



## Effect of Initial Plant Density on Growth and Nutrients Removal Efficiency of Duckweed (*Lemna minor*) from Leachate

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### Authors' contributions

This work was carried out in collaboration among all authors. Author JI designed and conducted this research and compiled the main draft of this research paper. Authors AJ and HJ contributed in laboratory analysis, data processing and formatting of manuscript. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AJEE/2021/v15i230225

#### Editor(s):

(1) Prof. Daniele De Wrachien, State University of Milan, Italy.

#### Reviewers:

(1) Consuelo Rojas Idrogo, Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Perú.  
(2) Kelli Evelin Müller Zortéa, Universidade do Estado de Mato Grosso Carlos Alberto Reyes Maldonado, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/66358>

Original Research Article

Received 01 January 2021

Accepted 06 March 2021

Published 15 June 2021

### ABSTRACT

An experiment was conducted by growing 25%, 50% and 100% initial densities of duckweed (*Lemna minor*) plants on dumpsite leachate under natural climatic conditions. *Lemna minor* (*L. minor*) growth and its ability to remove and absorb the nutrients (nitrogen and phosphorous) from leachate was investigated at each mat density. A simple mathematical model was developed to calculate the harvesting frequency (in days) of *L. minor* on leachate. The maximum growth rate ( $6.84 \pm 4.13 \text{ g m}^{-2}\text{day}^{-1}$ ) of *L. minor* was observed at 50% initial density of *L. minor* plants on leachate whereas, the nutrients removal from leachate was the highest at 100% initial cover of *L. minor* plants on leachate. At 100% density *L. minor* removed nitrogen at the rate of  $152.12 \pm 2.31 \text{ mgm}^{-2}\text{day}^{-1}$  total kjeldahl nitrogen (TKN) and phosphorous at the rate  $109.24 \pm 3.05 \text{ mgm}^{-2}\text{day}^{-1}$  total phosphorous (TP) from the leachate. Absorption of the nitrogen and phosphorous was also highest at 50% density when *L. minor* absorbed 86% of the total removed nitrogen and 77% of the

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total removed phosphorous into its biomass. At 100% density in addition to the absorption of nutrients by *L. minor*, factors such as nitrification/denitrification and, nitrogen and phosphorous assimilation by algae and microorganisms also account for the overall high rates of nutrients removal from leachate. Based on the results of this study, *L. minor* can be used as a potential aquatic plant for developing a cost-effective natural system of leachate treatment.

**Keywords:** Nutrients; phosphorous; nitrogen; nitrification; denitrification.

## 1. INTRODUCTION

Leachate is the concentrated form of wastewater [1]. At open dump sites, it is produced by the biochemical reactions within waste stream and percolation of rainwater through solid waste layers [2,3]. Depending on the nature of waste material, climatic conditions (temperature, sunlight and precipitation), solid waste management practices and age of landfill/dumpsites, leachate may contain a variety of pollutants including nitrogen, phosphorous and heavy metals [4]. Worldwide most of the parameters of leachate quality such as pH, Electrical Conductivity (EC), Chemical Oxygen Demand (COD), phosphate ( $\text{PO}_4^{3-}$ ), nitrate ( $\text{NO}_3^-$ ), chloride ( $\text{Cl}^-$ ) ions and heavy metals usually exceed the permissible limits of wastewater quality standards [5]. Heavily polluted leachate is one the emerging causes of surface and ground water contamination in developing countries [6].

Currently various physical-chemical processes such as coagulation-flocculation, chemical precipitation, floatation, adsorption, ion exchange, membrane filtration and electrochemical treatment are being used for the treatment of wastewater and leachate. Commonly used biological treatment methods are the activated sludge process, sequencing batch reactors, aerated lagoons, trickling filters, anaerobic filters, and phytoremediation etc. [7].

Phytoremediation is the use of plants to remove and detoxify the environmental contaminants. It is considered the least cost and efficient methods of wastewater treatment [8] having two-fold benefits: i) treatment of polluted media, and, ii) conversion of polluted nutrients into potentially useful biomass of aquatic plants [9]. Based on fate of contaminant in plant bodies, phytoremediation may take any one of the following forms [10,11]:

- Phytodegradation- breakdown of contaminants by enzymes or microorganisms (bacteria, fungi yeast etc.).

- Rhizofiltration is the uptake of contaminants by plant roots in wetlands areas.
- Phytoextraction- accumulation of contaminants into the roots and aboveground shoots or leaves of plants.
- Phytotransformation-uptake of organic pollutants from soil, water and sediments and transformed to less toxic, stable or less mobile forms.
- Phytostabilization-reduction in movement and migration of pollutants in environmental media.

Recently many types of aquatic plants particularly the duckweed have received attraction in wastewater treatment mainly due to the cost effectiveness and ease of the treatment operations [12]. Duckweed is a small floating macrophyte belonging to family Lemnaceae of monocotyledonous plants. Duckweed has 37 species belonging to 4 genera: i) *Lemna*, ii) *Spirodela*, iii) *Wolffia*, iv) *Wolffiella* [13]. Duckweed is the simple plant comprising a frond and one or more adventitious type roots [14].

Duckweed is amongst the promising aquatic plants having the ability to absorb large amounts of nutrients and trace metals from wastewater. and has high growth rates even under worse environmental conditions [15,16]. Duckweed can grow well at wastewaters with high nitrogen and phosphorus contents and has tremendous ability to uptake the nutrients from growth media while maintaining healthy plant growth [17]. Nutrient removal efficiencies and growth of duckweed plants are affected by many factors such as temperature, salinity, pH and nutrient concentration of growth media and, initial plant density [18]. The ammonium ion ( $\text{NH}_4^+$ ) is the most readily available form of nitrogen for duckweed. Nitrogen is fixed as protein in duckweed biomass. Assimilation of  $\text{NH}_4^+$  by duckweed fronds and roots appears to be the primary mechanism of nitrogen fixation in this plant [19]. Phosphorous (P) constitute about 1.5% of duckweed dry mass. Duckweed has the ability to accumulate high amounts of P in its

biomass through its fronds and roots. When duckweed dies, stored P in plant biomass is readily available in the water [20].

The *L. minor*, is the most widely spread specie of duckweed. *L. minor* under favorable conditions can double its biomass in two days forming dense mats on the surface of water body [17]. It is reported by Iqbal and Baig that *L. minor* can remove up to 62% nitrogen and 58% phosphorous from diluted leachate with the high rate of nutrients absorption into its body mass [21]. The *L. minor* can grow well at wastewaters with nitrogen and phosphorus levels as high as 240 mg NH<sub>4</sub>-N L<sup>-1</sup> and 31.0 mg PO<sub>4</sub>-P L<sup>-1</sup> respectively and can uptake the N and P from wastewater at the rates of 0.995 mg N L<sup>-1</sup>-h, and 0.129 mg P L<sup>-1</sup>-h, respectively while maintaining a steady growth rate of about and 1.33 g dry biomass/m<sup>2</sup>-h [22]. Bergmann et al. also concluded that *L. minor* species of duckweed are the best for treatment of high strength wastewaters with high biomass production and nutrient removal rates [23].

Efficient nutrient removal by *L. minor* is highly dependent on initial density of plants on growth medium and healthy cropping system [24]. Nitrogen and phosphorous removal from wastewaters by *L. minor*-based systems has strong relationship with initial density of *L. minor* mat and the overall performance of such systems can be predicted by the very judgmental role of incubation density [25,26]. Xu and Shen during a study on *L. minor*, identified that at 60% initial plant densities *L. minor* system was capable to remove about 83.7% of total nitrogen (TN) and 89.4% of total phosphorus (TP) from 6% swine lagoon water in eight weeks at a harvest frequency of twice a week [27].

Recently the *L. minor* based commercial wastewater treatment systems have successfully been practiced in many parts of the world such as Bangladesh and Argentina etc. *L. minor* based commercial treatment systems however require the efficient management of cultivation conditions and process optimization [24,28]. Having potential of wastewater treatment, *L. minor* can also be cost-effective and efficient means of leachate treatment [29].

Based on this fact, present study was designed to investigate the *L. minor* growth and its ability to remove nutrients (N and P) from dumpsite leachate at three different initial plant densities under the natural climatic conditions.

## 2. MATERIALS AND METHODS

Leachate used in this study was prepared in a plastic container of internal diameter, 1.5m and the vertical height of about 1.8 m. About 100 kg of solid waste was collected from each residential, commercial, and industrial dump site in Islamabad, Pakistan and mixed in the leachate container. Mixed solid waste was continuously agitated and sprayed with water for 60 days, after which the leachate was collected from bottom outlet. Table 1 provides the initial nutrients concentration and COD of the leachate used during this study.

Mixed culture of duckweed containing various species was collected from wastewater treatment pond located at National University of Sciences and Technology, Islamabad, Pakistan. Duckweed sample was identified by using duckweed guide of "Botanical Society of the British Isles" (BSBI), United Kingdome and *L. minor* plants were isolated from mixed culture. Isolated *L. minor* plants were acclimatized for 4-5 days under new environmental conditions.

Three different initial mat densities of *L. minor* (25, 50 and 100% plant cover) were grown on leachate with an initial concentration (in terms of COD) of about 1,563+6.53 mg L<sup>-1</sup>.

*L. minor* was grown in plastic containers of 300 ml volume capacity and surface area of 25.8 cm<sup>2</sup>. For each mat density, five (05) containers were used having 250 ml leachate in each container at an internal depth of 9.5 cm. *L. minor* containers were placed in open environment within a meshed iron rack under natural climatic conditions (average ambient air temperature: 34°C, solar radiations: 3.5-3.8 kWh m<sup>-2</sup> day<sup>-1</sup>, photoperiod: 11.8 hours). Climatic data for this study was obtained from Meteorological Department of Pakistan. The pH of leachate was maintained between 6.5 to 7.5 throughout the

**Table 1. Initial concentrations of nutrients and COD (mean ± SD, n=3) of leachate used as medium for *L. minor* growth**

TKN	Nutrients Concentration (mg L <sup>-1</sup> )			COD (mg L <sup>-1</sup> )
	NH <sub>4</sub> <sup>+</sup> -N	TP	o-PO <sub>4</sub> <sup>3-</sup> -P	
54.65 ± 2.59	32.77 ± 3.44	41.29 ± 4.49	9.29 ± 3.18	1,563 ± 6.53

experiment by using either 1M solution of hydrochloric acid (HCl) and sodium hydroxide (NaOH).

Experiment was lasted for seven (07) days during which samples from the leachate containers were analyzed for Total kjeldahl nitrogen (TKN), Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), Total phosphorous (TP), Orthophosphate ( $\text{o-PO}_4^{3-}\text{-P}$ ) and Chemical oxygen demand (COD) at the start and end of the experiment. The dry weight of *L. minor* was measured after drying the plants at 70 °C until constant weight. Dried *L. minor* mass was analyzed for total nitrogen (TN) with the help of total nitrogen test kit and TP content with the help of Phosphorus Portable Photometer - HI96706 – Hanna, before and after the experiment.

Data collected on all parameters was analyzed statistically using Fisher's analysis of variance (ANOVA) techniques under completely randomized design (CRD). Difference among treatment's means were compared using Least Significant Difference (LSD) test at 5% probability level. Microsoft Excel and Statistix-8.1softwares were used for statistical analysis.

### 3. RESULTS AND DISCUSSION

#### 3.1 *Lemna minor* Growth

*L. minor* exhibited a maximum growth rate of  $6.84 \pm 4.13 \text{ g m}^{-2}\text{day}^{-1}$  at 50% initial mat density (Table 2). At above and below the 50% density growth rate of duckweed was decreased. At 50% plant cover, *L. minor* plants have ample supply of nutrients in leachate and appropriate sunlight and temperature is available for the optimum plant growth. Comparatively lesser growth rate of *L. minor* at 25% initial plant density is possibly due to the inverse density dependence (Allee effect) which is probably due to the high rates of fluctuations in temperatures within partially filled and small sized *L. minor* containers in our experiment.

At 100% density crowding of *L. minor* plants within duckweed containers may retarded the overall growth rate. At high initial densities there may exist an increased competition among the available nutrients, sunlight, and other essential requirements for *L. minor* growth. Driever et al. reported that in a dense mat, *L. minor* fronds are piled up into several layers with two parts of each layer: an upper part with nutrient limitation and; a lower part with light limitation or  $\text{CO}_2$  limitation

resulting in overall decrease in growth rate of the *L. minor* plants [17]. A nonlinear inverse relationship between *L. minor* growth and high initial plant density has also been reported by Ziegler, et al. [28].

#### 3.2 Nutrient Removal from Leachate by *L. minor*

As shown in Table 3, the nitrogen and phosphorous removal was highest at 100% initial density of the *L. minor*. At 100% initial plant cover the *L. minor* removed TKN, TP and COD at the rate of  $152.12 \pm 2.31 \text{ mg m}^{-2}\text{day}^{-1}$ ,  $133.71 \pm 1.90 \text{ mg m}^{-2}\text{day}^{-1}$  and  $3.31 \pm 5.28 \text{ g m}^{-2}\text{day}^{-1}$  respectively from the leachate. As can be seen in Table 3, the removal rates of all nutrients increase with an increase in initial plant density of the *L. minor* on leachate suggesting a positive relationship between the *L. minor* density and nutrient removal. Similar trend of COD removal can also be noticed which is increased with an increase in initial densities of *L. minor* plants on leachate.

#### 3.3 Nutrients Absorption by *L. minor*

Fig.1 shows that *L. minor* has ability to absorb significant amounts of nitrogen and phosphorous into its biomass at all three densities however; the maximum absorption of N (86% of the total removed from leachate) and P (77% of the total removed) was recorded at 50% initial density of *L. minor* plants on leachate. This is consistent with the *L. minor* growth which is also high at 50% mat density. A comparison of Table 3 and Fig 1 reveals that the overall rates of nitrogen and phosphorous removal from leachate by *L. minor* is high at 100% mat density whereas, the uptake of these nutrients into the *L. minor* body mass is high at 50% initial density of *L. minor* on leachate. It is reported by (Cheng et al. that in the natural *L. minor* -leachate systems, beside the absorption by *L. minor* plants, a significant amount of nutrients and COD may also be removed by the processes such as; microbial and algal assimilations of nitrogen and phosphorous, removal of nitrogen by nitrification/denitrification and ammonia volatilization which seem more prominent in our experiment at 100% initial densities of *L. minor* plants in leachate containers [22]. Ammonia volatilization seems less prominent in our experiment as pH of the leachate was maintained at below 8 throughout the experiment. At 50% initial density there is also comparatively a less inter plant competition

among Lemna minor plants for space and sunlight etc. resulting in high nutrients absorption by *L. minor*.

### 3.3 Lemna minor Harvest Frequency Modeling

In view of the commercial application of *L. minor*-based leachate treatment system, it is necessary to adopt a harvesting strategy. Harvesting is necessary to maintain the healthy plat growth on leachate and to avoid the crowding effect. Crowding inhibits the *L. minor* growth by increasing the competition for light and nutrients among standing plants. Harvested *L. minor* may be used as the potential source of nitrogenous fertilizer for plant growth and as the food supplement by livestock and other animals provided it is free from heavy metals and other dangerous contaminants.

Harvesting frequency (HF) in days for any *L. minor* based natural leachate treatment system,

can be calculated with the help of following simple mathematical calculations.

$$HF \text{ (days)} = \left[ \frac{(100/D) \text{ Initial } L. \text{ minor density (\%)} \times (DM) \text{ Initial mass of } L. \text{ minor (g m}^{-2}) - \text{Initial mass (g m}^{-2})}{\{(GR) L. \text{ minor growth rate (g m}^{-2} \text{ day}^{-1})\}} \right] \dots\dots\dots 1$$

*L. minor* mass (DM) in g m<sup>-2</sup> can be calculated by following relationship:

$$DM \text{ (g m}^{-2}) = \frac{\text{Total mass of } L. \text{ minor plants grown on leachate (g)}}{\text{Surface area of growth container (m}^2)} \dots\dots\dots 2$$

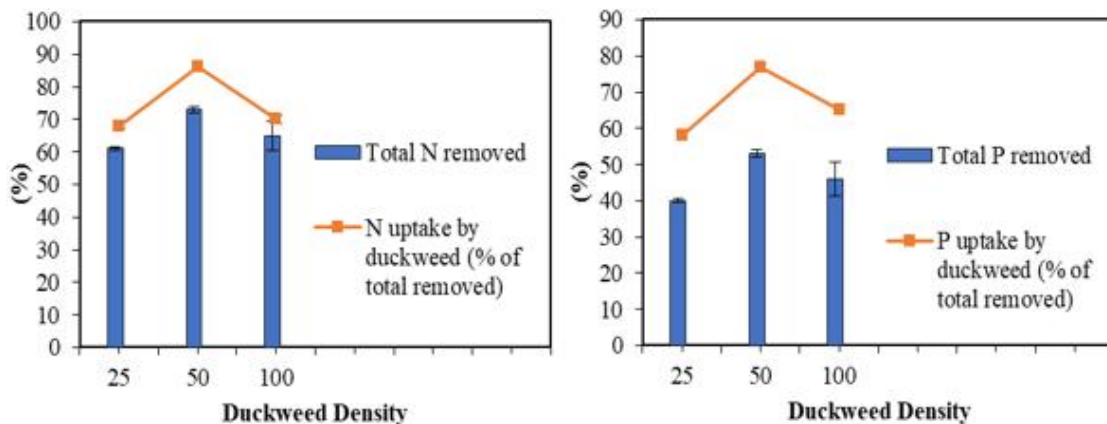
It is important to note that equations (1) and (2) can be applied for any system of units provided they are uniform in the numerators and de numerators. For example, initial *L. minor* mass may be taken as mgcm<sup>2</sup> instead of gm<sup>-2</sup>. Similarly, the day<sup>-1</sup> can be replaced with h<sup>-1</sup> (per hour), m<sup>-1</sup> (per minute) or year<sup>-1</sup> etc.

**Table 2. Growth rates of L.minor (mean ± SD, n=5) at three different initial plant densities on leachate under natural conditions**

L. minor density (% Cover)	L. minor mass mg (g)	Growth rate (g m <sup>-2</sup> day <sup>-1</sup> )
25	30 (0.03)	5.98 ± 2.23
50	60 (0.06)	6.84 ± 4.13
100	120 (0.12)	6.14 ± 2.22

**Table 3. Rates of nutrients removal and COD reduction (mean ± SD, n=5) from leachate at different stocking densities of L. minor under the natural climatic conditions**

L. minor Density (%)	Removal rates of nutrients (mg m <sup>-2</sup> day <sup>-1</sup> )				COD (g m <sup>-2</sup> day <sup>-1</sup> )
	TKN	NH <sub>4</sub> <sup>+</sup> -N	TP	o-PO <sub>4</sub> <sup>3-</sup> -P	
25	134.74 ± 2.91	117.04 ± 3.13	93.85 ± 3.57	30.10 ± 1.45	3.19 ± 7.81
50	141.69 ± 3.32	119.37 ± 1.91	97.60 ± 2.62	33.03 ± 2.54	3.24 ± 6.45
100	152.12 ± 2.31	133.71 ± 1.90	109.24 ± 3.05	38.78 ± 2.55	3.31 ± 5.28



**Fig. 1. Mass balance of total N and P removal and uptake by L. minor from leachate under natural climatic conditions.**

#### 4. CONCLUSION

Under the natural climatic conditions, *L. minor* maintained a steady growth on leachate at 25%, 50% and 100% initial densities. However, *L. minor* exhibited a maximum growth rate ( $6.84 \pm 4.13 \text{ g m}^{-2} \text{ day}^{-1}$ ) at 50% initial plant cover on leachate. Nutrients and COD removal from leachate were highest at 100% plant density of *L. minor* on leachate. *L. minor* absorbed highest amounts of nitrogen and phosphorous into its body mass at 50% mat density. This study recommends a 50% initial density for healthy growth and optimum removal of nutrients from leachate by *L. minor*. Through continuous harvesting and maintaining steady supply of nutrients in leachate, *L. minor* can be used as a potential aquatic plant for establishing a cost effective and easy to operate system of leachate treatment under natural conditions.

#### ACKNOWLEDGEMENTS

Authors are thankful to Higher Education Commission (HEC) of Pakistan for providing financial resources to conduct this study. We are thankful to Water and Wastewater Laboratory at Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology (NUST) for providing state of the art analytical facilities during this study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

- Brennan RB, Healy MG, Morrison L, Hynes S, Norton D, Clifford E. Management of landfill leachate: The legacy of European Union Directives. *Waste Manag.* 2016;55:355-63.
- Kalčíková G, Vávrová M, Zagorc-Končan J, Žgajnar Gotvajn A. Seasonal variations in municipal landfill leachate quality. *Manag. Environ. Qual.: An Int. J.* 2011;22:612-619.
- Vaccari M, Vinti G, Tudor T. An analysis of the risk posed by leachate from dumpsites in developing countries. *Environ.* 2018;5:99.
- Lombardi F, Costa G, Sirini P. Analysis of the role of the sanitary landfill in waste management strategies based upon a review of lab leaching tests and new tools to evaluate leachate production. *Revista Ambiente y Agua.* 2017;12: 543-555.
- Iqbal H, Baig MA, Hanif MU, Ali SU, Flury M. Leaching of metals, organic carbon and nutrients from municipal waste under semi-arid conditions. *Int. J. Environ. Res.* 2015;9:187-196.
- Xu Y, Xue X, Dong L, Nai C, Liu Y, Huang Q. Long-term dynamics of leachate production, leakage from hazardous waste landfill sites and the impact on groundwater quality and human health. *Waste Manag.* 2018;82:156-166.
- Chemlal R, Azzouz L, Kernani R, Abdi N, Lounici H, Grib H, Drouiche N. Combination of advanced oxidation and biological processes for the landfill leachate treatment. *Ecol. Eng.* 2014;73:281-289.
- Bove D, Merello S, Frumento D, Arni SA, Aliakbarian B, Converti AA. Critical review of biological processes and technologies for landfill leachate treatment. *Chem. Eng. Technol.* 2015;38:2115-2126.
- Saleem ZB, Laffray X, Al-Ashoor A, Ayadi H, Aleya L. Metals and metalloid bioconcentrations in the tissues of *Typha latifolia* grown in the four interconnected ponds of a domestic landfill site. *J. Environ. Sci.* 2017;54:56-68.
- Vidali M. Bioremediation. An overview. *Pure and Applied Chemistry.* 2001;73:1163-1172.
- Shinde S. Bioremediation. An overview. *Recent Research in Science and Technology.* 2013;5(5):67-72.
- Lasfar S, Monette F, Millette L, Azzouz A. Intrinsic growth rate: A new approach to evaluate the effects of temperature, photoperiod and phosphorus–nitrogen concentrations on duckweed growth under controlled eutrophication. *Water Res.* 2007;41:2333-2340.
- Sree KS, Bog M, Appenroth KJ. Taxonomy of duckweeds (Lemnaceae), potential new crop plants. *Emir. J. Food Agric.* 2016;28:291-302.
- Pandey A, Verma RK. Nutritional composition, Taxonomical and phytoremediation status of Duckweed (*Wolffia*). *Ann. Plant Sci.* 2018;7:1928-1931.
- Zhao Y, Fang Y, Jin Y, Huang J, Bao S, Fu T, et al. Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: A pilot-scale

- comparison with water hyacinth. *Bioresour. Technol.* 2014;163:82-91.
16. Ohlbaum M, Wadgaonkar SL, Van Bruggen JJ, Nancharaiah YV, Lens PN. Phytoremediation of seleniferous soil leachate using the aquatic plants *Lemna minor* and *Egeria densa*. *Ecol. Eng.* 2018;120:321-328.
  17. Driever SM, Van Nes EH, Roijackers RM. Growth limitation of *Lemna minor* due to high plant density. *Aquat. Bot.* 2005;81:245-251.
  18. Lu Q, He ZL, Graetz DA, Stoffella PJ, Yang X. Phytoremediation to remove nutrients and improve eutrophic storm waters using water lettuce (*Pistia stratiotes* L.). *Environ. Sci. Pollut. Res.* 2010;17:84-96.
  19. Leng RA, Stambolie JH, Bell R. Duckweed-a potential high-protein feed resource for domestic animals and fish. *Livest. Res. Rural. Dev.* 1995;7(1):36.
  20. Campbell NA, Reece JB, Mitchell LG. *Biology*; 2002.
  21. Iqbal J, Baig MA. Nitrogen and phosphorous removal from leachate by duckweed (*Lemna minor*). *Environ. Prot. Eng.* 2017;43:123-134.
  22. Cheng J, Landesman L, Bergmann BA, Classen JJ, Howard JW, Yamamoto YT. Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. *Transactions of the ASAE.* 2002;45:1003-1010.
  23. Bergmann BA, Cheng J, Classen J, Stomp AM. Nutrient removal from swine lagoon effluent by duckweed. *Transactions of the ASAE.* 2000;43:263-269.
  24. Ge X, Zhang N, Phillips GC, Xu J. Growing *Lemna minor* in agricultural wastewater and converting the duckweed biomass to ethanol. *Bioresour. Technol.* 2012;124:485-488.
  25. Liebhold A, Bascompte J. The Allee effect, stochastic dynamics and the eradication of alien species. *Ecol. Lett.* 2003;6:133-140.
  26. Frederic M, Samir L, Louise M, Abdelkrim A. Comprehensive modeling of mat density effect on duckweed (*Lemna minor*) growth under controlled eutrophication. *Water Res.* 2006;40:2901-2910.
  27. Xu J, Shen G. Growing duckweed in swine wastewater for nutrient recovery and biomass production. *Bioresour. Technol.* 2011;102:848-853.
  28. Ziegler P, Adelman K, Zimmer S, Schmidt C, Appenroth KJ. Relative in vitro growth rates of duckweeds (*Lemnaceae*)—the most rapidly growing higher plants. *Plant Biology.* 2015;17:33-41.
  29. Gatidou G, Oursouzidou M, Stefanatou A, Stasinakis AS. Removal mechanisms of benzotriazoles in duckweed *Lemna minor* wastewater treatment systems. *Sci. Total Environ.* 2017;15:596-597.

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