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Phytoremediation of industrial wastewater by culturing aquatic macrophytes, *Trapa natans* L. and *Salvinia cucullata* Roxb.

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Abstract

Treatment of industrial wastewater was conducted using *Trapa natans* L. and *Salvinia cucullata* Roxb. to determine phytoremediation ability through the evaluation of removal efficiencies of pollutants and nutrients. The experiment was designed in batch cultures for a period of 45 days and consisted of 3 sets of half-spherical earthen containers (0.5 meter diameter) with depth of 0.3 meter each and capacity of 40 liter wastewater. One set contained undiluted wastewater as control culture consisting of without macrophyte and other two sets of containers were employed for treated cultures with *T. natans* and *S. cucullata*. The removal efficiencies of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), nitrate–N, ammonium–N and total phosphorus (TP) in cultures of *T. natans* and *S. cucullata* were 55.00%, 33.32%, 50.00%, 31.25% and 77.27%; and 43.02%, 31.04%, 20.00%, 5.26% and 81.25%, respectively. Phytoremediation ability of test species was assessed comparing the results of control culture with the cultures of treatment and the findings indicate significant reduction in BOD_5, COD, nitrate–N and ammonium–N after growth of macrophytes for 45 days. Dissolved oxygen (DO) increased significantly in both plant cultures comparing with control culture. The pH in culture of *T. natans* increased from 7.21 to 8.17. BOD_5, nitrate–N, ammonium–N and total nitrogen (TN) were reduced significantly in culture of *T. natans* comparing with culture of *S. cucullata*. Implication of the finding indicates that *T. natans* and *S. cucullata* can be utilized as efficient phytoremediators in treatment of industrial wastewater.

Key words: Industrial wastewater, phytoremediation, removal efficiency, *Trapa natans*, *Salvinia cucullata*.

INTRODUCTION

Treatment of industrial wastewater is essential before disposal to prevent any significant undesirable or harmful effect (Pelczar *et al*., 1993; Mishra *et al.*, 2012). Disposal of inadequately treated industrial wastewater leads dissemination of pathogenic microorganisms, increased danger of using natural water bodies for drinking supplies and swimming, contamination of aquatic organisms making them unsafe for human consumption, large losses of waterfowl population, diminished value of water for recreational purposes, depletion of oxygen supply of water by unstable organic and inorganic matter, killing of aquatic life, accumulation of toxic chemicals and creation of miscellaneous objectionable condition such as offensive odors (Loan *et al.*, 2014; Shah *et al.*, 2014).

Available technologies for wastewater treatment can help in re-establishing and preserving physical, chemical and biological integrity of water. These technologies can be classified in two basic groups (Nevena & Jovanovic, 2005) – (i) conventional methods for

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purification of wastewater (treatment is carried out by physical, chemical and biological processes) and (ii) alternative methods for purification of wastewater (treatment is carried out by self-purification process in natural wetlands). The conventional wastewater treatment facilities fail in satisfying all demands of ecologically aware societies (Abbasi, 1999). Phytoremediation is ecofriendly technology for treating wastewater. Floating aquatic macrophyte based treatment systems have potential for removing pollutants and nutrients from wastewater. The high productivity and nutrient removal capability of floating aquatic plants have created substantial interest in their use for wastewater treatment and re-source recovery (Reeta & Ann, 2004; Mishra *et al*., 2012; Nabi *et al*., 2016).

Biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) could be greatly reduced and that the resulting water is suitable for use in irrigation and aquaculture (Rose, 1999; Gamage & Yapa, 2001). Floating macrophytes are well-suited to treat biodegradable wastes with BOD_5 of the order of 200 mg/L. Aquatic macrophytes have ability of transporting atmospheric oxygen to wastewater through their leaves, stems and roots (Reddy & DeBusk, 1984; Sagova *et al.*, 1993). A portion of oxygen transported is consumed during root respiration, the rest enters the water column and is utilized by aerobic bacteria for oxidation. This ability of some plants to pump in oxygen can be strong enough to enable oxidation of even strongly reduced sediments in eutrophic water bodies (Flessa, 1994). Total Nitrogen (N) and phosphorus (P) can be removed from wastewater by aquatic macrophytes (Dolan *et al*., 1981; Russel *et al*., 1994; Weisner *et al*., 1994).

In Bangladesh, a few experiments have focused on the treatment of domestic wastewater (Nabi *et al.*, 2016) and a few sporadic studies on treatment of industrial wastewater (Momtaz *et al.*, 2010; Momtaz *et al.*, 2013; Jahan *et al.*, 2014) with aquatic macrophytes have been performed. With these backgrounds, the experiment was conducted using batch cultures of two aquatic macrophytes - *Trapa natans* L. (Synonym: *Trapa bispinosa* Roxb.) and *Salvinia cucullata* Roxb. in industrial wastewater for assessing phytoremediation ability with the evaluation of removal efficiency of pollutants and nutrients. Therefore, chemical parameters and nutrients of wastewater before and after treatment were determined and removal efficiency was evaluated in this study.

MATERIALS AND METHODS

Collection of industrial wastewater: Industrial wastewater was collected from the point sources of discharges of two textile industries namely Doel Complex and H. R. Textile at Savar, Dhaka. Wastewater was colored and obnoxious unpleasant odor was found during collection.

Selection of aquatic macrophytes as test species: To carry out aquatic macrophyte based treatment system (AMS) for industrial wastewater, two locally available floating macrophytes were selected as test species – *Trapa natans* L. (Family – Trapaceae) and *Salvinia cucullata* Roxb. (Family – Salviniaceae). Test species were collected from a lake of Jahangirnagar University, which had no connection with industrial and domestic wastewater. Taxonomic identification of two test species *T. natans* L. and *S. cucullata* Roxb. was confirmed following Khan & Halim (1987) and compared with herbarium specimen preserved in Bangladesh National Herbarium.

Batch cultures: The cultures were performed for a period of 45 days. The experiment consisted of 3 sets of half-spherical earthen containers (0.5 m diameter) with a depth of 0.3 m each and a capacity of 40 L wastewater. A set contained undiluted wastewater as control culture (C) without macrophyte. Other set of containers were employed for treated culture of *T. natans* (T) and the remaining set was employed for treated culture of *S. cucullata* (S). Both control and treated cultures of each comprised of three replications. Losses in culture volume due to evapotranspiration were countered by addition of deionized water to the original level. Water sampling was performed on the second day following the volume adjustment with addition of deionized water. The earthen containers were initially stocked with plants such that half of the surface area was covered.

Sampling of wastewater: The treatments were assessed by measuring chemical parameters in grab samples which were collected by dipping a 100 ml graduated cylinder at three places across the surface of earthen container and mixed sample was collected from each culture (Nabi *et al.*, 2016). Wastewater in each container was sampled 2 times over 45 days of duration: one on the day of starting of the experiment and another on the 45th day. From this composite sample 0.5 liter sample was acidified with concentrated HCl to maintain the pH below 2 and used for the determination of nutrient parameters (APHA, 1998). However, fresh samples (unacidified) were used to determine the pH. BOD bottles were used to collect water sample for determining BOD_5.

Determination of chemical characteristics of wastewater: Dissolved oxygen (DO) content of sample water was determined by using DO meter (Hanna Instruments: Hi9143. DO Meter, Portugal). The sample bottle (250 ml) was completely filled up with water to avoid the existence of air and DO was measured instantly after the collection of water. Biochemical oxygen demand (BOD_5) was evaluated by measuring oxygen concentration in sample before and after incubation in BOD incubator at 22˚C for 5 days. Chemical oxygen demand (COD) reflux unit consisting of flat bottom flask with ground glass mouth was used for determining COD. BOD_5 and COD of water were measured using Winkler method (De, 1996). The pH of water was determined by using a glass electrode pH meter (Griffin pH meter, Model No. 40). Nitrate–N, ammonium–N and total nitrogen (TN) in wastewater were determined using Micro-kjeldhal distillation method as described by Jackson (1973). Total phosphorus (TP) content in wastewater was determined by ascorbic acid blue colour method (Murphy & Riley, 1962).

Data Analysis:

Evaluation of pollutant/nutrient removal efficiency: We determined pollutant/nutrient load in wastewater before and after treatment and evaluated removal efficiency. The percentage removal efficiencies (PRE) of pollutant/nutrient in wastewater after 45 days of treatment were evaluated by the following equation,

$PRE(\%) = \frac{A-B}{A} \times 100$, where A = concentration at starting and B = concentration at the end.

Statistical Analysis: Data from the experiment was analyzed using the Statistical Package for Social Science (SPSS) for Windows. The values were expressed as Mean±SEM in which triplicates data was used (n=3). Phytoremediation ability of test species was assessed comparing the results of control culture with the cultures of treatment. Unpaired *t* test was conducted as the test of significance to evaluate variation in removal efficiency between control and plant cultures. The variation in removal efficiency between test plant species was also determined by unpaired *t* test.

RESULTS AND DISCUSSION

Dissolved oxygen (DO) in untreated wastewater was 1.99 mg/L and after treatment the amount of DO was 1.90 mg/L in control culture (C) (Fig. 1a). The initial amount of DO in cultures of *T. natans* (T) and *S. cucullata* (S) were 1.55 mg/L and 1.78 mg/L, respectively whereas after treatment DO were 3.28 mg/L and 3.33 mg/L in the cultures of *T. natans* and *S. cucullata*, respectively. In control culture biochemical oxygen demand (BOD_5) were 188 mg/L and 164 mg/L before and after treatment, respectively (Fig. 1b). In the cultures of *T. natans* and *S. cucullata* BOD_5 were 160 mg/L and 172 mg/L before treatment whereas after treatment BOD_5 were 72 mg/L and and 68 mg/L, respectively. Before treatment chemical oxygen demand (COD) in cultures of control, *T. natans* and *S. cucullata* were 71.60 mg/L, 71.60 mg/L and 122.75 mg/L, respectively (Fig. 1c). After treatment corresponding COD levels of those cultures were 61.38 mg/L, 51.15 mg/L and 93.67 mg/L, respectively. The pH levels of untreated and treated wastewater in control culture (C) were 8.25 and 8.06, respectively (Fig. 1d). The initial and final pH of untreated and treated cultures of *T. natans* and *S. cucullata* were 7.21 and 7.94; and 8.17 and 7.93, respectively.

Nitrate–N in untreated cultures of control, *T. natans* and *S. cucullata* were 1.96 mg/L, 1.68 mg/L and 1.40 mg/L, respectively (Fig. 1e). After treatment, the observed corresponding amounts of nitrate–N were 1.68 mg/L, 0.84 mg/L and 1.12 mg/L, respectively. Ammonium-N in untreated and treated cultures of control, *T. natans* and *S. cucullata* were 8.40 mg/L, 8.96 mg/L and 5.32 mg/L; and 8.12, 6.16 and 5.04 mg/L, respectively (Fig. 1f). The initial values of TN in cultures of control, *T. natans* and *S. cucullata* were 0.0224%, 0.0252% and 0.0224%, respectively (Fig. 1g). The amounts of TN after treatment in the corresponding cultures were 0.0154%, 0.0175% and 0.01785%, respectively. The initial levels of total phosphorus (TP) in untreated cultures of control, *T. natans* and *S. cucullata* were 240 mg/L, 220 mg/L and 160 mg/L, respectively (Fig. 1h). After treatment, TP were 62.5 mg/L, 50 mg/L and 30 mg/L in cultures of control, *T. natans* and *S. cucullata,* respectively.

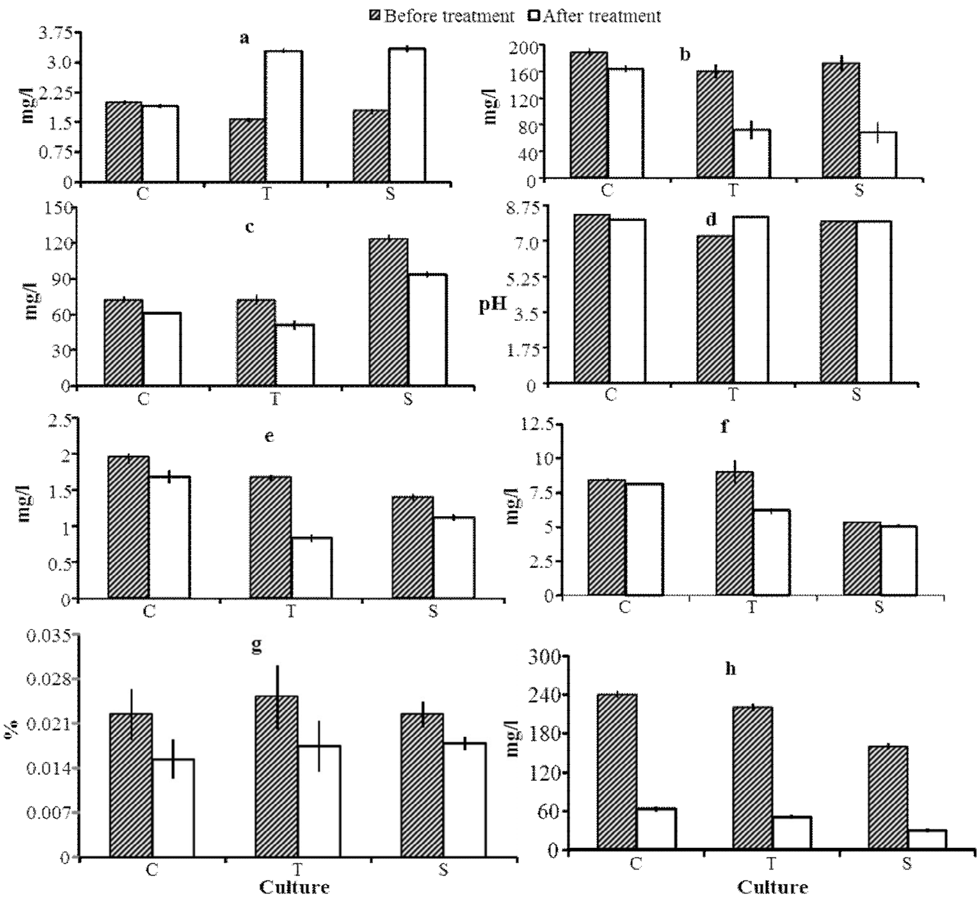

Fig. 1. Chemical characteristics (Mean±SEM) of wastewater before and after treatment in the cultures of control (C) and treatment with *T. natans* (T) and *S. cucullata* (S); a = dissolved oxygen (DO), b = biochemical oxygen demand (BOD_5), c = chemical oxygen demand (COD), d = pH, e = nitrate–N, f = ammonium–N, g = total nitrogen (TN), h = total phosphorus (TP). Vertical bar indicates standard error (SEM)

Removal efficiencies of pollutants and nutrients: Dissolved oxygen (DO) was decreased (4.52%) after treatment in control culture. DO was increased in both the cultures of *T. natans* (111.61%) and *S. cucullata* (100%) after treatment. The amount of DO varied in plant cultures due to their different rate of oxygen exchange from aerial tissue into the root zone (Reeta & Ann, 2004). Removal efficiency for biochemical oxygen demand (BOD_5) was 55.0% and 43.02% in cultures of *T. natans* and *S. cucullata*, respectively. BOD_5 removal efficiency was recovered in control culture (12.76%). Chemical oxygen demand (COD) removal efficiency in control culture was 16.65%. COD removal efficiency in cultures of *T. natans* and *S. cucullata* were 33.32% and 31.04%, respectively. In control culture the value of pH decreased slightly (2.3%). The value of pH in culture of *T. natans* increased from 7.21 to 8.17. The reduction of wastewater pH could be attributed to adsorption or absorption of pollutants by the test plants (Mahmood *et al*., 2005; Dipu *et al*., 2011).

Nitrate–N was removed 14.28% by the control culture. In plant cultures the nitrate–N removal efficiency of *T. natans* was 50% and *S. cucullata* was 20%. The removal efficiency of ammonium–N by the cultures of control, *T. natans* and *S. cucullata* were 3.33%, 31.25% and 5.26%, respectively. Total nitrogen (TN) removal efficiency in cultures of *T. natans* and *S. cucullata* were not noticeable comparing to control culture. Nitrogen is removed from AMS by a number of mechanisms - (a) uptake by plants, (b) volatilisation of ammonia, and (c) bacterial nitrification/denitrification. Among them, bacterial nitrification/denitrification have the most dominant influence on nitrogen removal (Russel *et al*., 1994; Weisner *et al*., 1994). In control culture, total phosphorus (TP) removal efficiency was 73.96%. This may be due to higher algal growth. TP removal efficiency in cultures of *T. natans* and *S. cucullata* were 77.27% and 81.25%, respectively. In AMS the removal of phosphorus can be performed in wastewater by plant-uptake, microbial assimilation, precipitation with cations such as calcium, magnesium, iron, manganese and adsorption by clay and organic matter (Dolan *et al*., 1981).

Comparison of removal efficiencies between the control culture and plant cultures of *T. natans* and *S. cucullata*: Dissolved oxygen (DO) increased significantly in both cultures of *T. natans* (*p*=0.000***) and *S. cucullata* (*p*=0.000***) comparing with control culture (Table 1). The percentage removal efficiency of BOD_5 and COD was highly significant in both cultures of *T. natans* (*p*=0.000***, *p*=0.001***) and *S. cucullata* (*p*=0.000***, *p*=0.0001***). Water pH increased significantly in the culture of *T. natans* (*p*=0.009**) and decreased in *S. cucullata* (*p*=0.000***) comparing with control culture. Nitrate–N and ammonium–N were reduced significantly by the cultures of *T. natans* (*p*=0.000*** and *p*=0.000**) and *S. cucullata* (*p*=0.003** and *p*=0.048*) comparing with control culture (Table 1). No significant variation was recorded in total nitrogen (TN) and total phosphorus (TP) content in the cultures of *T. natans* and *S. cucullata* comparing with control culture.

Comparison of removal efficiencies between the cultures of *T. natans* and *S. cucullata*: Dissolved oxygen (DO) decreased significantly (*p* = 0.001***) in the culture of *S. cucullata* comparing with the culture of *T. natans* (Table 2). The removal efficiency of BOD_5 in the culture of *S. cucullata* was significantly lower (*p*=0.018*) than that of *T. natans*. No significant variation was recorded in removal efficiency of COD. In culture of *T. natans* pH increased significantly (*p*=0.005**) whereas total nitrogen was reduced significantly (*p*=0.035*) in culture of *S. cucullata*. The removal efficiency of nitrate–N and ammonium–N in culture of *T. natans* was high significantly (*p*=0.000***) comparing with the culture of *S. cucullata*. Total nitrogen (TN) removal was significant (*p*=0.035*) in culture of *T. natans* comparing with the culture of *S. cucullata* (Table 2).

Table 1. Comparison of percentage removal efficiencies (PRE) between control culture with cultures of *T. natans* and *S. cucullata* (Mean±SEM, t/p)

Culture	DO	BOD_5	COD	pH	Nitrate–N	Ammonium–N	TN	TP
Control	4.520±1.002	12.760±0.116	16.650±2.009	2.300±0.173	14.280±5.000	3.330±0.000	31.250±0.000	73.960±1.002
T. natans	111.540±0.180	55.000±2.887	33.320±1.790	13.310±2.344	50.000±1.732	31.250±1.559	30.550±1.328	77.270±3.880
t/p	-594.862/**0.000*****	-14.621/**0.000*****	-9.313/0.**001*****	-4.684/0.**009****	-20.623/0.**000*****	-17.911/0.**000*****	0.527/0.626	-0.853/0.442
S. cucullata	100.000±1.155	43.023±1.169	31.040±1.467	0.013±1.002	20.000±0.866	5.260±0.687	20.310±2.973	81.250±3.031
t/p	-82.684/**0.000*****	-25.760/**0.000*****	-9.811/**0.001*****	12.501/**0.000*****	-6.605/**0.003****	-2.809/**0.048****	3.679/0.021	-2.405/0.0.074

Boldface indicates significance at * p<0.05 level, ** p<0.01 level and *** p<0.001 level; DO =Dissolved oxygen, BOD_5 = Biochemical oxygen demand, COD = Chemical oxygen demand, TN = Total nitrogen, TP = Total phosphorus

Table 2. Comparison of percentage removal efficiencies (PRE) between culture of *T. natans* and *S. cucullata* (Mean±SEM, t/p)

Culture	DO	BOD_5	COD	pH	Nitrate–N	Ammonium–N	TN	TP
T. natans	111.540±0.180	55.000±2.887	33.320±1.790	13.310±2.344	50.000±1.732	31.250±1.559	30.550±1.328	77.270±3.880
S. cucullata	100.000±1.155	43.023±1.169	31.040±1.467	0.013±1.002	20.000±0.866	5.260±0.687	20.310±2.973	81.250±3.031
t/p	9.875/**0.001*****	3.845/0.**018***	0.985/0.380	5.623/**0.005****	15.492/**0.000*****	15.257/**0.000*****	3.145/**0.035***	-1.74137931

Boldface indicates significance at * p<0.05 level, ** p<0.01 level and *** p<0.001 level; DO = Dissolved oxygen, BOD_5 = Biochemical oxygen demand, COD = Chemical oxygen demand, TN = Total nitrogen, TP = Total phosphorus

Treatment of industrial wastewater with aquatic macrophytes *T. natans* and *S. cucullata* can be a promising technology for removing pollutants and nutrients from industrial wastewater. The findings indicate the significant reduction in BOD_5, COD, nitrate–N and ammonium–N after growth of macrophytes for 45 days. Dissolved oxygen (DO) increased significantly in both plant cultures comparing with control culture. The pH in culture of *T. natans* increased from 7.21 to 8.17. BOD_5, nitrate–N, ammonium–N and total nitrogen (TN) were reduced significantly in culture of *T. natans* comparing with culture of *S. cucullata*. Implication of the finding indicates that *T. natans* and *S. cucullata* can be utilized as efficient phytoremediators in treatment of industrial wastewater. Further research should be conducted to determine phytoremediation ability of heavy metal in industrial wastewater using *T. natans* and *S. cucullata* as test species.

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