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Evaluation of *Lemna minor* phytoremediation performance for the treatment of dairy wastewater

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ABSTRACT

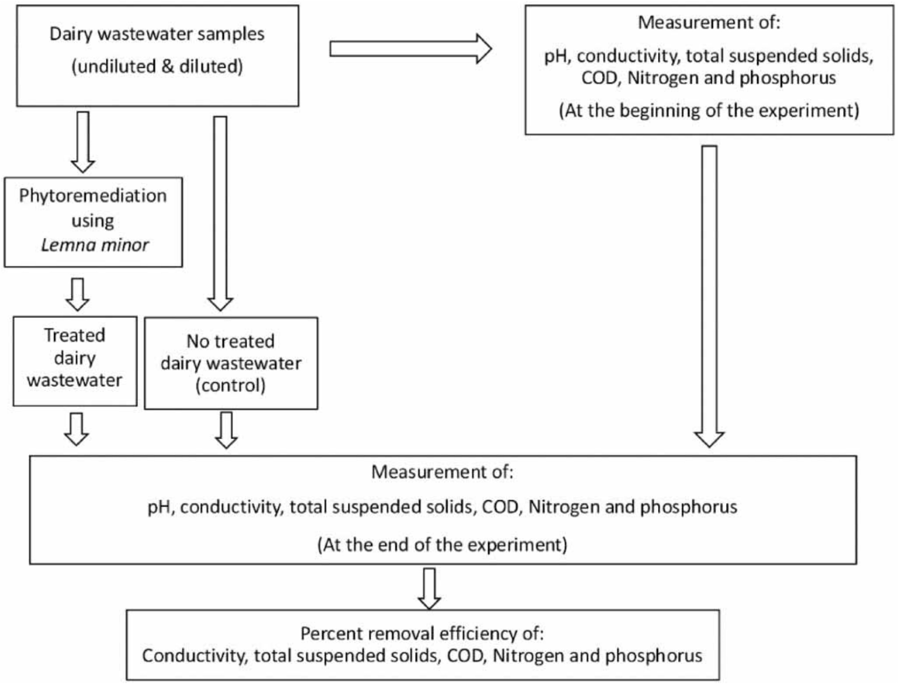
The aim of the study was to evaluate the efficiency of *Lemna minor* to purify synthetic dairy wastewater (SDW) and real dairy wastewater (RDW), both diluted and undiluted. Results showed that *Lemna* significantly reduced electrical conductivity (EC); the removal efficiency for EC was 19.4% in RDW and 9.8% in SDW. Higher values were recorded for diluted real dairy wastewater (DRDW) (41%) and for diluted synthetic dairy wastewater (DSDW) (35.8%). Total suspended solids were slightly reduced in RDW (14%) and SDW (13.5%), compared to DSDW (25%) and DRDW (29%). Greater removing efficiency of chemical oxygen demand was recorded in DRDW (60%) and DSDW (55.5%) compared to RDW (27.6%) and SDW (27%). In RDW, nitrogen and phosphorus removal were 24.8 and 37.4% and in SDW were 25 and 48.6%, respectively, compared to much higher results in diluted effluents, and nitrogen removal was 65.4% in DRDW and 63.5% in DSDW, but the best results were recorded for phosphorus removal in DSDW (78.8%) and DRDW (87%). The higher elimination of all the parameters investigated in the units populated with duckweed compared to the control attests to the effectiveness of *L. minor* in the phytoremediation of dairy wastewater, especially when reducing the primary pollutant load.

Key words: dairy wastewater, duckweed, effluent, *Lemna minor*, phytoremediation, removal efficiency

HIGHLIGHTS

- Untreated dairy wastewater poses a major threat to the environment.
- Conventional techniques for treating dairy wastewater are less practical and expensive.
- Phytoremediation is an eco-friendly method and a good alternative to treat dairy wastewater.
- Phytoremediation is a solution from nature itself.
- *Lemna minor* has shown promising outcomes in treating dairy wastewater.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The dairy industry is one of the most important components of the food industry worldwide. In recent years, this sector has flourished due to population growth and increased demand for dairy products in many parts of the world (Schierano *et al.* 2020); moreover, this flourishing has led to excessive consumption of water and consequently the production of huge amounts of wastewater. It was estimated that the processing of 1 l of milk generates 0.2–10 l of effluent (Silva *et al.* 2019)**,** which means that 4–11 million tons of wastewater are discharged into nature without prior treatment, causing a serious threat to biodiversity (Garg *et al.* 2020).

A thorough understanding of the characterizations of the derived wastewater is essential to the deployment of a given treatment technique and ensuring its performance. Dairy effluent is made up of milk fractions (2% of total processed milk) or processed products (Ashekuzzaman *et al.* 2019), and is typically characterized by unpleasant odors resulting from the whey protein's quick degradation (Kushwaha *et al.* 2011), high organic matter levels, relatively high biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved and suspended solids, nutrients including nitrogen (N), phosphorus (P), and various cleaning agents of an acidic and alkaline nature, leading to large variations in potential hydrogen (pH) values. It may also contain fats and oils that could create a film on the water's surface, preventing the transfer of oxygen to aquatic life and thereby endangering their survival (Ahmad *et al.* 2019).

The typical method of treating dairy wastewater is physical–chemical treatment (Ritambhara *et al.* 2019; Silva *et al.* 2019). Due to their effectiveness in removing colloidal and suspended materials, coagulation and flocculation are still the most frequently used methods at the step level (Carvalho *et al.* 2014). However, they may cause second-level pollution due to the use of chemicals such as aluminum sulfate, so they are applied as a pre-treatment (Wang & Serventi 2019).

In addition to physicochemical techniques, biological technologies are thought to be the most common way to refine dairy effluents, which are known for having easily biodegradable, nontoxic organic pollutants, or nutrients (Ritambhara *et al.* 2019). Biological methods involve both aerobic and anaerobic treatment techniques, which have some drawbacks. For example, while some nutrients are partially degraded by anaerobic treatments, aerobic treatments such as activated sludge, trickling filters, and membrane bioreactor procedures require a lot of energy, high operational and maintenance costs, as well as physicochemical methods (Ahmad *et al.* 2019).

To rid aquatic ecosystems of dairy contaminants, it was crucial to use phytoremediation, a new low-cost cleaning technology for wastewater. It is an economical, straightforward, and biological solution that is safe for the environment (Ghaly & Farag 2007). According to Sadowsky (1999), current non-biological remediation methods cost 4–1,000 times as much per volume as biological methods like phytoremediation. Several authors define phytoremediation as the process by which specific plants and the microorganisms that they are associated with can naturally bioaccumulate, degrade, or render less dangerous pollutants in water, air, and soil, as well as change the associated soluble chemicals into less soluble or less toxic forms to prevent movement or toxicological effects.

In the literature, it has been noted that phytoremediation has been used to treat a variety of wastewater types, including dairy wastewater, and that various aquatic plants, also called macrophytes, were applied to treat industrial wastewater. According to research, duckweed is one of the most promising aquatic families for phytoremediation and is widely dispersed in aquatic habitats around the world. It is applied in both domestic and industrial wastewater treatment systems due to its very high phytoremediation potential for a variety of toxins, including organic and inorganic pollutants (Ekperusi *et al.* 2019).

The purpose of this study is to assess the effectiveness and appropriateness of aquatic duckweed, *Lemna minor*, to treat dairy wastewater.

2. MATERIALS AND METHODS

2.1. Preparing and collecting dairy wastewater

The present study was carried out in two sorts of dairy wastewater: real dairy sewage and synthetic dairy sewage. Synthetic dairy wastewater (SDW) based on dairy wastewater composition was prepared in the laboratory by dissolving 4 g of dried milk powder of the Loya brand per liter of tap water, and this method was adopted by Kushwaha *et al.* (2011). According to Ahmad *et al.* (2019), and Ashekuzzaman *et al.* (2019), the SDW has the same properties as dairy wastewater at this rate. The real dairy wastewater (RDW) was collected from the dairy industry unit of Beni Tamou (Lactalis), located in the industrial area, 10 km north of Blida city, Algeria. The selected dairy unit has a milk processing capacity of 340,000 l/day and an average wastewater generation of 46,000 l/day. Thus, 20 l of SDW and RDW samples were kept at a temperature of 4 °C in plastic containers.

The COD of the samples was found to be 2,098 and 3,608 mg/l for the RDW and the SDW, respectively. Munavalli & Saler (2009) reported that aquatic plants cannot tolerate organic COD loading beyond 1,000 mg/l, so from each dairy wastewater, two effluents were prepared, one without dilution and the other diluted to 50% with tap water (w). Initial concentrations of the following effluent parameters were measured: pH, EC, total suspended solids (TSS), COD, total nitrogen (TN), and total phosphorus (TP). The findings are shown in Table 1.

Table 1 | Initial sample characterization and quality requirements for wastewater discharges from the dairy industry to the environment

Parameters	RDW	DRDW	SDW	DSDW	Discharge limit
pH	5 ± 0.1	7 ± 0	6.4 ± 0	7.9 ± 0.1	6–9
EC (μS/cm)	4,723 ± 3.2	2,523 ± 3.1	4,073 ± 5	2,930 ± 2.5	
TSS (mg/l)	1,087 ± 3.1	899 ± 2.5	1,065 ± 5	891 ± 4.2	50
COD (mg/l)	2,099 ± 1.6	1,121 ± 3	3,608 ± 4	1,141 ± 4.5	250
TN (mg/l)	593 ± 2.3	342 ± 0.3	782 ± 6.2	415 ± 2	10
TP (mg/l)	293 ± 3.2	135 ± 3.2	63 ± 2.6	26 ± 5.1	2

Mean ± SD, $n = 3$. Discharge limit, according to the 2007 World Bank report.
RDW, real dairy wastewater; DRDW, diluted real dairy wastewater; SDW, synthetic dairy wastewater; DSDW, diluted synthetic dairy wastewater; pH, potential hydrogen; EC, electric conductivity; TSS, total suspended solids; COD, chemical oxygen demand; TN, total nitrogen; TP, total phosphorus.

2.2. Supply and preparation of the duckweed

Duckweed *(L. minor)* colonies were collected from an artificial freshwater pond in the Test Garden of Hamma, Algiers, Algeria. The plant was identified through morphology observation with one, two, or three green oval-shaped fronds, each having a single root hanging in the water.

The plant was selected as a good model plant for phytoremediation because of its small size, rapid growth, and ease of cultivation, as evidenced by Tkalec *et al.* (1998). *L. minor* is a small floating plant with a single white central rootlet that is up to 2 cm tall and one or more green leaves that are 2–4 mm in diameter, known as fronds. It reproduces vegetatively by simply splitting into separate individual plants to create a dense layer in nutrient-rich fresh and brackish waters (Al-Snafi 2019). The frond doubling time, according to Frick (1985), is around 1.4 days.

Lemna was brought to the laboratory in plastic bags, cleaned thoroughly to remove dust and insect larvae, disinfected by soaking it in a 0.5% sodium hypochlorite solution for 1–2 min, and then gently rinsed with water. *Lemna* was then grown for 15 days in a bucket of fresh tap water for acclimatization and to normalize its growth in a thermostatic chamber (at 22 ± 2 °C) with a photoperiod of 16 h of light (4,000 Lux) and an 8 h dark cycle.

2.3. Experimental setups

The experiments were setup in the plant improvement laboratory of the Nature and Life Sciences faculty at Blida University, Algeria, while the dairy wastewater's physicochemical characteristics were assessed in the Chenoua wastewater treatment plant laboratory in Tipaza, 70 km west of Algiers, Algeria.

Duckweed (*L. minor*) was tested to see how effective it was at purifying dairy wastewater under controlled conditions. In total, 40 duckweed colonies were transferred to glass flasks holding 400 ml of culture medium: SDW, RDW, diluted synthetic dairy wastewater (DSDW), and diluted real dairy wastewater (DRDW), with SDW* and RDW* as controls without duckweed.

To avoid excessive water evaporation, all flasks were wrapped at the bottom with plastic wrap with holes and placed in a thermostatic chamber with the same settings as during the pre-treatment test. Every culture medium setup was duplicated three times for each collection day. This experiment used a total of 72 setups.

SDW* and RDW* were designed to examine changes in effluent properties in the absence of *L. minor*, as well as to assess its uptake capability. For 10 days, water samples were taken on the first, fourth, seventh, and tenth days to measure the physicochemical parameters indicated above.

2.4. Analytical methods

pH and EC were measured using, respectively, the Hanna pH/ORP meter Model HI 2211 (accuracy ±0.01 pH and (Hanna HI 2315) conductivity meter (accuracy at 20 °C: ±1% of full scale except for probe error), the electrical conductivity and pH probes were calibrated, respectively, in the range 0–10 μS/cm, and pH 4 and 7, using standard solutions. TSS were quantified by U.S. EPA Method 160.2 (USEPA 1999) identical in technique to the filtration through glass-fiber method filters (ISO 11923:1997). COD was measured by the dichromate method (ISO 6060:1989). Organic nitrogen was measured by the macro-Kjeldahl method after mineralization with selenium (ISO 5663:1984) and phosphorus by the ammonium molybdate spectrometric method (ISO 6878:2004). The measurement was done in triplicate, and the mean value was taken.

Removal efficiency (R) in percent for EC, TSS, COD, TN, and TP was calculated based on the following equation used by Kamyab *et al.* (2017):

$$R(\%) = \left[\frac{(C_o - C_t)}{C_o}\right] \times 100$$

where C_o and C_t represent, respectively, the concentration at the beginning of the experiment and at the time of interest t.

2.5. Analysis of data

Statistical analysis was processed using SPSS software (IBM SPSS Statistics, Version 25). The significance of the role of *L. minor* in treating dairy wastewater was analyzed using a one-way analysis of variance (ANOVA). An LSD post hoc test with 95% confidence intervals ($p \leq 0.05$) was used for testing differences between the means. Besides, Pearson's correlation coefficient analysis was used to highlight potential relationships among the measured parameters studied. Graphs were drawn using Origin Pro-2021 (Origin Lab Corporation, Northampton, MA, USA), and all values shown in the figures are mean ± standard deviation ($n = 3$).

3. RESULTS AND DISCUSSION

3.1. pH and EC of treated wastewater

The variation of pH and EC values throughout the experiment, as well as the removal rate of EC, are shown in Figures 1 and 2, respectively.

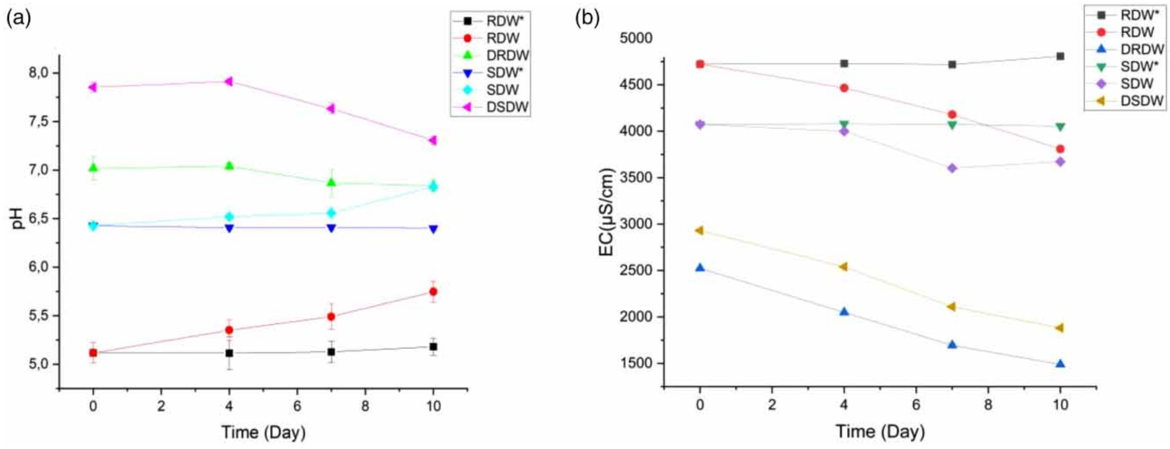


Figure 1 | Mean values of pH and EC of wastewater treatments and control. Initial concentrations are indicated at time (0).

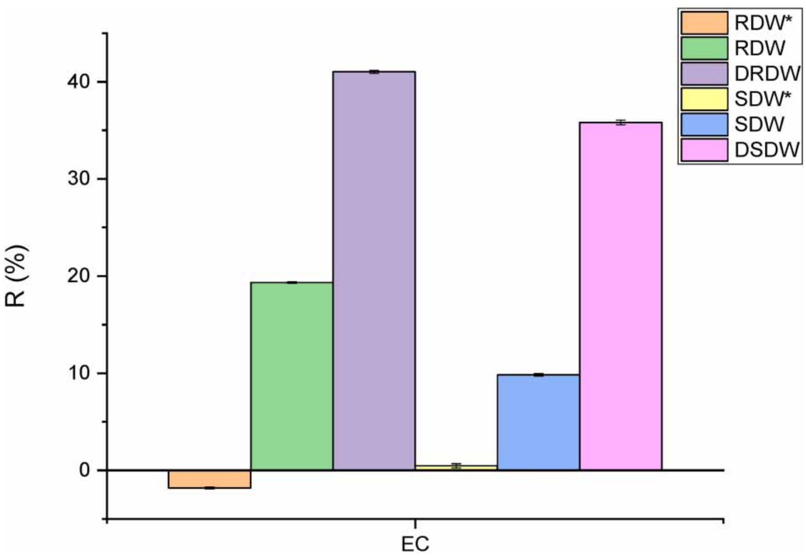


Figure 2 | Percentage removal for EC.

The pH level during phytoremediation appears to be a highly essential factor since it impacts the adsorption and desorption of pollutants as well as their availability to plant roots and their transport in cells, and it is also essential that the pH remains within the proper ranges for plant growth (Chen & Huang 2003). As is the situation in the current study, the pH value of the effluent to be treated is advantageous for the growth of *Lemna*, which requires, according to Leng *et al.* (1995), a pH range from 5 to 9.

Table 1 indicates that initial pH values for the undiluted effluents, real and synthetic, were in the acidic range (5 and 6.4, respectively), whereas for the diluted effluents, the values tended to rise toward neutral pH, DRDW (7), and DSDW (7.9), obviously as a result of using tap water with an alkaline pH equal to 8.

At the end of the experiment, we find that, unlike the *Lemna*-treated effluents, the values of the controls shifted toward the acidic range with minor variations. The RDW* pH increased to 5.2 and the SDW* pH decreased to 6.4, this was confirmed by the one-way repeated measures ANOVA post hoc test, which showed that the pH values of the *Lemna*-treated effluents samples were statistically different from those found for the controls ($p \leq 0.05$) and that there were significant differences between the diluted and undiluted treated effluents ($p \leq 0.05$), the pH increased in the undiluted effluents, RDW (5.8) and SDW (6.3) to always remain within the acidic range, and decreased in the diluted effluents DRDW (6.5) and DSDW (7.3) as shown in Figure 1(a).

EC is a general indicator of water quality that is related to the amount and kind of dissolved salts and can be used to track wastewater treatment procedures. The current study found that EC decreased in all treatment units compared to controls, along with a marked reduction in diluted effluents. One-way, repeated measures ANOVA analysis revealed a statistically significant difference between *L. minor* treatments and controls for the percent removal of EC ($p \leq 0.05$), and post hoc test comparisons revealed a statistically significant difference between the values of diluted and undiluted treated dairy effluents ($p \leq 0.05$).

According to the results shown in Figures 1(b) and 2, the conductivity of the control units remained quite stable, it showed very little fluctuation. EC increased slightly by 1.8% in RDW* and declined only by 0.5% in SDW*.

Higher removal rates were recorded in the treated units in RDW and SDW, respectively, EC decreased by 19.4 and 9.8%. However, the highest removal rates were found in the DRDW (41%), and DSDW (35.8%), which are comparable to the outcomes reached by Amare *et al.* (2018) for the same test duration. Levlin (2010) reported that the variation of conductivity in wastewater can be caused by variations in the ion content (hydrogen $H+$, hydroxide $OH-$, and nutrients such as phosphate and nitrate), and the main processes that reduce it are biological nutrient removal. Moreover, Dipu *et al.* (2011) attested that the decrease in EC that occurs during phytoremediation is caused by the heavy uptake of nutrients by the duckweed. It was also noted that the conductivity value's gradual decline was influenced by the initial conductivity value and the initial low conductivity value fell more than the initial high conductivity value.

3.2. TSS and COD removal

The efficiency of *L. minor* in removing TSS, and COD is observed. The results are shown in Figures 3 and 4.

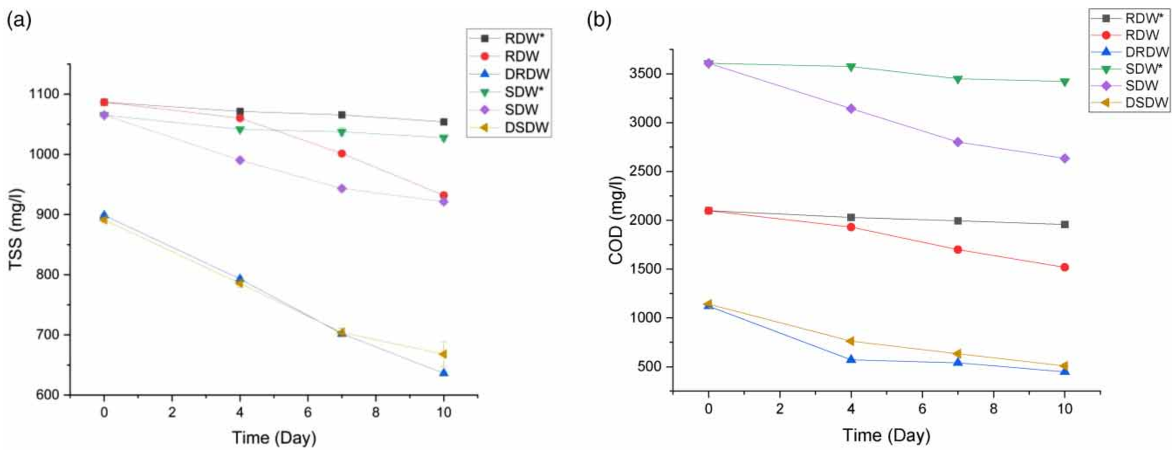


Figure 3 | Mean values of TSS and COD of wastewater treatments and control. Initial concentrations are indicated at time (0).

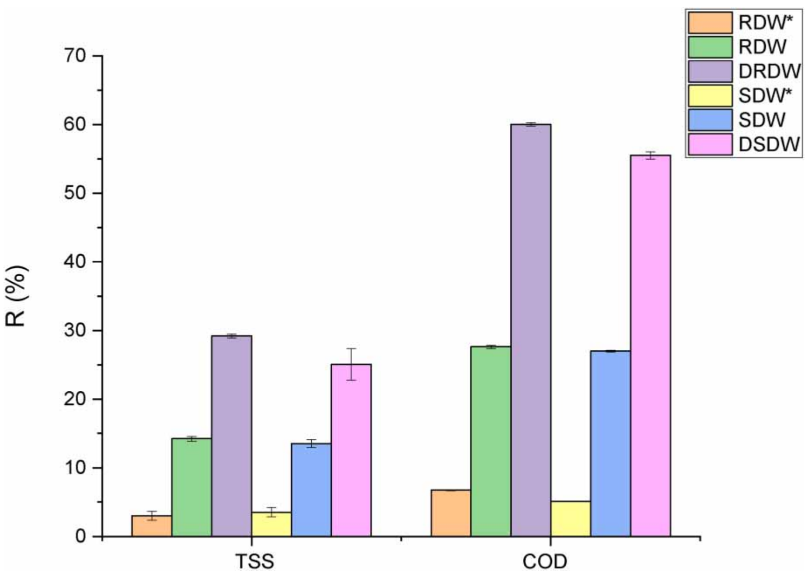


Figure 4 | Percentage removal for TSS and COD during the experimental period.

According to Figure 3(a), the reductions in the values of TSS in both real and simulated dairy effluents had a very identical course. A very small and almost similar decrease was recorded for the controls, RDW* (3%) and SDW* (3.5%), which are considerably different from the results observed in the treated effluent, as confirmed by the one-way repeated measures ANOVA post hoc test, which showed that *Lemna* was able to remove significant values of TSS from the dairy effluent ($p \leq 0.05$); furthermore, statistical analysis revealed that dilutions had a significant impact on *Lemna*'s capability to reduce TSS from water samples ($p \leq 0.05$). As indicated in Figure 4, TSS decreased by 13.5 and 14.2%, respectively, in undiluted SDW and RDW while decreasing by 25 and 29.2%, respectively, in DSDW and DRDW. These findings are corroborated by Krishna & Polprasert (2008), who claim that duckweed roots serve as filters by trapping suspended particles in the water and thereby lowering their levels. However, these findings appear to be insufficient given the high level of organic matter in the dairy effluent, which reduces duckweed's effectiveness. Additionally, it was stated by Akansha *et al.* (2020) that phytoremediation should be preceded by conventional techniques, such as membrane filtration or sand filtration, or, in extremely high TSS concentrations, by the use of electrocoagulation, which is an effective option that offers high removal at various concentrations of TSS.

The variations in COD as a function of time for each treatment (duckweed and control) are shown in Figure 3(b), and the efficiency of removal is shown in Figure 4.

COD is a critical indicator in wastewater monitoring and pollution studies. It shows the organic strength of the effluent (Ritambhara *et al.* 2019) as well as the amount of oxygen needed to cleanse it. Logically, these data are used to track and enhance water treatment performance.

Compared to initial concentrations, a slight and similar decrease in the COD was recorded in *Lemna*-free units (6.7% for RDW* and 5.1% for SDW*, respectively), and the one-way repeated measures ANOVA post hoc test indicated that the results obtained are significantly different from those found for the effluents treated with *Lemna*. Additionally, the test revealed that highly significant differences were found between diluted and undiluted treated effluent ($p \leq 0.05$).

Undiluted treated effluent with a high initial COD showed a slight decrease in RDW during the first 4 days, followed by a gradual and more noticeable decrease until the end of the experiment when the COD reached 2,099 mg/l, whereas a steady and progressive decline was seen in SDW throughout the course of the study, reaching 2,635 mg/l. The recorded removal rates of 27.6% for RDW and 27% for SDW were much lower than those found by Amare *et al.* (2018).

The COD variation in the diluted effluent followed the same pattern, with a bigger decline during the first 4 days, followed by a lesser decrease until the experiment ended. At the end of the study, the highest percent removal was recorded for DRDW (60%), followed by a rate of 55.5% for DSDW. It is noted that these results are very similar to those observed by Dipu *et al.* (2011) for the same treatment duration and quite similar initial organic loads.

A strong positive correlation that was statistically significant ($p \leq 0.05$) was observed between COD and TSS, which was also confirmed by Krishna & Polprasert (2008). This is most probably explained by the fact that *Lemna* participated in the removal of suspended particles, which are most of the time loaded and thus induce a decrease in the COD load in the water.

3.3. TN and TP removal

Losses of nitrogen compounds and phosphorus from the dairy industry present a serious problem as they contribute to increased eutrophication in receiving water bodies. They have been estimated by Ritambhara *et al.* (2019) to be in the order of 4.2–6% for TN and 0.6–0.7% for TP.

Several authors have reported that the ability of floating plants to remove nutrients by adsorption is governed by many factors, including the plant's growth performance, total biomass per unit area, effluent composition, the chemical forms in which the nutrients are available, as well as various physicochemical and biochemical mechanisms related to the water–root interface.

Table 2 displays the variations of nitrogen and phosphorus over time for each treatment (duckweed and control), while Figure 5 illustrates the effectiveness of nitrogen and phosphorus removal.

Changes in nitrogen and phosphorus from the initial concentrations were minor in the control effluent. RDW* had a very small decrease in phosphorus and nitrogen content (4.4 and 6.4%, respectively), whereas SDW* showed very stable values with a removal rate of 1.5% for N and 2% for P. The one-way repeated measures ANOVA post hoc test revealed that these results differed significantly from those observed in the *Lemna*-treated

Table 2 | Nutrient concentration of the effluent samples during the experiment period (mg/l)

		Parameters (mean ± SD) ($n = 3$)	
Treatment	**Time (day)**	**TN**	**TP**
RDW*	0	592.7 ± 2.3	292.7 ± 3.2
	4	588 ± 2	285 ± 5.3
	7	574.3 ± 1.2	279 ± 1.7
	10	566.3 ± 3.1	274 ± 4
RDW	0	592.7 ± 2.31	292.7 ± 3.2
	4	533.7 ± 0.6	222 ± 3.6
	7	510.3 ± 1.5	190 ± 5.3
	10	446 ± 3	183.3 ± 4.
DRDW	0	341.7 ± 2.9	134.7 ± 3.2
	4	233 ± 4.4	85 ± 2.7
	7	186.3 ± 3.1	43.3 ± 3.1
	10	118.3 ± 3.2	17.3 ± 2.1
SDW*	0	782 ± 6.2	63 ± 2.6
	4	775 ± 3.6	62.3 ± 2.
	7	770.7 ± 2.1	62 ± 1
	10	770 ± 6.1	61.7 ± 1.5
SDW	0	782 ± 6.2	63 ± 2.7
	4	695.3 ± 5	56 ± 3.2
	7	618 ± 8.2	41.3 ± 2.5
	10	586 ± 5.6	32.3 ± 21
DSDW	0	415 ± 2	25.7 ± 5.1
	4	372 ± 2.6	19.3 ± 2.1
	7	230.3 ± 3	8.7 ± 2.1
	10	151.7 ± 2.1	5.3 ± 0.6
Discharge limit		10	2

Discharge limit, according to the World Bank report (2007).
RDW, real dairy wastewater; DRDW, diluted real dairy wastewater; SDW, synthetic dairy wastewater; DSDW, diluted synthetic dairy wastewater; DRW* and SDW*, control samples; TN, total nitrogen; TP, total phosphorus.

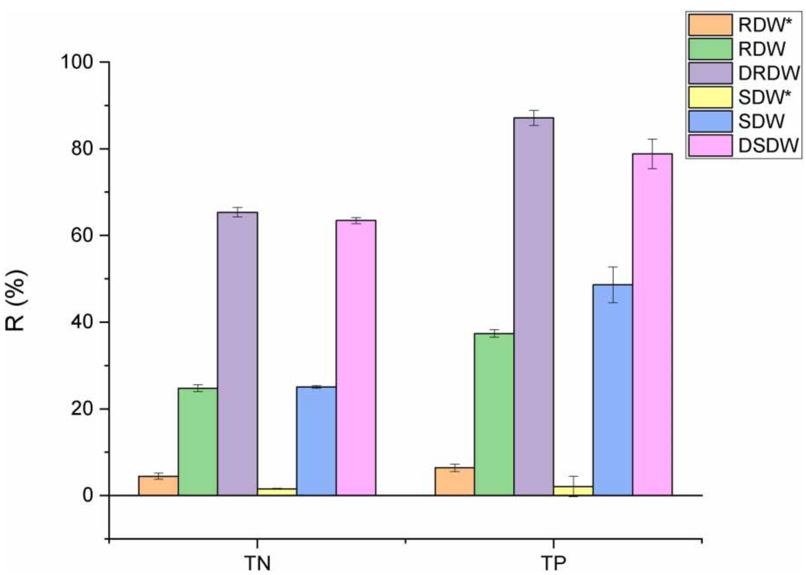


Figure 5 | Percentage removal for TN and TP during the experimental period.

units ($p \leq 0.05$), confirming *Lemna*'s high nutrient uptake capacity. The statistical study ($p \leq 0.05$) also confirmed the dilution effect, as we observed higher P removal values in the diluted effluents. In the case of undiluted effluents, a progressive decrease in N concentration was observed, with relatively low removal rates recorded (24.8% at RDW and 25% at SDW), whereas P removal rates were higher (37.4% at RDW and 48.6% at SDW). The

Lemna adsorption of both nutrients was valuable in diluted effluents. The removal of N was fast and followed the same pattern at DRDW and DSDW, where removal efficiencies were quite close (65.4 and 63.5%) respectively, and comparable with those reported by Amare *et al.* (2018). A drastic decrease in P was observed in both effluents, with removal rates comparable to those achieved by Amare *et al.* (2018). The highest removal efficiency was recorded in DRDW (87%), followed by 78.8% in DSDW, where the content of P reached 5.3 mg/l, which is probably due to the low initial levels of P in the simulated effluent. It should be noted that these interesting P uptake values are most likely related, according to Farrell (2012), to the phosphorus hyper-accumulation of duckweed.

A statistically significant positive correlation between EC, TN, and COD was observed, since COD removal is significantly influenced by various variables, including pH, suspended particulate matter, retention time, high organic loading rates, and TN and P percentage removals (Tekerlekopoulou *et al.* 2020). Furthermore, floating plants promote the precipitation phenomenon, which leads to increased nutrient uptake (Tanner & Headley 2011).

4. CONCLUSION

The current study was able to demonstrate the water purifying ability of the small macrophyte *L. minor*, since it reaches very promising absorption rates, especially for COD, nitrogen, and phosphorus.

Both previous works and current results showed that phytoremediation by *Lemna*, an eco-friendly and low-cost process, is very effective to polish dairy wastewater; however, it has the drawbacks of being slow and requiring relatively long periods to reach the desired removal rates, which makes it more suitable to be used as a secondary or tertiary treatment after having applied techniques that allow reducing the polluting load. As reported by Jing *et al.* (2002), the efficiency of a system based on live plant treatment is generally improved by lowering the initial pollutant load.

Additional research and larger-scale, long-term studies should be conducted to determine *Lemna*'s maximum capacity as a treatment agent for dairy wastewater and shift phytoremediation from a laboratory-scale theoretical model backed by scientific proof to feasible, field-practiced technologies.

ACKNOWLEDGEMENTS

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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