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Original Research Article

Phytoremediation of Sewage Water for Raising Potted Plants using *Lemna* sp.

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Abstract: A large number of plants have the potential to remediate polluted water. The phytoremediated water, if not considered too fit for drinking as per WHO standards, can be used for non-potable purposes such as watering of plants or washing. The primary concern in water used for irrigation, is its salinity level. Since salts in water can affect both the soil structure and crop yield, it is important to maintain a certain level of salts, an indicator of suitability of water for irrigation purposes. The present study was therefore undertaken at Kirori Mal College, University of Delhi to remediate sewage water generated at the college campus, for irrigation purposes using duckweed (*Lemna sp),* easily available in and around the study site. Duckweed was grown for two weeks in sewage, pond and tap water taken from Kirori Mal College site. The light and dark cycle with 6 hrs of natural sunlight was provided. After 15 days the water samples were tested for hardness, alkalinity, boron, Ca^{2+} and Na^{+} concentration, Sodium Absorption Ratio (SAR), and Residual Sodium Carbonate (RSC) at Shri Ram Institute of Industrial Research. The untreated samples served as control. The duckweed showed maximum growth in terms of harvested biomass in sewage water during the second week. The alkalinity in all samples showed a decrease with respect to their control. The preliminary studies have shown that sewage water can be phytoremediated for irrigation purposes using duckweed. The results are discussed in light of the literature available on phytoremediation by duckweed.

Keywords: Duckweed, Phytoremediation, Residual Sodium Carbonate (RSC), Sewage water, Sodium Absorption Ratio (SAR), Total Salt Concentration (TSC)

Introduction

Water supports all human activities, making water pollution one of the biggest health issues around the globe. Discharge of untreated sewage water into water bodies is common all over the world. Even when subjected to treatment, the remediated water may not meet the standards of potable water because either the treatment plants are less efficient or the level of pollutants is too high. However, treated waters in such cases can be made fit for agriculture through physical (e.g., aeration), chemical (oxidative and reductive) or biological means (bioremediation). Phytoremediation is a biological process (bioremediation) in which plants are used to accumulate, degrade or eliminate a range of pollutants such as heavy metals, pesticides, crude oils from a water body rendering clean water for potable or non- potable consumption. Besides *Lemna,* other aquatic phytoremediators are *Azolla, Eichhornia, Wolfia, Potamogeton* and *Spirodela* (Ansari *et al.*, 2020). *Lemna,* one of the most effective macrophytes has shown great potential for the phytoremediation of a range of substances namely, organic pollutants, heavy metals, agrochemicals, pharmaceuticals and personal care products radioactive waste, petroleum hydrocarbons, dyes, toxins, nanomaterials and other pollutants (Ekperusi *et al*., 2019). The subfamily (Lemnoideae) with 36 species includes the world's smallest and fastest growing flowering plants with *L. minor* known for its role in phytoremediation (Bokhari*et al*., 2016). The plant is composed of one or few leaves called fronds and a single root or rootlet with no stem. Vegetative reproduction is a common mode of increasing the number of individuals and the plant simply divides forming separate individuals (Correll and Correll, 1972). *L. minor* about 2-4mm across, forms aggregates or colonies on the surface of water (Rusoff *et al*., 1980). The frond doubling time of *L. minor* is about 1.4 days (Frick, 1985). The ease with which duckweed can be handled in the laboratory, and the high rate of multiplication makes it popular in studies on phytoremediation. Commonly grown in stagnant or slow-flowing, nutrient-enriched water throughout tropical and temperate zones, duckweed growth conditions include temperatures ranging from 6 to 33°C and a wide pH range with optimal growth between pH 5.5 and 7.5 (Mkandawire and Dudel, 2005a,b).

As a result of a short life cycle, large duckweed biomass is generated which can be used as cattle feed. However, the mother frond usually dies after production of six generations (Ziegler *et al*., 2015). The plant absorbs metals namely, Cr, Al, Ni, Zn, U and other rare earth elements (Mkandawire and Dudel, 2007 a, b; Forni *et al*., 2016). Duckweeds, in general have the ability to quickly absorb nitrogen, phosphorus and other nutrients besides removing pathogens. With a growth rate of 13–38 dry tons/hectare/ year in water treatment lagoons, the duckweeds are currently being explored to convert agricultural, industrial and municipal wastewater into clean water and a high-protein animal feed while providing biofuels and even plastics (Fourounjian *et al.,* 2020). To make water potable, extensive and intensive methods are required as it must be disinfected also before water is released for consumption. Since it is easier to get non-potable water upon phytoremediation than potable or drinking water, it is therefore important to understand the parameters set for use of such water for irrigation purposes. According to (Zaman *et al.,* 2018) there are four parameters to assess the irrigation water quality i.e., Residual Sodium Carbonate (RSC), Sodium Absorption Ratio (SAR), Total Salt Concentration (TSC) and Boron concentration (Table 1) on the basis of which water can be classified into low, medium, high and very high salinity water. Low salinity water can be used in all the type of crops but very high salinity water should be only used for salt stress tolerant crops.

Total Salt Concentration (TSC)

Salt concentration is a measure of salinity which is measured according to standard protocol IS 11624-2019 in terms of total salt concentration (TSC) and is expressed as electrical conductivity. Excessive salt concentration decreases the osmotic potential of water and in turn causes oxidative stress on plants growing in such conditions. Irrigation is a major human activity in agriculture which can cause soil salinity if salt concentration in water is high which in turn affects the yield (Zaman *et al.,* 2018).

Table 1. Water quality rating based on electrical conductivity (Ec), Residual Sodium complex (RSC) and Boron concentration (Zaman *et al*., 2018).

S. No.	Class	Ec (electrical conductivity in μS/cm)	Sodium Absorption Ratio (SAR) values	Residual Sodium Complex (RSC) values	Boron Concentration (in ppm)
1	Low	<1,500	10	<1.5	<1
2	Medium	1,500-3,000	10-18	1.5-3.0	1-2
3	High	3,000-6,000	18-26	3.0-6.0	2-4
4	Very high	>6,000	>26	>6.0	>4

Sodium Absorption Ratio (SAR)

SAR or sodium absorption ratio is defined as the ratio of the Na concentration to Calcium and Magnesium concentration and is calculated by the equation:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

(Where, all concentrations are in meq/L)

Sodium Adsorption Ratio is a measure of the amount of sodium (Na) relative to calcium (Ca) and magnesium (Mg) in the water extracted from saturated soil paste. Higher value of Sodium Absorption Ratio means higher are the chances of sodium replacing calcium and magnesium from soil which will result in soil infiltration and degradation of soil (Zaman *et al.,* 2018).

Residual Sodium Carbonate (RSC)

The **Residual Sodium Carbonate (RSC) index** of irrigation water or soil water indicates the alkalinity of the sample. The RSC index is used to find the suitability of the water for irrigation in clay soils which have a high cation exchange capacity. When dissolved sodium in comparison with dissolved calcium and magnesium is high in water, clay soil swells or undergoes dispersion which drastically reduces its infiltration capacity (Hopkins *et al.,* 2007).

Residual Sodium Carbonate is sum of carbonate and bicarbonates subtracted by calcium and magnesium concentration i.e.,

$$RSC = ([HCO_3^-] + [CO_3^{2-}]) - ([Ca^{2+}] + [Mg^{2+}])$$

(Where, all concentrations are measured in meq/L)

Boron concentration

Boron is the micronutrient and required for uptake and utilization of Ca^{2+}, membrane functioning, cell elongation, cell differentiation, and carbohydrates translocation. It is absorbed as BO_3^{3-} or $B_4O_7^{2-}$ but if boron is present in concentration more than the required amount, it may cause toxicity. Duckweed can remove boron from water under salt stress conditions (Liu *et al.,* 2018).

Alkalinity and hardness

Alkalinity, the measure of buffering capacity primarily depends on carbonate and hydroxide content, whereas hardness measures precipitation power of water which depends on calcium and magnesium concentration. Their value reflects soil infiltration may occur during irrigation.

Material and methods

Collection of water samples

Three different samples of water were sourced in the month of March 2022. Tap water was obtained from the laboratory of Kirori Mal College (KMC) itself. Pond water (in which *Lemna* was growing in nature) was procured from Sarpakar Lake located at Kamla Nehru Ridge, University of Delhi, while the sewage water was collected from the KMC sewage pipes. The grab collection was made in labeled, wide mouthed glass reagent bottles of a liter capacity. These bottles were maintained at room temperature away from direct light (Kalkhajeh *et al.*, 2019). *Lemna* was also collected from the ridge and cleaned of algae and other debris before being used in the experiment.

Phytoremediation of samples

The collected water samples were divided into two sets, Set A with 2L water of three different sources, served as control and was set aside for 0-day analysis of water quality. The Set B, 25L each from three different sources was subjected to phytoremediation with *Lemna* kept in three glass containers each with surface area of $0.23m^2$. In each of the three glass containers, 50 g of duckweed was added (Fig. 1). The three containers were maintained under natural conditions. The pH of the water samples recorded with a pen type digital pH meter was noted initially i.e., 0 day and after every two days.

On the seventh day, the duckweed was harvested from the three tanks and excess water was removed by gently pressing plants in layers of blotting sheets. While the duckweed harvested from the sewage tank was discarded as the plants appeared unhealthy and senescing, the biomass collected from the tap water and pond water tank was weighed and pooled for the second round of phytoremediation. In each of the three tanks, 50g of duckweed was added and the containers were again kept

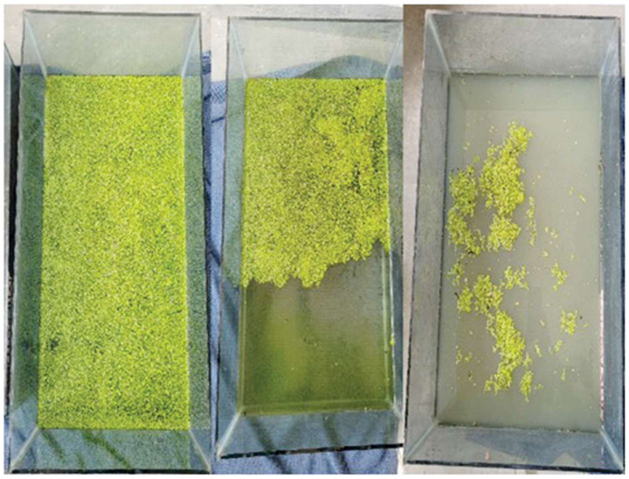

Fig. 1. A-C: Duckweed growth after fourteen days in tap water (A), pond water (B) and sewage (C).

under natural conditions of light and dark period as in the first round of remediation. On the fourteenth day, duckweed was harvested from each of the three tanks and weighed. The pH of the water sample was again recorded.

Samples of day 0 (untreated) and day 14 (phytoremediated) were tested for hardness (IS: 3025 Pt-21-2009, RA 2019), alkalinity (IS: 3025 Pt-23- 1986, RA 2019), boron (IS: 3025 Pt-2-2019), Total Salt Concentration (IS: 11624-2019), SAR (IS: 11624-2019), RSC (IS: 11624-2019), Ca^{2+} (IS:3025 Pt-40-1981, RA 2019) and Na^{+} (APHA 23rd Ed., 3111) concentration at Shriram Institute of Industrial Research, University Road, Delhi, an ISO-9001, 14001 & 45001 Certified Institute (Table 2).

Statistical analysis

The plant growth is directly related to the water quality, i.e., if the water quality is improved, *Lemna* would generate greater biomass when harvested. The percent increase in biomass in the second phase over the first phase was used to perform one tailed t-test using MS EXCEL 2021.

Results

Salt concentration in sewage water is significantly high even after fifteen days of phytoremediation (Table 2). The Sodium Absorption Ratio (SAR) was also high in sewage water. Calcium and sodium content increased in sewage water resulting in increase in alkalinity, hardness, salt concentration, and SAR. There is decrease in calcium and sodium content in pond water which results in decrease in alkalinity, hardness, salt concentration and SAR. However, in the tap water, there was a decrease in sodium and an increase in calcium content in water which resulted in only slight variations (if any) in SAR, salt concentration, alkalinity, and hardness. The pH changes in the pond and sewage water were not significant.

There was a decrease in boron concentration in sewage water, on the contrary, the boron concentration increased in tap and pond water after phytoremediation for fifteen days (Table 2). This might be due to high salt concentration in sewage water which caused damage to cell membranes hence there was no restriction in uptake of boron while in tap water and pond water salt concentration was less

Table 2. Water quality parameters of day 0 and day 14 (phytoremediated) water samples. Water samples were analysed by Shriram Institute for Industrial Research, University Road, Delhi.

	Water sample					
	Sewage		Pond		Tap	
	0 day	14 days	0 day	14 days	0 day	14 days
Salt concentration (µS/cm)	6080	7170	1817	1651	465	484
SAR (mmol /L)	8.0	9.7	3.4	2.8	0.8	0.8
RSC (meq./L)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Boron (mg/L)	0.5	0.3	0.1	0.2	0.1	0.3
Hardness	1550	1760	496	472	164	180
Alkalinity (in terms of $CaCO_3$)	824	717	249	185	113	109
Ca^{2+} Concentration	714	940	172	142	23	34
Na^{+} Concentration	312	357	120	51	39	23

and thus the cell membrane was not damaged which restricted the uptake of boron.

The growth rate of duckweeds was measured in terms of percentage increase in biomass which was 36.8%, 85.2%, 112.6% in sewage, pond and tap water, respectively in the first phase (Fig. 2). During the second phase there were 95.8%, 168.2% and 174% in sewage, pond and tap water, respectively, with p value of 0.05.

Discussion

The present study reveals interesting facts about *Lemna sp.* as a phytoremediator. As the results show, the growth of the duckweed in terms of biomass produced increases initially

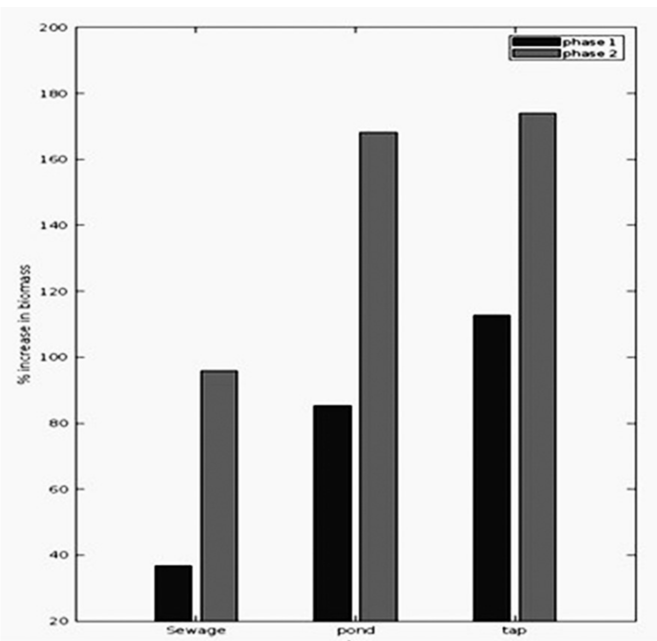

Fig. 2. Percentage increase in fresh weight of duckweed during first phase (day 0 to day 7; black) and during second phase (day 7 to day 14; grey)

but after three to four days it starts disintegrating in sewage water. After harvesting and adding fresh duckweed, the growth is resumed. *Lemna* has a life span, under optimal environment conditions, of about 1.5 days - 4.5 days and each mother plant undergoes a maximum six cycles of multiplication (Zeigler *et al.*, 2015). After that they disintegrate adding the pollutants back into the water body, and this might be the reason for rise in salt concentrations and hardness of water samples tested after fifteen days. Further, the salt and other nutrient concentrations are significantly high in sewage water; it allows *Lemna* to multiply for one or two cycles but if not harvested it starts disintegrating and releases all the pollutants back into water. Thus, to fully remediate the water, fresh duckweed is to be added after 4-5 days. Phytoremediation is promising, cost effective and easy to set up for cleaning water. The potential of the duckweed to phytoremediate water is also marred by the fact that the fragile plants can spread the pollutants to uncontaminated water bodies (Mkandawire & Dudel, 2007). *Lemna* is known to create anaerobic conditions when it multiplies fast and makes a mat over the water body.

The present study was done in the month of March when temperature ranges from 15 to 30^{0}C. Optimum growth of the duckweed occurs only in a small temperature range 10 to 30^{0}C (Mkandawire and Dudel 2005b). Hence months of extreme winters and summers are not conducive for such studies and the cleaning process gets restricted (Landesman *et al.*, 2005).

Thus, *Lemna* can be effective in the cleansing process if the water body which is subjected to phytoremediation is small. Phytoremediation mechanisms are needed to design effective and commercially viable treatment of the sewage water that is remediated to irrigate the potted plants. The plants have to be subjected to drying after they accumulate pollutants and this incurs additional energy and equipment costs. This limits the potential of the duckweed to be used as a phytoremediator on a commercial basis (Mkandawire and Dudel, 2007). Further optimization of the protocol and conditions to remediate water are required as the variation in the season does not allow the remediation to be done throughout the year.

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References

Ansari AA, Naeem M, Gill SS and AlZuaibr MF. 2020. Phytoremediation of contaminated waters: An eco-friendly technology based on aquatic macrophytes application, The Egyptian Journal of Aquatic Research. 46(4): 371-376. https://doi.org/10.1016/j.ejar.2020.03.002

BIS 1987. Indian standards: Guidelines for the quality of irrigation water IS 11624. Bureau of Indian Standards, New Delhi. https://law.resource.org/pub/in/bis/S06/is.11624.1986.pdf

Bokhari SH, Ahmad I, Mahmood-ul-Hassan M and Mohammad A. 2016. Phytoremediation potential of *Lemna minor* L. for heavy metals. International Journal of Phytoremediation. 18: 25-32. https://doi.org/10.1080/15226514.2015.1058331

Correll DS and Correll HB. 1972. Aquatic and wetland plants for Southwestern United States. Washington, DC: Environmental Protection Agency.https://doi.org/10.5962/bhl.title.4197

Ekperusi AO, Sikoki FD and Nwachukwu EO. 2019. Application of common duckweed (*Lemna minor*) in phytoremediation of chemicals in the environment: State and future perspective. Chemosphere. 223: 285-309. https://doi.org/10.1016/j.chemosphere.2019.02.025

Forni C and Tommasi F. 2016. Duckweed: A tool for ecotoxicology and a candidate for phytoremediation. Current Biotechnology. 5(1): 2-10. https://doi.org/10.2174/2211550104666150819190629

Fourounjian P, Fakhoorian T and Cao H. 2020. Importance of Duckweeds in Basic Research and Their Industrial Applications. In: The Duckweed genomes. Compendium of plant genomes. Eds. Cao X, Fourounjian P and Wang W. Springer, Cham. Pp: 1-17. https://doi.org/10.1007/978-3-030-11045-1_1

Frick H. 1985. Boron tolerance and accumulation in the duckweed: *Lemna minor*. Journal of Plant Nutrition. 8(12): 1123-1129. https://doi.org/10.1080/01904168509363411

Hopkins BG, Horneck DA, Stevens RG, Ellsworth JW and Sullivan DM. 2007. Managing Irrigation Water Quality for Crop Production in the Pacific Northwest. Covallis, Or, Oregon State University, Extension Service. https://ir.library.oregonstate.edu/concern/administrative_report_or_publications/w3763710v

Kalkhajeh YK, Amiri BJ, Huang B, Khalyani A H, Hu W, Gao H and Thompson ML. 2019 . Methods for Sample Collection, Storage, and Analysis of Freshwater Phosphorus. Water. 11(9):1889-1912. https://doi.org/10.3390/w11091889

Landesman L. 2000. Effects of Herbivory and Competition on Growth of Lemnaceae in Systems for Wastewater Treatment and Livestock Feed Production. A dissertation submitted to the University of Louisiana at Lafayette, Louisiana, 150 pp. https://www.proquest.com/docview/304674372?pq-origsite=gscholar&fromopenview=true

Landesman L, Parker NC, Fedler C and Konikoff M. 2005. Modeling duckweed growth in wastewater treatment systems. Livestock Research for Rural Development. 17(6):61.https://pubs.er.usgs.gov/publication/70029561

Liu C, Gu W, Dai Z, Li J, Jiang H, and Zhang Q. 2018. Boron accumulation by *Lemna minor L.* under salt stress. Scientific Reporter. 8(1): 8954. https://doi.org/10.1038/s41598-018-27343-y

Mkandawire M and Dudel EG. 2005a . Accumulation of arsenic in *Lemna gibba L.* (duckweed) in tailing waters of two abandoned uranium mines in Saxony, Germany. Science of the Total Environment. 336: 81-89. https://doi.org/10.1016/j.scitotenv.2004.06.002

Mkandawire M and Dudel EG. 2005b. Assignment of *Lemna gibba L.* (duckweed) bioassay for in situ ecotoxicity assessment. Aquatic Ecology 39: 151-165. https://doi.org/10.1007/s10452-004-5411-1

Mkandawire M and Dudel EG 2007. Are *Lemna* spp. effective phytoremediation agents?. Bioremediation, Biodiversity and Bioavailability. 1: 56-71.

Rusoff LL, BlakeneyEWJr, CulleyDDJr. 1980. Duckweeds (Lemnaceae family): a potential source of protein and amino acids. Journal of Agriculture and Food Chemistry. 28 (4): 848–850. https://doi.org/10.1021/jf60230a040

Zaman M, Shahid SA and Heng L. 2018. Irrigation Water Quality. In: Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Eds. Zaman M, Shahid SA and Heng L. Springer, Cham. 113-131.https://doi.org/10.1007/978-3-319-96190-3_5

Ziegler P, Adelmann K, Zimmer S, Schmidt C and Appenroth KJ. 2015. Relative in vitro growth rates of duckweeds (Lemnaceae)-The most rapidly growing higher plants. Plant Biology. 17(s1):33-41. https://doi.org/10.1111/plb.12184