

Evaluation of the Efficiency of Duckweed (*Lemna minor* L.) as a Phytoremediation Agent in Wastewater Treatment in Kashmir Himalayas

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Abstract

In the present study, the efficiency of duckweed (*Lemna minor* L.) as an effective natural biological tool in wastewater treatment was examined in an outdoor aquatic system. Duckweed plants were inoculated into wastewater and tap water systems for treatment over fifteen day's retention periods under local outdoor natural conditions. Water samples were taken below duckweed cover after fifteen days to assess the plant's efficiency in purifying waste water from different pollutants. For comparison, the plants were also grown in tubs containing tap water. The results show that concentrations of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Copper (Cu), Zinc (Zn), Nickel (Ni), Chromium (Cr), Cadmium (Cd) and Lead (Pb) decreased by 93.4%, 99.9%, 93.9%, 98.5%, 91.9%, 85.0%, 95.0%, 90.0%, 99.8%, 99.5% and 95.0% respectively in waste water and subsequently these elements exhibited an increasing concentration in the plant body. Almost similar results were obtained when the plants were grown in tubs containing tap water. Biochemical parameters viz. chl-a, b, total-chl, carbohydrates and proteins as well as nutrient status of the macrophyte increased after the completion of the retention period both in waste and tap water. Results confirm that duckweeds can effectively be used for wastewater treatment systems.

Keywords: *Lemna minor*; Wastewater; Retention period

Introduction

Multiple environmental factors in association with anthropogenic activities have significantly altered our aquatic ecosystems. Over use of chemical fertilizers and intensification of industrial activities contaminate watercourses and water-bearing stratum with heavy metals and other pollutants. Toxic elements and heavy metals are an important category of pollutants and as such have major detrimental impacts on both human health as well as the health of terrestrial and aquatic ecosystems. The discharge of harmful chemical compounds into the aquatic environments disturbs the structure and functioning of the natural ecosystems. Restoration of water contaminated with potentially toxic metals and metalloids is of major global concern and thus there is a need to reduce their concentrations to a protective level in order to prevent eutrophication and other metallic enrichment. It is an established fact that in comparison to chemical treatments, the biological methods for the removal of contaminants from the environment are cheaper than the conventional remediation technologies [1]. In recent years, there has been a growing struggle to provide efficient, inexpensive, and environmentally friendly options for the remediation of trace elements and other contaminants in waste water. Moreover, aquatic plants are of special interest unlike the terrestrial plants, because they are capable of bio concentrating many nutrients and heavy metals in large quantities [2-4]. In Kashmir valley Anchar lake is one of the lakes severely suffering from nutrient enrichment and duck weed (*Lemna minor* L. belonging family Lemnaceae) is one of the common aquatic free floating macrophytes found in this water body. In order to evaluate the phytoremediation potential of duck weed a laboratory experiment was conducted in 2014-15 to analyze the nutrient and heavy metal absorption potential to assess its efficacy for waste water treatment.

Materials and Methods

Preparation steps

Waste water from a nearby ditch was transferred to the laboratory after filtering it to get rid of large suspended solids. The filtered water was immediately collected into four opaque tubs (as replicates) to prevent light entering except at the top [5]. Each tub was 50 cm long, 35 cm wide

and 25 cm deep and was filled with 10 L waste water. Duckweed (*Lemna minor* L.) plants were collected from a local ditch. The plants were cleaned by tap water followed by distilled water and were transferred to waste water systems for aquatic treatment. For comparison, similar set of tubs was also kept wherein duckweed was grown in tap water. The experiment was kept under outdoor local environmental conditions for fifteen days retention time.

Subsurface (under plant mat) water samples were collected in polyethylene bottles from all sides of each tank and then mixed after the completion of 15 days for chemical analysis. Similarly, plant samples were also collected from each tub for the analysis purposes.

Analysis of the water and plant samples

Macro, micro and heavy metal status in the water were carried out according to standard methods for examination of water and wastewater [6]. In plants except for the estimation of chlorophyll (a, b and total) where fresh samples were used, the plant samples were air dried and grounded in a grinder. The Photosynthetic pigments (Chlorophyll and Carotenoids) were analyzed by following the methods of Strain et al. [7] and Duxbury and Yentsch [8] respectively. Similarly, carbohydrate and proteins were analyzed by following the methods of Dubios et al. [9] and Lowry et al. [10]. Dried plant was analysed for total N by Kjeldahl method [11], total P (Vanadatemolybdate method), Ca, Mg and K were determined using ammonium acetate method [12]. Cu, Zn, Fe, Mn, Ni, Cd, Cr and Pb were analyzed by using AAS (Atomic Absorption

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Spectrophotometer). ELEMENTAS AAS4141-Electronics Corporation of India Limited).

Results and Discussion

Duckweed plant were inoculated into a wastewater system for aquatic treatment over 15 day's retention time periods to assess the plant's efficiency in improving macronutrients, micronutrients and heavy metal characteristics of wastewater and tap water. The wastewater and the plants that were used in the experiment were taken from the nearby ditch.

Macronutrients (N, P, K, Ca, Mg)

The results show that macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in wastewater reached their minimum concentrations of 0.5, 0.001, 1.10, 0.4 and 2.6 mg L⁻¹ respectively after growing *Lemna minor* for 15 days in it with a reduction percentage of 93.4%, 99.9%, 93.8%, 98.4% and 91.8% respectively (Table 1). However, these macronutrients have increased in the plant body during the retention period with an increasing percentage of 23.4%, 7.14%, 5.0%, 1.74% and 37.5% respectively (Table 2). Similar results have been observed in tap water that was used for comparison purposes where macronutrients had decreased in concentration after *Lemna minor* was grown in it for 15 days with a reduction percentage of 100%, 100%, 60.52%, 94.50% and 97.22% (Table 1) respectively and subsequently in plant body these nutrients have shown increasing percentage of 8.13%, 4.44%, 0.59%, 0.91% and 17.86% (Table 2) respectively during the experimental period. Fast growing duckweed in nutrient rich water is a highly efficient sink for both phosphorus and potassium; little of each, however, is required for rapid growth. In concurrence with the present findings, Verma and Suthar [13] have also reported that the nutrient load in wastewater reduced significantly by 83-89% of NO₃⁻ and 67-72% of total phosphorus. Pandey [14] reported that in the duckweed treatment system at Halisahar, nitrogen and phosphorus removals were in the range of 50-75% and 17-35% respectively. In the support of present findings Zaltauskaite et al. [15] found that *Lemna minor* efficiently removes N, P, K and also Ca and Mg from wastewaters compared to other treatment methods. The cause of reduction in nitrogen, phosphorus, potassium, calcium and magnesium was utilized by the *Lemna minor* as a nutrient as also reported out by Patel and Kanungo [16].

Micronutrients (Cu, Zn, Ni)

Micronutrients such as Copper (Cu), Zinc (Zn) and Nickel (Ni) reached their minimum concentrations of 0.6, 0.01 and 0.01 mg L⁻¹ respectively after *Lemna minor* was grown in it for 15 days, with a

reduction percentage of 85.0%, 95.0% and 90.0% respectively (Table 1). On the other side, these micronutrient (Cu, Zn and Ni) show high concentration in the plant body during the retention time with an increasing percentage of 34.69%, 69.44%, and 66.67% respectively in the plant body (Table 2). Similar results have been observed in tap water that was used for comparison purposes where micronutrients zinc and copper had decreased in concentration after *Lemna minor* was grown in it for 15 days with a reduction percentage 100% and subsequently in plant body these nutrients have shown increasing percentage of 2.22% and 0.71% respectively (Table 2) however nickel has shown no change as it was not observed in tap water during initial analysis. In the line of present study Waffa et al. [17] reported that duckweed treatment system reduced zinc by 93.6% and copper by 100% during eight days retention period. Zaltauskaite et al. [15] also found that Zn was most efficiently removed, depending on the initial Zn concentration *Lemna minor* removed between 42.3-77.8% of Zn. They also found that after the 7 days of effluents exposure to *Lemna minor* treatment, the concentration nickel was reduced significantly.

The major cause of micronutrient reduction in waste water / tap water was utilization by the *Lemna minor* plant for body formation and development. Duckweeds require a number of macro and micronutrients for their normal growth. Nutrients are absorbed through all surfaces of the duckweed leaf. DWRP [18] reported that the highest growth rate for Lemnaceae under optimal laboratory conditions is about 0.66 generations per day that is equal to a doubling time of 16 hours. It has been reported that duckweeds generally double their mass in 16 hours to 2 days under optimal conditions which further helps in uptake of nutrients from the growing media for developing body tissue. As also reported by Korner and Vermaat [19] that the nutrients removed by duckweeds from growing media are mainly realized by newly grown tissue of the plants. Duckweed has a high mineral absorption capacity and can tolerate high organic loading as well as high concentrations of micronutrients.

Heavy metals (Cd, Cr and Pb)

The concentrations of heavy metals such as Cd, Cr and Pb reduced after *Lemna minor* was grown on wastewater for 15 days retention period. These metals have reached their minimum concentration of 0.001, 0.001 and 0.01 mg L⁻¹ respectively with a reduction percentage of 99.5%, 99.8% and 95.0% respectively, however in plant body their concentration has increased during the study period with an increasing percentage of 92.06% 43.59% and 0.52% respectively. In case of tap water cadmium and lead were not observed during initial analysis however chromium had decreased in concentration from 0.20 ppm to 0.00 ppm with a reduction percentage of 100% (Table 1) and subsequently

Parameters (ppm)	Waste water			Tap water			P ≤ 0.05
	Initial	Final	% Decrease	Initial	Final	% Decrease	
Nitrogen(N)	7.600	0.500	93.421	0.400	0.000	100.00	0.01
Phosphorus(P)	1.100	0.001	99.909	0.110	0.000	100.00	0.28
Potassium (K)	18.000	1.100	93.889	3.800	1.500	60.52	0.57
Calcium (Ca)	25.900	0.400	98.456	9.100	0.500	94.51	0.02
Magnesium (Mg)	32.000	2.600	91.875	3.600	0.100	97.22	0.06
Copper (Cu)	4.000	0.600	85.000	0.300	0.000	100.00	0.00
Zinc (Zn)	0.200	0.010	95.000	0.100	0.000	100.00	0.54
Nickel (Ni)	0.100	0.010	90.000	0.000	0.000	0.000	0.04
Cadmium (Cd)	0.200	0.001	99.500	0.000	0.000	0.000	0.02
Chromium (Cr)	0.600	0.001	99.833	0.200	0.000	100.00	0.38
Lead (Pb)	0.200	0.010	95.000	0.000	0.000	0.000	0.02

Table 1: Mean values of various parameters of waste water and tap water before and after treatment by *Lemna minor*.

Parameters	Waste water			Tap water			P≤ 0.05
	Initial	Final	%increase	Initial	Final	%increase	
N (%)	1.44	1.88	23.40	1.13	1.23	8.13	0.351
P (%)	0.52	0.56	7.14	0.43	0.45	4.44	0.06
K (%)	1.71	1.80	5.00	1.69	1.70	0.59	0.05
Ca (%)	1.13	1.15	1.74	1.09	1.10	0.91	0.04
Mg (%)	0.45	0.72	37.50	0.46	0.56	17.86	0.00
Cu (mg/kg)	24.85	38.05	34.69	25.08	25.26	0.71	0.11
Zn (mg/kg)	3.27	10.70	69.44	3.97	4.06	2.22	0.04
Ni (mg/kg)	0.02	0.06	66.67	0.01	0.01	0.00	0.15
Cr (mg/kg)	0.22	0.39	43.59	0.30	0.31	3.23	0.06
Cd (mg/kg)	0.05	0.63	92.06	0.05	0.05	0.00	0.55
Pb (mg/kg)	25.09	25.22	0.52	25.02	25.02	0.00	0.05
Carbohydrate (%)	15.28	20.01	23.64	15.33	17.54	15.19	0.08
Proteins (%)	12.23	17.61	30.55	12.15	13.20	7.95	0.04
Chl'a' (mg/g)	2.88	4.06	29.06	2.98	3.96	24.75	0.81
Chl'b' (mg/g)	1.13	2.01	43.78	1.40	1.93	27.46	0.59
Total chlorophyll (mg/g)	4.10	6.08	32.57	4.30	5.45	21.10	0.57
Carotenoids (mg/g)	1.48	1.80	17.78	1.49	1.59	6.29	0.08

Table 2: Mean values of various parameters in *Lemna minor* before and after growing them in Waste water and Tap water.

in plant body chromium have shown increasing percentage of 3.23%, whereas cadmium and lead have shown no change in their initial concentrations (Table 2). In concurrence with the present findings, Waffa [17] studied that duckweed aquatic treatment system performed 100% lead and 66.7% of cadmium removal from wastewater after 8 days treatment period. Similar results have also been found by Kara et al. [20] and concluded that duckweed *Lemna minor* L. take up Pb, Cu, Fe, Cd and Ni from contaminated solutions. Removal of heavy metals from wastewater was also studied by Zayed et al. [21] and the results confirm that duckweed (*Lemna minor* L.) is excellent accumulators of Cd, Se and Cu.

Biochemical parameters

The photosynthetic pigments chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids and other biochemical parameters such as total carbohydrates and total proteins, also reported an increase in their concentrations in *Lemna minor* during the experimental period.

The final result obtained after growing *Lemna minor* in the wastewater show that photosynthetic pigments such as Chl 'a' has increased in concentration from 2.88 mg/g to 4.06 mg/g exhibiting an increase of 29.06% ; Chl 'b' level has increased from 1.13 mg/g to 2.01 mg/g depicting an increase of 43.78%; total chlorophyll level has increased from 4.10 mg/g to 6.08 mg/g reporting an increase of 32.57% and carotenoid level has increased from 1.48 mg/g to 1.80 mg/g showing a increase of 17.78% in the tissues of *Lemna minor*. Also, other biochemical parameters such as total carbohydrate level has increased from 15.28% to 20.01% showing an increase of about 23.64% and total protein level have increased in concentration from 12.23% to 17.61% exhibiting an increase of about 30.55% in the tissues of *Lemna minor*. Similarly, the result obtained after growing *Lemna minor* in the tap water showed that photosynthetic pigments such as Chl 'a', Chl 'b', total chl-, carotenoids, carbohydrate and protein contents increased by 24.75%, 27.46%, 21.10% 6.29%, 15.19% and 7.95% respectively in the plant tissues of *Lemna minor*. Zaltauskaite et al. [15] have also found that wastewater treatment resulted in higher content of photosynthetic pigments in *L. minor* exposed to untreated wastewater. Similarly, Benerjee and Matai [22] have also reported concentration of carbohydrates in *Lemna minor* increased by 15.19% and 23.64% on dry weight basis when grown on tap water and wastewater respectively.

An earlier study on the biochemical composition of aquatic plants of Dal Lake in Kashmir valley revealed carbohydrates content in *Lemna minor* was 46.41-85.74% on dry weight basis [23]. However, the carbohydrate content observed during the present study remained within the findings of Mishra and Jha, Prasannakumari et al. and Mini [24-26]. Hammouda et al. [27] also found that protein content in duckweed increased when grown on wastewater and reached a maximum concentration of 47.1% with a maximum increase in all amino acids. Pandey, 2001 reported that duckweed had high nutrient value in the dried biomass; 20-31% protein, 0.5-2.2% fat, 0.008-0.01% vitamin C and 0.003-0.007% iron who recommended its use as a food supplement for fish, poultry, and cattle. The increase in concentration of biochemical parameters may be mainly due to increased absorption of macronutrient and micronutrients, which in turn increase the tissue proteins and carbohydrates.

Conclusion

Chemical analysis recorded that nutrients and heavy metals in wastewater and tap water were significantly reduced by *Lemna minor* over 15 days treatment. Therefore, this aquatic weed helps in a continuous gradual decrease in the nutrients and heavy metals from the wastewater. *Lemna minor* has been shown to be a potential scavenger of nutrients and heavy metals from wastewater and may be used in wastewater treatment systems. Further, there were no significant difference between duckweed treatment systems in waste water and tap water. Duckweeds efficiently remove nutrients and heavy metals from both the systems. Duckweed grows rapidly and is capable of nutrient uptake under a wide range of environmental conditions. Compared to most other aquatic plants, it is less sensitive to low temperatures, very high nutrient levels, pH fluctuations, pests and diseases.

It may be conclude that *Lemna minor* can be used as wastewater phytoremediation agent. Though protocols need to be developed and further studies are required to understand the mechanisms involved in the uptake of pollutants so that maximum potential can be utilized for use in phytoremediation technology.

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