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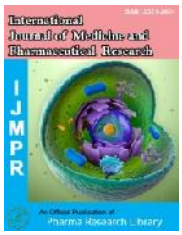

Research Article **Open Access**

Dissolved Oxygen Enrichment and Phytotoxicity Recovery of Factory Effluent by *Lemna Minor* and *Sphagnum Squarrosum*

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A B S T R A C T

Fast growing industrialization has put a serious threat on environmental safety. Phytoremediation is emerging as a cutting edge area of research gaining commercial significance in the contemporary field of environmental science. Phytoremediation is the common, *in situ*, eco-friendly, cost effective, easily deployable, solar energy driven and aesthetically pleasing method for remediation of polluted soils, sediments, and wastewaters contaminated with single or multiple pollutants. Factory effluent toxicity was studied by analytical method (hydro-chemical variables), by phytosociological method (community structure) and by assessment of toxicity on *Cicer arietinum*. The diurnal variation in dissolved oxygen concentration in aquatic ecosystem was also studied. The study fairly demonstrates the role of *Lemna minor* and *Sphagnum squarrosum* mosaic as efficient and viable phytoremediator tool as a cleanup option for surface water contamination.

Keywords: Factory effluent, *Lemna*, *Sphagnum*, phytoremediation

A R T I C L E I N F O

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1. Introduction

Due to demographic pressure, mushroom growth of industrial units and indiscriminate use of pesticides, the waste water amelioration and disposal has become one of the major and serious environmental problem (Dhar *et al.*, 2004). The

actual toxicity of wastewater is due to synergistic effect or interference of different constituents. It is, therefore, advisable to assess its toxic potential on organism than the quantitative identification of toxic ingredients (Doust *et al,*. 1994; Singhal and Mahto, 2004). Biological treatment of industrial effluent through aquatic macrophytes has emerged as a simple, cost-effective, sensitive, accurate, rapid, self-sustaining and eco-friendly alternative than the mechanical devices. Moreover, it requires less manpower and low maintenance (Buss *et al.*, 2005; Eapen and D'Souza, 2005). However, most of these studies are restricted to aquatic macrophytes only such as water hyacinth, duckweed and cattails (Tripathi and Upadhyay, 2003; Miretzky *et al.*, 2004) and cryptogam and angiosperm simulation treatment data are scarcely available. The simulation treatment of *Lemna* and *Sphagnum* for bioremediation study is a successful strategy because:

a. *Lemna* and *Sphagnum* help in oxygenation of water through photosynthesis.
b. The ability of *Sphagnum* to turn and maintain the pH of water body slightly acidic helps in bringing down the COD by increasing the solubility of acid soluble toxicants, which are absorbed by aquatic plants. This increases the phytoremediation potential of the plants.

The purpose of present study was to study the diurnal variation in dissolved oxygen (DO), phytotoxicity assessment of factory effluent using three methods, an analytical method based on hydro-chemical variables (chemical oxygen demand, dissolved oxygen, total suspended solids, total dissolved solids, pH, phosphorus and nitrogen content), a phyto-sociological one, based on survey of aquatic macrophyte community and a biological method by assessing the effluent toxicity on *Cicer arietinum* L. and to study the phytoremediation potential of *Lemna minor* and *Sphagnum squarrosum* simulation treatment.

2. Materials and Methods

The factory effluent is released through a nallah (width approximately 8 ft.), which joins with two separate nallahs (narrow water channel), one of Camphor factory and another drain out from residential colony of C B Gunj at station 'b'. The non-polluted nallah of C B Gunj is marked as station 'a'. The common nallah joins river Shankha. The station located on the common nallah is marked as station 'c' which is approximately 5 km away from junction point of station 'b'. The effluent disposed off from factory was collected at about 10 cm depth in plastic bottles and analysed physico-chemically through analytical methods suggested by American Public Health Association (1989). Temperature was analysed *in situ* while dissolve oxygen (idiometric method), BOD, TDS, TSS, pH, COD (bichromate method), total nitrogen (Micro-Kjeldhal method), total phosphorus (titration method), chloride content, temporary and permanent hardness were analysed in the laboratory. All the parameters were analysed within 24 hours of collection except BOD. For diurnal variation, dissolved oxygen of the effluent was tested at each successive 4 hours for three consecutive days. A phyto-sociological method, based on aquatic plant community analysis was adopted for the assessment of toxicity. For phytoremediation studies, 2000 ml effluent collected from station 'b' was incubated for seven days in glass aquarium with 100 g thoroughly cleaned, washed, green and identical sized *Sphagnum squarrosum* Crome Samml. (5 cm from apex), collected from Mukteswar, Uttranchal, India (2380 msl.) and *Lemna minor* (50 g) collected from local water bodies under laboratory conditions (65 mE^2S^{-1} for 14 hours and 10 hours dark cycle and temperature 20 ± 2 ^oC).

Seeds of *Cicer arietinum* L. were procured from authorized dealer at local market. They were surface sterilized with 0.01% $HgCl_2$ for 2 minutes and washed with double distilled water. Twenty seeds were tested for germination in sterilized Petridishes. 25 ml of water sample was poured in Petridishes lined with filter paper. Germination test was conducted in BOD incubator at 20 ^oC and 50% relative humidity. Protrusion of radical was considered as an index of successful germination. *In vivo* nitrate reductase (E.C.1.6.6.1) activity was measured in accordance to the method of Srivastava (1975) by spectrophotometrically quantifying the nitrite released into the incubation medium. The colour developed due to the formation of diazo compound with sulphanilamide and nitrite is coupled with NED. For peroxidase (E.C.1.11.1.7) estimation the method of Putter (1974) was followed using guaiacol as a dye. For Nitrogen, estimation micro-Kjeldhal method of Jackson (1962) was followed. Proline content was estimated following the method of Bates *et al.* (1973). Protein was estimated following the Folin-phenol method of Lowry *et al.* (1951) using Bovin Serum albumin as standard. Chlorophyll was estimated according to modified Arnon (1949) method by extracting the pigment in 80% acetone. For chlorophyll synthesis experiment, the 7 days old young leaves were floated separately on tap water + Hoagland, untreated effluent + Hoagland and *Lemna* and *Sphagnum* treated effluent + Hoagland in a ratio of 3 : 1. Effluent of station 'b' was used for all the biochemical, physiological and phytoremediation studies.

Each experiment was conducted thrice, each time in triplicates. The data presented are the average of all the treatments with standard error. For statistical analysis of data first ANOVA was applied to identify whether the treatment had any significant influence on the parameters measured followed by Dunken Multiple Range test (mean separation test).

3. Results and Discussion

Hydrilla verticillata, *Hydroryza aristata*, *Potamogeton sp.*, *Lemna minor* and *Azolla pinnata* were found throughout the channel at station 'a' representing the submerged and floating form. *Polygonum barbatum, Ranunculus sclaretus* and *Jussiaea repens* were also frequently distributed at the periphery of the water channel and sometimes found mixed with other erect species (Table 2).

Due to high turbidity from floating debris and suspended solids, the water at station 'b' was not conducive for the

development of biotic community. Blue green algae *Phormidium sp.*, *Lyngbya sp.*, *Microcystis sp.*, *Anabaena sp.* and *Oscillatoria sp.* were present at station 'b' that cover marginal rocks in slippery layers and give foul odour upon seasonal decay. Green algae that accommodate themselves to the putrid zone of active decomposition include *Spirogyra sp.* and *Stigeoclonium sp*. At this station no aquatic macrophyte was observed. The occurrence of macrophytes started from + 0.80 km from station 'b' and the population increased further progressively. *Eichhornia crassipes*, a dominating floating species was found all along the peripheral zone and in patches or at shallow zones. *Lemna minor* and *Spirodela polyrhiza* were also found frequently at 1.0 km from station 'b'. *Nymphaea stellata* and *Nymphoides sp.* were of frequent occurrence among rooted floating species. *Polygonum barbatum*, *Jussiaea repens*, *Limnophila aquatica* were common emergent hydrophytes (Table 2).

At station 'c' the pigmented flagellates were represented by *Euglena sp.* and *Pandorina sp.*, green algae by *Cladophora sp.*, *Ankistrodesmus sp.*, *Rhizoclonium sp.*, diatoms by *Meridion sp.* and *Cyclotella sp.* rooted hydrophytes by *Elodea sp.* and *Potamageton sp.* Aquatic microphytes and meadows serve as an excellent natural food for the aquatic animals and also provide shelter to them. The pollution tolerant plants like *Eichhornia sp.*, *Trapa sp.*, *Phragmatis sp.*, *Hydrilla sp.* and *Typha sp.* and Sludge worms *Tubifex sp.*, *Limnodrilus sp.* were found in nallah water from station 'b' to 'c'. There was, therefore, a progressive growth in fauna with increasing density and diversity in flora.

Maximum dissolved oxygen was observed at 4 pm in non-polluted water stream of station 'a', representing 77% saturation. A classical diurnal rhythm in the dissolved oxygen level was observed in the non-polluted nallah of station 'a' (6.1 mg L^{-1} at 8 am to 10.8 mg L^{-1} at 4 pm). The minimum early morning dissolved oxygen rises up to maximum during afternoon (4 pm) and then fall steadily during night when photosynthesis ceases and biotic communities exert a respiratory oxygen demand (Table 3).

Thus, the oxygen production by aquatic plants induces a significant diurnal variation of the dissolved oxygen concentration (Banjongproo and Wett, 2002). No significant rhythmic variation in DO was observed at station 'b' (2.0 mg L^{-1} at 8 am and the maximum 2.5 mg L^{-1} at 4 pm). This low amplitude may be due to low or nil aquatic flora. The fluctuation in dissolved oxygen in an aquatic system may be due to continuous exchange of molecular oxygen through air-water interface, DO depletion by oxygen demanding wastes and consumption of oxygen by aerobic bacteria, plants and animals in the water. The aquatic plant community add DO in photosynthesis during day while deplete it during night. The phenomenon of super saturation can be created with the help of aquatic plants (Wilcock and Nagels, 2001). Thus, the community structure of an aquatic ecosystem plays a crucial role in dissolved oxygen level. The effluent was found to be toxic, as it did not harbor any macrophyte vegetation near the discharge point. The potential toxicity of factory effluent was evident by its brownish black color, foul odour, high BOD (187 mg L^{-1}), TSS (1240 mg L^{-1}), TDS (922 mg L^{-1}), total Kjeldahl nitrogen (12.14 mg L^{-1}) and phosphorus (14.88 mg L^{-1}) content. Study revealed that simulation treatment with *Lemna minor* and *Sphagnum squarrosum* has significantly removed the total nitrogen (41%) and phosphorus (43%) from the effluent water (Table 1). The increase in total phosphorous in untreated effluent was either due to inorganic or organic phosphorous discharged in the factory effluent. Normally, phosphorus in the range of 1.5 -3.0 mg L^{-1} and nitrogen around 4 mg L^{-1} in water is accepted for agriculture purposes. The DO of any water body is an indicator of water quality (Saxena, 1995). As dissolved oxygen drops below 4 mg L^{-1}, most of the living forms begin to reduce. Factory effluent has very low dissolved oxygen (1.8 mg L^{-1}). The simulation treatment with *Sphagnum squarrosum* and *Lemna minor* has enriched the DO of the effluent up to 155%.

The percentage germination of *Cicer arietinum* L. seeds was inhibited significantly in untreated effluent (37% at 120 hours of soaking). However, better germination behavior was observed in treated effluent, both in terms of germination time and germination percentage (Figure 1, Table 4) which indicate a depletion of pollutants upon treatment with *Sphagnum squarrosum* and *Lemna minor*. The alkaline pH may have inhibitory effect on seed germination (Augusthy and Mani, 2001) and therefore, the change in pH of the effluent towards slightly acidic range (*i.e.* around 6.5) after incubation with *Sphagnum squarrosum* has enhanced the seed germination.

The pronounced phytotoxicity of untreated effluent of station 'b' was further evident by reduced dry matter, shoot length, root length, chlorophyll and protein content of *Cicer arietinum* grown in untreated effluent than control. Both in treated and untreated effluent the shoot were found to be more sensitive and responsive than roots (Figure 2, Table 5). A substantial recovery in productivity and biochemical parameters *i.e.* dry matter, chlorophyll, protein content and nitrate reductase activity was observed in seedlings established in treated effluent of station 'b' (Table 6).

The maximum root length and shoot length of the germinating seedling were observed in tap water while minimum in untreated effluent. Maximum chlorophyll genesis was observed in leaves floated in Hoagland followed by Hoagland + *Lemna* and *Sphagnum* treated effluent and minimum by Hoagland + untreated effluent (Fig. 3). The reduced chlorophyll genesis in untreated effluent may be due to reduced - aminolevulinic acid dehydratase, an enzyme for chlorophyll biosynthesis (Prasad and Prasad, 1987; Vajpai *et al.*, 2000).

Duckweeds have an important potential in nutrient recovery from waste water because of their rapid multiplication and high protein content in biomass. The growth rate of duckweed biomass has a direct relationship with nutrient removal and recovery (Chong *et al.*, 2004). Simplicity in testing the effluent toxic potential and thereby remediation through hydrophytes make these plants an ideal tool for phytoremediation studies (Srivastava *et al.*, 2000; Kramer, 2005). It is known that *Sphagnum* has natural capacity for ion

exchange with metallic toxicants such as Cu, Pb, Ni etc. (Saxena *et al.*, 1999) and it is related to the pH of the medium. At pH above 8.0 *Sphagnum* peat itself is not very stable and at low pH below 3.0 most metals are leached out from peat. Between these values, it is known that peat can absorb most metals in a very efficient manner. Increased germination percentage, chlorophyll, protein content and nitrate reductase activity of *Cicer arietinum* in *Sphagnum squarrosum* treated effluent strongly support the purification efficiency of *Sphagnum squarrosum*. The study fairly demonstrates feasibility of simulation treatment of *Lemna minor* and *Sphagnum squarrosum* Crome Samml. for dissolved oxygen enrichment and phytoremediation of polluted water bodies.

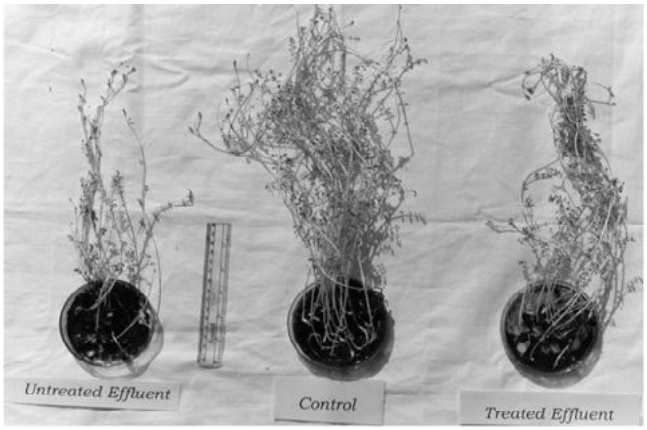

Figure 1: Lab crop of *Cicer aeritinum* in different experimental sets

Figure 2: Phytotoxicity of factory effluent on root and shoot length of *Cicer aeritinum*

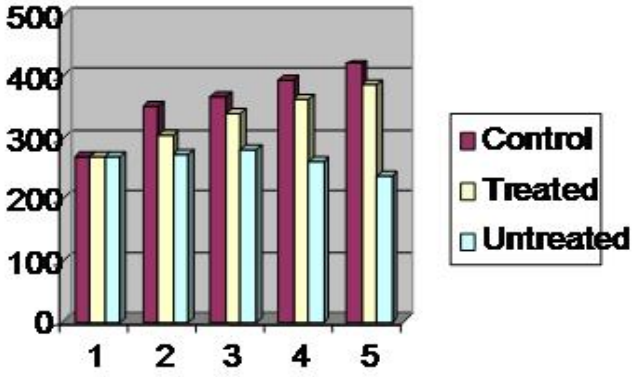

Figure 3: Time course of chlorophyll genesis in greening *Cicer aeritinum* leaf segments incubated in ¼ Hoagland (control), treated effluent and untreated effluent

Table 1: Physio-chemical parameters of factory effluent before and after treatment with *Lemna* and *Sphagnum*

Parameters	Tap water	Station 'a'	Station 'b'	Station 'b' treated	Station 'c'
Colour	Colourless	Transparent	Deep brown	Light Pale	Transparent
Total alkalinity	62.4 ± 5.2	95.4 ± 6.3	387.0 ± 17.6	123.8 ± 14.2	104.31 ± 7.5
D.O.	3.0 ± 0.09	8.6 ± 0.7	1.8 ± 0.2	4.6 ± 0.3	4.8 ± 0.6
B.O.D.	-	51.74 ± 7.9	187 ± 13.8	46.8 ± 2.9	48.81 ± 3.4
T.S.S.	46 ± 5.2	213 ± 11.7	1240 ± 19.3	282 ± 17.1	206.43 ± 14.6
T.D.S.	91 ± 6.1	102 ± 14.5	922 ± 11.3	114 ± 7.6	94.74 ± 6.2
Total Nitrogen	8.13 ± 0.6	14.87 ± 1.1	12.14 ± 0.9	7.16 ± 0.8	11.35 ± 1.0
Total Phosphorus	0.3 ± 0.03	2.37 ± 0.03	14.88 ± 0.08	8.43 ± 0.09	5.82 ± 0.06
pH	6.8 ± 0.9	7.2 ± 0.5	8.3 ± 0.4	6.4 ± 0.8	7.3 ± 0.6
Total Hardness	76.9 ± 8.3	112.63 ± 10.4	142.84 ± 11.3	90.36 ± 7.3	93.57 ± 6.8
COD	40.45 ± 2.9	274 ± 6.7	1054 ± 11.4	438 ± 9.6	304.85 ± 9.3
Chloride	2.1 ± 0.01	4.1 ± 0.03	7.8 ± 0.02	4.9 ± 0.02	5.7 ± 0.04

All the parameters are in mg L^{-1}, except pH, unless specified. Values are the average of at least three determinations with standard error.

Table 2: Aquatic flora at three marked stations of undertaken study area

Plants	Station 'a'	Station 'b'	Station 'c'
Ranunculus	+	-	-
Phormidium	-	+	+
Lyngbya	-	+	-
Microcystis	-	+	-
Anabaena	-	+	-
Oscillatoria	-	+	-
Spirogyra	+	+	-

Stigeoclonium	+	+	-
Cladorhoya	-	-	+
Ankistrodesmus	-	+	-
Meridion	-	+	-
Cyclotella	-	+	-
Elodea	+	+	-
Potamogeton	+	+	-
Spharotilus	-	-	+
Eristalis	-	+	+
Eichhornia	-	-	+
Typha	-	-	+
Trapa	-	-	+
Phragmatis	+	-	+
Hydrilla	+	+	+
Lemna	+	-	-
Azolla	+	-	-
Hydroryza	+	-	-
Nymphaea	-	-	+
Nymphoides	-	-	+
Spirodela	-	+	+
Elecharis	+	-	+
Polygonam	+	-	-
Jussiaea	+	-	-

Table 3: Diurnal variation in dissolved oxygen (mg L^{-1}) concentration

Water body	**Time**				
	8 am	**12 noon**	**4 pm**	**8 pm**	**4 am**
Station'a' (Up Stream)	6.1 ± 0.05	7.6 ± 0.05	10.8 ± 0.08	9.3 ± 0.07	5.2 ± 0.06
Station 'b' (Discharge point)	2.0 ± 0.02	2.3 ± 0.03	2.5 ± 0.02	2.1 ± 0.02	1.7 ± 0.01
Station 'c' (Down stream)	4.1 ± 0.04	6.9 ± 0.05	8.2 ± 0.06	7.4 ± 0.06	5.1 ± 0.4

Values are the average of at least three determinations with standard error.

Table 4: Effect of factory effluent of station 'b' (untreated and treated with *Lemna* and *Sphagnum*) on seed germination in *Cicer arietinum*

Time (Hours after soaking)	**Seed germination (%)**		
	Tap water	**Untreated effluent**	**Treated effluent**
24	14	3	10
48	95	8	49
96	98	34	81
120	98	37	92

Table 5: Effect of factory effluent of station 'b' (untreated and treated with *Lemna* and *Sphagnum*) of root length (cm) and shoot length (cm) of *Cicer arietinum*

Exposure (in days)	**Tap water**		**Untreated effluent**		**Treated effluent**	
	Shoot length	**Root length**	**Shoot length**	**Root length**	**Shoot length**	**Root length**
1	0	0	0	0	0	0
2	1.3 ± 0.08	0.4 ± 0.05	0.3 ± 0.02	0.6 ± 0.05	1.2 ± 0.02	0.4 ± 0.04
3	3.9 ± 0.9	0.8 ± 0.06	1.0 ± 0.06	1.4 ± 0.08	3.6 ± 0.12	1.0 ± 0.07
4	6.4 ± 0.23	1.3 ± 0.80	2.8 ± 0.09	2.8 ± 0.15	5.8 ± 0.19	1.5 ± 0.08
5	8.5 ± 1.4	1.9 ± 0.07	3.5 ± 0.09	3.7 ± 0.12	7.9 ± 0.31	2.3 ± 0.12
6	11.4 ± 1.7	2.2 ± 1.3	4.0 ± 0.17	5.4 ± 0.16	10.8 ± 0.26	2.8 ±0.09
7	13.2 ± 1.4	2.6 ± 0.9	4.2 ± 1.2	6.5 ± 1.9	12.4 ± 0.27	3.2 ± 0.13

Values are the average of at least three determinations with standard error.

Table 6: Effect of factory of station 'b' (untreated and treated with *Lemna* and *Sphagnum*) on growth and productivity parameters of *Cicer arietinum* seedlings

Parameter	Tap water	Untreated effluent	Treated effluent
Chlorophyll (g g^{1} fr.wt)	281 ± 18^{a}	134 ± 9.6^{b}	264 ± 15^{a}
Protein (mg g^{-1} dr.wt.)	25.7 ± 1.6^{a}	18.9 ± 0.09^{b}	24.4 ± 1.4^{a}
Nitrate reductase activity (n mole NO_2 h^{-1} g^{-1} fr. wt.)	3296 ± 27^{a}	1927 ± 14^{b}	2811 ± 19^{c}
Na+ (mg g-1 fr. wt.)	3.28 ± 0.05^{a}	6.9 ± 0.04^{b}	10.45 ± 0.09^{c}
K+ (mg g^{-1} fr.wt.)	9.4 ± 0.06^{a}	25.60 ± 1.7^{b}	16.45 ± 1.4^{a}
Ash (%)	6.11 ± 0.3^{a}	6.31 ± 0.4^{a}	6.18 ± 0.8^{a}
Fresh weight (gm per seeding)	0.93 ± 0.07^{a}	0.364 ± 0.03^{b}	0.586 ± 0.06^{b}
Proline content (mg g^{-1} fr.wt.)	0.512 ± 0.06^{a}	0.618 ± 0.06^{b}	0.548 ± 0.04^{c}

Values are the average of at least three determinations with standard error. Values with the same superscript are statistically the same (DMR test).

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