment with water hyacinth recorded a maximum of 22.8°C.

pH

The highest pH value of 7.50 was recorded in treatment with water hyacinth and the lowest pH value of 7.25 has been observed in the sample after filtering of the raw wastewater.

Alkalinity

The highest value of 145mg/L of alkalinity has been observed in the unfiltered water sample and lowest value of 120 mg/L of alkalinity has been observed in the water hyacinth treated water sample.

Percentage Transmission

The highest percentage transmission 92% has been observed in treated sample and the lowest percentage transmission 60% has been observed in unfiltered sample has been very much reduced due to the treatment.

Electrical Conductivity

The unfiltered sample recorded on EC of 98 μ s cm⁻¹ and this gradually decreased to 84 μ s cm⁻¹ after the filtration and to 75 μ s cm⁻¹ after water hyacinth treatment.

Hardness

The height value of 560 mg/L of hardness was recorded in the unfiltered sample and the lowest value of 410 mg/L of alkalinity has been observed after the water hyacinth treated wastewater.

Chlorides

The height value of 85.72 mg/L of chloride has been recorded in unfiltered sample and the lowest value

of 34.04mg/L of chloride was observed after water hyacinth treated sample.

Total Nitrogen

The unfiltered sample had the height nitrogen content of 0.06% and the lowest level of 0.04% after the water hyacinth treated sample has been observed.

Total Carbon

The unfiltered sample has the height value of 0.143 after treatment with water hyacinth the carbon content was lowered to 0.125.

Sulphates

The height of 174.58 mg/L of sulphate was recorded in unfiltered sample and the lowest value of 152.84 mg/L of sulphate was recorded after water hyacinth treatment.

Calcium

Both unfiltered sample and the water hyacinth treated wastewater recorded 1mg/L.

Potassium

The unfiltered sample recorded 18mg/L and after water hyacinth treatment the potassium level reduced to 14mg/L.

Sodium

The height value of 40 mg/L was recorded in unfiltered samples and lowest value of 37mg/L in water hyacinth treated sample was observed.

Biological Oxygen Demand

In the unfiltered wastewater sample the Biological Oxygen Demand 625 mg/L which gradually decreased with each treatment and reached a minimum of 50mg/L after the water hyacinth treatment.

Chemical Oxygen Demand

The height value of 246.4 mg/L was recorded in unfiltered samples and the lowest value of 73.6mg/L after the water hyacinth treatment was observed.

Total Solids

The height value of 180mg/L total solids was recorded in unfiltered sample and the lowest value of 140mg/l after the water hyacinth treatment was observed.

Total Dissolved Solids

The unfiltered sample has the highest dissolved solids with a value of 120gm/L and after the water hyacinth treatment the total dissolved solids reduced to 100 gm/L.

Total Suspended Solids

The unfiltered sample has the highest suspended solids with a value of 60gm/L and after the water hyacinth treatment the total suspended solids lowered to 40gm/L.

Total Plate Count

The unfiltered sample has the highest colony count of 92×10^5 bacteria/ml and the water hyacinth treated sample has lowest colony count of 50×10^5 bacteria/ml.

Enumeration Of Coliforms

All the three treatment recorded in MPN of coliforms as more than 2,400/100ml of the sample.

Table -1 Physicochemical parameters of wastewater collected from Ladies Hostel in GRI.

S.No	Parameters	Values
1.	Temperature	25.8°C
2.	pH	7.5
3.	Alkalinity (mg/L)	145
4.	Electrical conductivity (μ s/cm ⁻¹)	98
5.	Percentage of Transmission (OD at 580nm)	60
6.	Hardness (mg/L)	560
7.	Chlorides (mg/L)	85.72
8.	Carbon (%)	0.143
9.	Nitrogen(%)	0.06
10.	Sulphates (mg/L)	174.58
11.	Calcium(mg/L)	1
12.	Potassium (mg/L)	18
13.	Sodium (mg/L)	40
14.	Biological Oxygen Demand	625
15.	Chemical Oxygen Demand	625.4
16.	Total solids (mg/L)	180
17.	Total Dissolved solids (mg/L)	120
18.	Total suspended solids (mg/L)	60

Table -2 Bacteriological Examination of wastewater collected from Ladies Hostel in GRI

S.No	Parameters	Microbial Load
1.	Enumeration of coliforms	> 2,400 coliforms /100ml
2.	Total plate count (Bacteria)	92×10^5 CFU/ml

Table -3 Physicochemical parameter of wastewater of filtration

S.No	Parameters	Values
1.	Temperature	23.2°C
2.	pH	7.3
3.	Alkalinity (mg/L)	125
4.	Electrical conductivity (μ s/cm ⁻¹)	84
5.	Percentage of Transmission (OD at 580nm)	76
6.	Hardness (mg/L)	450
7.	Chlorides (mg/L)	50.04
8.	Carbon (%)	0.138
9.	Nitrogen(%)	0.05
10.	Sulphates (mg/L)	160.84
11.	Calcium(mg/L)	-
12.	Potassium (mg/L)	15
13.	Sodium (mg/L)	38
14.	Biological Oxygen Demand	327
15.	Chemical Oxygen Demand	169.7
16.	Total solids (mg/L)	160
17.	Total Dissolved solids (mg/L)	110
18.	Total suspended solids (mg/L)	50

Table -4 Bacteriological examination of wastewater of filtration

S.No	Parameters	Microbial Load
1.	Enumeration of coliforms	> 2,400 coliforms /100ml
2.	Total plate count (Bacteria)	78×10^5 CFU/ml

Table -5 Physicochemical parameters of wastewater after water hyacinth treatment.

S.No	Parameters	Values
1.	Temperature	22.8°C
2.	pH	7.25
3.	Alkalinity (mg/L)	120
4.	Electrical conductivity (μ s/cm ⁻¹)	75
5.	Percentage of Transmission (OD at 580nm)	92
6.	Hardness (mg/L)	410
7.	Chlorides (mg/L)	34.04
8.	Carbon (%)	0.125
9.	Nitrogen(%)	0.04
10.	Sulphates (mg/L)	152.84
11.	Calcium(mg/L)	1
12.	Potassium (mg/L)	14
13.	Sodium (mg/L)	37
14.	Biological Oxygen Demand	50
15.	Chemical Oxygen Demand	73.6
16.	Total solids (mg/L)	140
17.	Total Dissolved solids (mg/L)	100
18.	Total suspended solids (mg/L)	40

Table -6 Bacteriological Examination of wastewater after water hyacinth treatment.

S.No	Parameters	Microbial Load
1.	Enumeration of coliforms	> 2,400 coliforms /100ml
2.	Total plate count (Bacteria)	50x10 ⁵ CFU/ml

DISCUSSION

According to Winter and Kickuth^[4] the Root-Zone method of wastewater has the ability to remove Nitrogen and Phosphorous, and to reduce the level of Biological Oxygen Demand and sulphur. The untreated wastewater can cause many health hazards to human being as well as to the environment, so that the treatment of the wastewater is

very essential.

After the treatment of wastewater, the chemical and biological load of the wastewater will be reduced and could be used for irrigation. The characteristic features of wastewater has already been reported by Seth et al^[5].

In this study after the treatment with Root-Zone method, alkalinity, hardness, chlorides, nitrogen, carbon and sulphates had been reduced in the wastewater. The total solids, dissolved solids and suspended solids are also decreased in the samples.

Bacteriological examination of wastewater is indispensable to determine the quality of water^[6]. Pollution of inland water bodies is not restricted to industrial countries but is a growing problem throughout the developing world where pollution control is either non-existent or unable to keep with increasing production and consumption.

These results are similar to that reported by Researchers^[7-11] who did similar experiment of wastewater through Root-Zone Method and observed it as best treatment for the recycling of wastewater.

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REFERENCES

1. Sinha SN, Sinha L P. "Studies on use of water hyacinth cultures in oxidation ponds treating digested sugar wastes and effluents of septic tanks", *Environ Health*, 1969; 11: 197-207.
2. McDonald RC, Wolverton B C. Comparative Study of Wastewater Lagoon with and without Water Hyacinth, *Economic Botany*. 34:101-110.
3. Ajithkumar CR, Biju CR, Thomas R. *Plecostomus multiradiatus* an armoured catfish from freshwater ponds near Kunnankulam, Kerala and its possible impact on indigenous fishes. LAK News, Limnological Association of Kerala, 1-2. 1998.
4. Winter M, Kickuth R. Elimination of sulphur compounds from wastewater by the root zone

- process. 1: Performance of a large-scale purification plant at a textile finishing industry. 11: Mode of formation of sulphur deposits. *Water Res.* 1989;23: 535-546 (1) & 547-560 (11).
5. Seth R, Goyal S K, Handa B K. Fixed film biomethanation of distillery spent wash using low cost porous media. *Resources, conservation and recycling.* 1995; 14:79-89.
 6. Patralekh. Bacterial enumeration of perennial pond of Bhagalpur, *journal of ecobiology,* 1991; 3(1):85-88
 7. Bijukumar A, Sushama S. Ichthyofauna of Ponnani estuary, Kerala. *T. Mar. Bioi. Ass. India,* 2000;42 (1 and 2) : 182-189.
 8. Singaram .Removal of chromium from tannery effluent by using water weeds. *Indian journal of environmental health.* 1994; 36(3):197-199.
 9. Chandra P, Sinha SU, Rai N. Bioremediation of chromium from water and soil by vascular aquatic plants. 1997; 274-282.
 10. Pandey JS, Deb SC, Khanna P. Research: Issues Related to Greenhouse Effect, Productivity Modeling, and Nutrient Cycling: A Case Study of Indian Wetlands, *Environmental management.* 1997; 21 (2), 219-224.
 11. Narayanan , Somashekar. Heavy Metal Composition In The Sediment And Plant Of The River Cauvery. *Journal of environment and pollution.* 1997;4(1):727.