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Application of Aquatic Plant *Ceratophyllum demersum* (L.) in Phytoremediation of Wastewater

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ABSTRACT

Background: Phytoremediation, plant usage for remediating natural resources, is being sought as a modern method to clean up polluted lands and streams, including water from ground. Bioremediation assisted by plant, also known as phytoremediation and includes the involvement of microbes associated with certain root systems to remediate soils comprising elevated organic compound concentrations. These strategies could provide cheaper methods for remediating polluted lands and streams with radionuclides, chemicals and different types of organics, with less secondary contaminants and less environmental effect than traditional methods of remediation can produce. All plants consume essential nutrients from their soil and water conditions like metals.

Methods: The domestic wastewater quality was determined by analyzing physico-chemical parameters like pH, TDS, TS and electrical conductivity, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, calcium, potassium, magnesium, sulphate, phosphate, nitrogen, iron and manganese and contaminant removal indices were determined during the year 2018-19.

Result: The present study was conducted to check the phytoremediation capability of aquatic plant *Ceratophyllum demersum* (L.). Wastewater was characterized using standard methods and pollutant concentrations were measured in sample of wastewater initially and after phytoremediation. The result of present study revealed maximum increase in value of DO (127.89%) while the other parameters *viz*. pH (7.24%), TDS (46.77%), TS (51.00%), BOD (60.79%), COD (58.88%), EC (49.89%), Ca (45.41%), K (46.58%), Mg (43.57%), S (45.21%), P (43.73%), N (42.80%), Fe (46.30%) and Mn (47.06%) recorded a decrease in 60 days at 40% value due to absorption of nutrients during phytoremediation.

Key words: *Ceratophyllum demersum* (L.), Phytoremediation, Wastewater.

INTRODUCTION

Phytoremediation, plant usage for remediating natural resources, is being sought as a modern method to clean up polluted lands and streams, including water from ground. All plants consume essential nutrients from their soil and water conditions like metals. Some plants, known as hyperaccumulators, have the capacity to hold vast quantities of metals, including those metals that may not seem to be appropriate for plant activity. Aquatic plants have played a part in the management of wastewater since early 1960 (Wooten and Dodd, 1976). Plants are grouped into emerging, floating leaves, submerged and free (not substratum) depending on their leaf relationship and growth form with water (Sood *et al*., 2012).

Ceratophyllum demersum (L.), widely known as rigid hornwort, BSBI List (2007) coon tail or coontail is a genus of *Ceratophyllum*. This is a submerged marine with a cosmopolitan range, stems of an aquatic plant, *Ceratophyllum demersum* (L.), exceed 1 to 3 m lengths, with several side shoots that render one specimen show as a wide, bushy mass. Leaves with 6 to 12 whorls are 8 mm to 40 mm long, forked or simple, into 2 to 8 thread-like segments; they are brittle and stiff. The fruit is a 4 to 5 mm long small seed, typically with 3 spines, 2 basal spines and 1 apical spine, 1 to 12 *Ceratophyllum demersum* (L.) grows with summer temperatures of water between 15 to 30°C and a rich nutrient condition in reservoirs, ponds and still streams. *Ceratophyllum demersum* (L.) develops in water which is still or very slow-moving. It is usually favored by conditions rich in nitrate where it grows more abundantly (Goulder and Boatman, 1971).

C. demersum is a cosmopolitan underwater marine species that has probably destroyed a significant portion of its fascinating ability as of now. This has the benefits of becoming a constant lasting great winter in deeper water and being both by the asexual reproduction of damaged or completed stems and by the sexual reproduction of other plants. It has strong biological resistance and is growing reasonably quick. The water body alarming effect contributes to improvements in growth by changes in distribution of nutrients, but in addition to faster dispersal across water sources making for more impressive competition of less daunting organisms (Mohan *et al*., 2017).

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MATERIALS AND METHODS

Study area

Kanpur urban city was visited repeatedly during 2016-19 to exact information related to wastewater sites and aquatic plants. After survey for collection of pond water, wastewater and aquatic plants of the urban sites of Kanpur was collected during the months of January to March, 2018 for experimental purpose.

Wastewater sampling and preparation

The selection criteria for each site were based upon anthropogenic activities, presence or absence of aquatic plants and accessibility at the site. Water sampling from ponds was done in January and was taken from 15 to 30 cm below the water surface. The waste water were collected from a point where drainage nala of polluted water mixing with Gangas water near Ganga Barrage, Azad Nagar, Kanpur. Samples were collected in wide mouth large plastic container and cork immediately and all the samples were brought all same day to settle in an open plastic tub in the research lab for one week to allow microorganisms to break down the solid organic water. The waste water then filtered and stored at 4°C temperature in a refrigerator till before treatment analysis was completed. Care was taken to prevent under shaking of the sample and also against the sun light while transporting them to the laboratory.

Aquatic plant sample

Ceratophyllum demersum (L.) plants which serve as tool of phytoremediation were collected from natural sites. In research laboratory of Botany department, they were rinsed with tap water to remove possible contamination were cultured into three different plastic tubs. After a week plants were transferred to three different small plastic containers filled with pond water. Approximately 4 gm of *Ceratophyllum demersum* (L.) were inoculated in each disc. Three replicate were taken for each treatment. Experiment was carried out from February 2018. The treatment level for experiment were:- (i) Control (pond water), (ii) 20 per cent waste water, (iii) 40 per cent waste water, (iv) 60 per cent waste water, (v) 80 per cent waste water , (vi) 100 per cent waste water.

For analysis, samples were drawn and estimations were made for physico-chemical characteristics of waste water before and after treatment of 15, 30 and 60 days growth of *Ceratophyllum demersum* (L.) plants.

Sample analysis

Ecological efficiency of *Ceratophyllum demersum* (L.) in phytoremediation of nutrients from domestic wastewater was studied *in vitro*. The domestic wastewater quality was determined by analyzing physico-chemical parameters. Instrumental analysis was done for physical parameters like pH, TDS, TS and electrical conductivity (Tripathi and Govil, 2001). Chemical parameters *viz.* dissolved oxygen (Winkler,1888), biochemical oxygen demand (Greenberg *et al*., 1980) chemical oxygen demand (Tripathi and Govil, 2001) were analyzed titrimetrically while, calcium (Wallace, 1951), potassium (Wallace, 1951), magnesium (Mason, 1950), sulphate (Chesnin and Yen, 1951), phosphate (Wallace, 1951), Nitrogen (Kjeldahl, 1883), iron (Humphries, 1956) and manganese (Nicholas and Fischer, 1950) contents were determined by spectrophotometric method. contaminant removal indices (Liu *et al*., 2016) were determined by using prescribed formula.

RESULTS AND DISCUSSION

pH

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the pH value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 4.07%, 6.10% and 7.24%. The results indicated that pH value was significantly decreased when treated with *Ceratophyllum demersum* (L.) (Beheary *et al*. 2019) (Table 1).

Total dissolve solids (TDS)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the TDS value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 19.16%, 33.13% and 46.77%. TDS value was observed between 261.40 and 563.80 ppm after phytoremediation with *Ceratophyllum* (Patel and Kanungo, 2010). Saha *et al*. (2015) reported that the phytoremediation technique has the capacity to reduce TDS levels in wastewater. When comparing the reduction percent of TDS among treatments and control, we found that treatment with *Ceratophyllum demersum* (L.) reduced TDS by 33.8% (Beheary *et al*. 2019) (Table 1).

Total solids (TS)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the TS value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 25.13%, 41.05% and 51.00%. Our findings are in support with Beheary *et al*. (2019) that showed reduction in TS from 483.25 to 319.75 (Table 1).

Biochemical oxygen demand (BOD)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the BOD value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 35.22%, 49.19% and 60.79%. The BOD was 10 to 20 mg/l after treatment with *Ceratophyllum demersum* (L). The drop was between 94.6 and 97.5 mg/l. The oxidation phase reduced by 78.3 to 83.8 (Kulasekaran *et al*., 2014). The findings were in accordance with the White and Cousins (2013) indications that floating wetlands would benefit from improved particle sedimentation and promoting the removal of nutrients and metals.

Chemical oxygen demand (COD)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the COD value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 40.36%, 51.55% and 58.88%. The calculated COD was decreased from 664 mg/L to 152.75 mg/l and from 260.5 mg/L to 64.5 mg/l in each year (Foroughi *et al*., 2010). In the design calculations for anticipated disposal of toxins by aquatic floating plants (Zimels *et al*., 2009).

Table 1: Effect on physico-chemical parameters of wastewater by *Ceratophyllum demersum* (L.).

Parameters		Days	Percentage wastewater					
			C	20	40	60	80	100
pH	B.T.		7.30± 0.02	7.59±0.03	7.87±0.04	8.13±0.06	8.42±0.07	8.65±0.04
	A.T.	15	7.21± 0.03	7.31±0.06	7.55±0.07	7.83±0.08	8.16±0.06	8.52±0.04
		30	7.15±0.03	7.27±0.06	7.39±0.06	7.68±0.07	8.08±0.05	8.47±0.03
		60	7.10±0.03	7.18±0.05	7.30±0.06	7.55±0.07	8.01±0.04	8.41±0.03
TDS	B.T.		340.77±0.05	546.91±0.06	748.03±0.07	940.17±0.08	1130.30±0.09	1321.43±0.06
	A.T.	15	317.34±0.07	450.04±0.07	604.74±0.08	765.45±0.08	952.15±0.05	1184.85±0.06
		30	307.77±0.05	403.99±0.06	500.19±0.07	646.41±0.07	848.61±0.05	1142.83±0.04
		60	293.16±0.04	352.08±0.06	398.20±0.06	528.22±0.07	762.24±0.04	1102.26±0.03
Total solid (mg/l)	B.T.		417.25±0.04	839.08±0.06	1255.91±0.06	1646.73±0.07	2069.57±0.09	2476.39±0.06
	A.T.	15	384.15±0.07	651.24±0.08	940.34±0.08	1255.43±0.07	1752.53±0.05	2186.00±0.06
		30	370.45±0.06	555.39±0.07	740.33±0.08	1075.27±0.07	1466.21±0.05	2023.15±0.04
		60	348.84±0.05	488.52±0.06	615.39±0.07	832.18±0.07	1314.95±0.04	1900.43±0.03
B.O.D. (mg/l)	B.T.		6.69±0.02	42.61±0.04	77.54±0.06	110.47±0.08	143.40±0.09	176.32±0.03
	A.T.	15	6.36±0.06	30.11±0.07	50.23±0.08	76.49±0.08	104.18±0.05	158.88±0.04
		30	6.16±0.05	24.39±0.06	39.40±0.07	62.40±0.07	86.75±0.05	152.80±0.04
		60	5.96±0.03	21.25±0.06	30.40±0.07	54.55±0.07	75.70±0.04	145.87±0.03
C.O.D. (mg/l)	B.T.		50.17±0.03	191.25±0.04	312.46±0.06	445.27±0.07	576.80±0.09	704.55±0.06
	A.T.	15	47.20±0.06	132.87±0.07	186.35±0.07	305.23±0.08	412.26±0.08	630.56±0.06
		30	45.08±0.05	111.84±0.06	151.38±0.06	254.17±0.07	362.74±0.06	580.34±0.06
		60	43.24±0.04	92.32±0.05	128.50±0.05	202.43±0.06	325.34±0.06	528.58±0.03
EC (µS cm^{-1})	B.T.		605.07±0.03	1089.01±0.04	1562.93±0.06	2016.87±0.06	2470.79±0.08	2924.73±0.04
	A.T.	15	567.87±0.06	892.19±0.08	1153.24±0.08	1494.28±0.07	1995.90±0.06	2552.60±0.04
		30	547.40±0.05	817.64±0.06	1007.05±0.07	1346.46±0.07	1898.92±0.05	2368.58±0.04
		60	524.52±0.03	735.11±0.06	783.27±0.06	1075.19±0.07	1468.72±0.05	2184.36±0.04
DO (mg/l)	B.T.		5.94±0.03	4.96± 0.06	3.98±0.06	3.05±0.07	2.10±0.08	1.15 ±0.04
	A.T.	15	6.65±0.04	7.67±0.08	6.70±0.07	4.85±0.08	3.16±0.05	1.29±0.06
		30	6.90±0.04	8.85±0.06	7.95±0.07	5.62±0.07	3.53±0.05	1.34±0.04
		60	7.19±0.03	9.53±0.05	9.07±0.06	6.16±0.07	3.84±0.05	1.40±0.03
Calcium (mg/l)	B.T.		44.64±0.02	97.04±0.04	148.44±0.04	197.85±0.06	247.25±0.07	296.65±0.04
	A.T.	15	42.80±0.06	78.94±0.07	112.65±0.08	150.66±0.08	209.52±0.05	283.82±0.04
		30	41.27±0.03	71.14±0.06	93.75±0.07	126.36±0.07	189.45±0.05	273.96±0.03
		60	39.50±0.03	62.43±0.06	81.03±0.06	110.65±0.07	167.88±0.04	261.87±0.03
Potassium (mg/l)	B.T.		25.59±0.02	46.11±0.03	65.63±0.06	83.11±0.06	100.62±0.08	118.12±0.04
	A.T.	15	24.75±0.04	36.53±0.07	49.05±0.07	63.55±0.08	82.16±0.06	113.97±0.06
		30	24.15±0.03	33.17±0.07	42.76±0.07	54.35±0.07	76.12±0.05	111.34±0.04
		60	23.45±0.02	28.80±0.06	35.06±0.06	45.31±0.07	69.55±0.05	108.18±0.03

Table 1: Continue...

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Magnesium (mg/l)	B.T.		18.11±0.02	41.92±0.03	64.74±0.04	85.54±0.06	106.36±0.07	127.17±0.04
	A.T.	15	17.68±0.04	33.02±0.07	47.98±0.08	65.93±0.07	84.89±0.05	124.10±0.06
		30	17.23±0.03	29.33±0.06	41.63±0.07	55.95±0.07	78.25±0.05	120.98±0.04
		60	17.00±0.02	26.23±0.06	36.53±0.06	49.83±0.07	70.25±0.04	119.05±0.03
Sulphur (mg/l)	B.T.		20.86±0.03	34.05±0.04	52.25±0.06	65.43±0.06	78.63±0.08	91.82±0.03
	A.T.	15	20.16±0.04	28.53±0.07	39.53±0.08	50.53±0.07	66.15±0.06	88.52±0.06
		30	19.65±0.03	25.25±0.06	35.35±0.07	45.25±0.07	62.45±0.06	86.35±0.04
		60	19.05±0.02	22.64±0.06	28.63±0.06	37.61±0.07	56.36±0.05	83.81±0.04
Phosphorus (mg/l)	B.T.		0.74±0.03	1.92±0.04	3.11±0.06	4.29±0.07	5.45±0.09	6.61±0.04
	A.T.	15	0.71±0.04	1.52±0.06	2.31±0.06	3.22±0.07	4.84±0.06	6.33±0.06
		30	0.69±0.03	1.42±0.06	2.07±0.06	2.96±0.07	4.44±0.06	6.15±0.04
		60	0.68±0.02	1.34±0.05	1.75±0.06	2.52±0.07	4.08±0.05	6.05±0.03
Iron (mg/l)	B.T.		0.27±0.02	0.68±0.03	1.08±0.04	1.47±0.06	1.85±0.07	2.16±0.04
	A.T.	15	0.25±0.02	0.54±0.04	0.82±0.06	1.16±0.07	1.55±0.07	1.97±0.06
		30	0.23±0.02	0.47±0.04	0.71±0.05	1.00±0.06	1.42±0.06	1.82±0.04
		60	0.21±0.02	0.40±0.03	0.58±0.05	0.82±0.06	1.27±0.05	1.62±0.03
Manganese (mg/l)	B.T.		0.016±0.002	0.051±0.003	0.085±0.006	0.114±0.007	0.148±0.008	0.180±0.003
	A.T.	15	0.013±0.002	0.039±0.003	0.063±0.007	0.086±0.007	0.118±0.005	0.145±0.004
		30	0.012±0.002	0.036±0.003	0.054±0.007	0.080±0.006	0.109±0.005	0.134±0.004
		60	0.011±0.002	0.030±0.002	0.045±0.006	0.063±0.006	0.094±0.005	0.123±0.003

B.T.: Before treatment,; A.T.: After treatment; TDS: Total dissolve solids; TS: Total solid; BOD: Biochemical oxygen demand; COD: Chemical oxygen demand; EC: Electrical conductivity; DO: Dissolve oxygen.

have demonstrated that they can minimise the COD and increase dissolved oxygen. In another study, *Ceratophyllum demersum* (L.) treated wastewater contained between 30 and 55 mg/l and the percentage drop was between 93.3 and 96.8 (Kulasekaran *et al*., 2014).

Electrical conductivity (EC)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the EC value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 26.21%, 35.57% and 49.89%. Due to the absorption by *Ceratophyllum demersum* (L.) of dissolved solids during the phytoremediation, the EC value was lowers following culture (Patel and Kanungo, 2010). Our results are also in agreement with that of Foroughi *et al*. (2014), who found that the EC values in diluted fresh latex wastewater was reduced by the use of Cd from 0.75 to 0.65 ds/l.

Dissolve oxygen (DO)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the DO value increased maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 68.34%, 99.75% and 127.89%. After the phytoremediation with *Ceratophyllum demersum* (L.), DO value had an opposite pattern to other parameters, varies 5.3-8.3 mg/L. The dissolved oxygen content was improved after phytoremediation (Patel and Kanungo, 2010).

Calcium (Ca)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the Ca value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 24.11%, 36.84% and 45.41%. Also in the seven-days culture of aquatic plants Das, 2005 reported reductions in sewage calcium. Percentage reduction in value after the phytoremediation varies from 14.74 to 26.74 for calcium (Patel and Kanungo, 2010).

Potassium

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the potassium value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 25.26%, 34.85% and 46.58%. Analysis of K after 18 days indicates a statistically significant decrease in K (Foroughi *et al*., 2014).

Magnesium (Mg)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the Mg value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 25.89%, 35.70% and 43.57%. The magnesium content in sewage was also reduced after the seven days cultivation of aquatic plants (Das, 2005). Another research showed that after phytoremediation, the percentage decrease in value of magnesium was 11.77–53.65 per cent (Patel and Kanungo, 2010).

Sulphur (S)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the sulphur value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 24.35%, 32.35% and 45.21%.

Phosphorus (P)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the P value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 25.72%, 33.44% and 43.73%. Reddy *et al*., 1990 studied the deposition by aquatic plants of different nutrients according to the relative aquatic population. Similar results were also obtained by Patel and Kanungo (2010). In minimising P levels of enriched nutrient waters, submerged macrophytes may be used (Gao *et al*. 2009). *C. demersum* (L.) formation in shallow lakes with a heavy phosphorous load has been demonstrated (Mjelde and Faafeng, 1997).

Nitrogen (N)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the N value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 24.10%, 33.83% and 42.80%. *Ceratophyllum demersum* (L.) treatment was the best in removing ammonia content with 3.44% (Beheary *et al*., 2019).

Iron (Fe)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the Fe value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 24.07%, 34.26% and 46.30%. Based on results the most adsorption happened after six days (Foroughi *et al*., 2011). The nutrients taken from the water include unradicated submerged vegetation, including *Ceratophyllum demersum* (L.) (Mjelde and Faafeng, 1997).

Manganese (Mn)

After treatment with aquatic plant *Ceratophyllum demersum* (L.) the Mn value reduced maximum in 15, 30 and 60 days is at 40% of wastewater *i.e.* 25.88%, 36.47% and 47.06%. The quantity of Mn taken from *C. demersum* was 94.21% of RMW and 90.82% of TMW (Foroughi *et al*., 2011). It is long established that all live and dead aquatic plants are heavy metal accumulators, which has led to a high degree of involvement from use of aquatic plants to extract heavy metals from wastewater (Lacher and Smith, 2002).

CONCLUSION

Phytoremediation is still in a phase of development and research and requires immense efforts to overcome technical barriers. *Ceratophyllum demersum* (L.) was found to be efficient in reducing the concentration of pH, TDS, TS, BOD, COD, EC, Ca, K, Mg, S, P, N, Fe and Mn within 60 days of treatment. This study concludes that *Ceratophyllum demersum* (L.) has proved to be promising tool for wastewater treatment more attention is required to make it publically applicable and accepted.

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