

Original Research Articles

Treatment of leachate solid wastewater and protein-rich biomass production using *Ceratophyllum demersum* (Linnaeus, 1753)

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The present study, conducted in 2018, aimed to treat landfill leachate using the *Ceratophyllum demersum* plant and convert the wastewater nutrients into protein-rich biomass. The experiment was carried out for 45 days in an enriched medium with ½ Hoagland nutrient solution. Regarding the experimental setups, *C. demersum* cultivation was performed in four different dilution ratios (100%, 75%, 50%, and 25%) in the landfill leachate. The changes in the chemical properties of the wastewater (pH, NO_4^- -N, NO_2^- -N, NO_3^- -N, and o-PO_4^{3-}) were measured. The values in the leachate and after treatment were measured as follows: NH_4^+ -N ranged from 18.03 to 476.2 mg/L, NO_3^- -N ranged from 5.0 to 33.98 mg/L, NO_2^- -N ranged from 1.49 to 7.06 mg/L, and PO_4^{3-} -P ranged from 27.99 to 145.4 mg/L. The protein yield (CP prot) in the experimental groups at the end of the 45-day study was as follows: T1 = 20.90%, T2 = 36.68 %, T3 = 41.78%, T4 = 47.43% and T5c = 49.56%. These results demonstrate that aquatic plants can be utilized in wastewater treatment and as animal feed due to their high protein yield. In my research, a completely nature-friendly treatment technique was used to reduce water pollution without using any chemicals.

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INTRODUCTION

Rapid population growth and agricultural development resulting from industrial activities release numerous organic and inorganic pollutants into nature.¹⁻³ These pollutants can be discharged into both aquatic and terrestrial environments. Discharging wastewater into water bodies adversely affects water quality, the environment, and public health.^{4,5} Due to the scarcity and unequal distribution of water resources in many countries, coupled with energy consumption issues and global warming, people need to seek alternative solutions and develop clean and ecological technologies to address environmental concerns. The conservation and sustainability of water resources can only be achieved through the reduction of wastewater treatment plant loads, increased treatment efficiency, and wastewater recycling.⁶ Wastewater treatment processes conventionally involve different physical, chemical, and biological combinations, resulting in high energy consumption and greenhouse gas emissions. However, due to their inadequate treatment efficiency, they are not considered an environmentally and economically suitable solution.

Nevertheless, in the terrestrial environment, waste materials are being reduced through various methods such as incineration, composting, pyrolysis, landfilling, and landfilling in certain areas without causing harm to the environment.⁷ Some materials stored in a pile form can become liquid during landfilling, creating “leachate containing dissolved or suspended substances.” Leachates contain high amounts of organic and inorganic matter, including heavy metal loads such as mercury, iron, manganese, and copper, posing a significant threat to groundwater.⁸ These wastewaters must be treated before being discharged into the environment to prevent them from harming human health and the ecosystem.⁹ Due to the diversity of wastewater loads from domestic and industrial sources, even when meeting discharge standards, wastewater continues to have adverse effects on aquatic organisms. Therefore, it is essential to research to develop new methods and techniques that can enhance wastewater treatment performance.

In many countries, especially in less developed ones, water plants have been used for natural treatment for many years.¹⁰ It was stated in their study that water plants could remove nitrogen and phosphorus from wetlands, and they found that plant physiology, such as plant height and leaf

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shape, also plays a role in general nutrient removal in treatment systems.¹¹ While the costs of classical treatment systems (remediation techniques) are considerably high, green remediation (phytoremediation) technology, which uses natural methods, provides significant advantages such as low energy requirements, low-cost installation, lack of chemical usage, and production of high-quality products.¹² Commonly used in phytoremediation, hyperaccumulator plants consist of floating, submerged, and emergent plants. These plants can accumulate 50 to 500 times more metals in their leaf stems and stalks than in the soil¹³ and have 100-1000 times more organic matter in their above-ground organs than non-hyperaccumulator plants.¹⁴ The *Ceratophyllum demersum*, our study subject, belongs to emergent plants that entirely live in water and is observed in ponds, lakes, ditches, and quiet rivers with high nutrient levels. They obtain all the necessary nutrients from the surrounding water. Due to its ability to absorb high concentrations of elements such as phosphorus and nitrogen, it is one of the most important aquatic plants used in wastewater treatment.¹⁵ There are numerous studies on the use of *C. demersum* in wastewater treatment.¹⁵⁻²⁰

The present study aimed to determine the treatment efficiency, growth performance, and protein values of the biomass obtained through the phytoremediation method using *Ceratophyllum demersum* with municipal leachate wastewater.

MATERIALS AND METHODS

CULTURE MEDIA AND WASTEWATER TREATMENT SYSTEM SETUP

The study was conducted 2018 using *Ceratophyllum demersum* plants that were kept in an enriched medium with ½ Hoagland nutrient²¹ in the Aquatic Plants Cultivation Laboratory of Ege University Fisheries Faculty for 45 days. Healthy plants from the preliminary test were selected to form the experimental groups. These individuals were first subjected to a 3-4 minute bath using 1.5% sodium hypochlorite (NaClO) for sterilization and then rinsed with distilled water.

For the experimental groups, containers (15 cm height, 6 cm radius, and 2000 ml volume) were disinfected by immersing them in 1% NaClO for 3-5 minutes. Subsequently, Hoagland nutrient medium diluted with distilled water was added to prepare six experimental groups, containing 0% (control), 25%, 50%, 75%, 100% wastewater with *C. demersum*, and 100% wastewater without *C. demersum* for aquatic plant production in wastewater. The experiment was conducted in triplicate to produce *C. demersum* in wastewater (Table 1).

The leachate waters used in the experiments were obtained from the solid waste storage facility of Izmir Metropolitan Municipality Cigli Solidwaste Storage Facility. Izmir is a major industrial city and the third largest city in Türkiye. The physicochemical properties of the solid waste leachate water are given in Table 2. The leachate water intended for the experiment was collected in large plastic

Table 1. Experimental groups and concentrations

Experimental Groups	Experimental Concentrations
T ₁	100% wastewater + <i>Ceratophyllum demersum</i> ,
T ₂	75% wastewater + 25% distilled water + <i>Ceratophyllum demersum</i> ,
T ₃	50% wastewater + 50% distilled water + <i>Ceratophyllum demersum</i> ,
T ₄	25% wastewater + 75% distilled water + <i>Ceratophyllum demersum</i> ,
T ₅	0% wastewater + 100% distilled water + Hoagland media + <i>Ceratophyllum demersum</i> (Control)
T ₆	100% wastewater with no <i>Ceratophyllum demersum</i>

Table 2. Physicochemical composition of leachate

Chemical structure	Value
Ph	8.94
Electrical conductivity(ms/cm)	40.40
COD (mg/L)	1415
NH ₄ ⁺ -N (mg/L)	474.2
NO ₂ ⁻ -N (mg/L)	6.49
NO ₃ ⁻ -N (mg/L)	33.68
PO ₄ ⁻³ -P(mg/L)	142.83

containers and transported to the laboratory, where its chemical characteristics were measured.

The T6 group (100% wastewater with no *Ceratophyllum demersum* was utilized as the experimental control for result comparison. T5 (100% Hoagland medium with *C. demersum*) was employed as a separate control group for biochemical characterization. The stock density of *C. demersum* in each container was adjusted to 2.5 g/500 mL.²² The test groups were shaken twice a day to ensure uniform nutrient distribution, while the Hoagland medium in the control group was refreshed by adding nutrient solution once a week. The experiment was conducted under a photoperiod of 16 hours of light and 8 hours of darkness.^{22,23} For illumination, daylight LED lamps were utilized, and the light intensity just above the experimental containers was measured as 216 µmol m⁻² s⁻¹ using a light meter. The ambient temperature of the samples taken for the culture was maintained at 25±1°C through central heating.

ANALYTICAL METHODS

The temperatures of the experimental groups were measured using a thermometer with a sensitivity of 0.1°C. The pH was measured using an Orion 420 A pH meter, while electrical conductivity (EC) was measured using a YSI 30 conductivity meter. Nutrients (nitrite-nitrogen, nitrate-nitrogen, ammonium-nitrogen, phosphate-phosphorus) were determined spectrophotometrically with the HACH DR

2000 spectrophotometer, following standard methods. Chemical oxygen demand was determined according to standard methods as well.

To determine the growth in the experimental groups, individuals of *C. demersum* were collected using a sieve, and the total biomass of each experimental group was measured using a balance. The growth rate of *C. demersum* was calculated using the standard methodology described in the study by.²⁴

The productivity of different experimental setups was determined using the relative growth rate (RGR) through *C. demersum* density (g/m²). The formula for RGR includes variables as follows: RB, representing the percentage of biomass; N, representing the number of days in the period; and A, representing the surface area of the water.

The specific growth rate (SGR) was determined using the following formula: $TB / (N * D * A)$, where TB is the total biomass harvested during the period, N is the number of days in the period, D is the average biomass density (g/m²), and A is the surface area of the water.

$$RGR = \frac{RB/N}{A}, \quad SGR = \frac{TB/N}{D.A}$$

The dry biomass and total protein values of the biomass were analyzed to determine the role of *C. demersum* in nutrient removal from wastewater and its biochemical efficiency. Following the AOAC 1990 (934.01) method, the Dry Matter Analysis was employed to conduct the analysis, and the subsequent formula was utilized for this purpose.

$$DM\% = \frac{\text{Dried sample weight (g)}}{\text{Sample weight included in the analysis (g)}} \times 100$$

Raw Protein Analysis was calculated using the formula outlined in the method (AOAC-976.05) (AOAC, 1990). In this formula, V0 represents the volume of HCl used in the blank test (ml); V1 represents the volume of HCl used in the sample titration (ml); c represents the concentration of HCl (mol/l); and m represents the sample weight (g).

$$\text{Crude Protein} = \frac{(V_0 - V_1) \times c \times 0.014 \times 6.25}{m}$$

Each experimental group's biomass and protein yield (CP) were computed using the following formulas. In this context, B represents the percentage increase in biomass in the experimental groups, bf denotes the biomass at the end of the experiment, bi represents the biomass at the beginning of the experiment, C represents the percentage increase in protein level, Cf denotes the protein content at the end of the experiment, and Ci denotes the protein content at the beginning of the experiment.

$$B(\%) = \left(\frac{bf - bi}{bf} \right) \times 100, \quad C(\%) = \left(\frac{cf - ci}{cf} \right) \times 100$$

$$CP(\%) = B \times C$$

STATISTICAL ANALYSIS

The one-way analysis of variance (ANOVA) was applied to the experimental groups, and in the event of significant differences between the groups, Duncan's post hoc test was employed. A significance level of 0.05 was adopted for this study, and the analysis was conducted using the IBM SPSS 25.0 statistical package.

RESULTS

THE EFFICIENCY OF WASTEWATER ON BIOMASS

The biomass yield of *C. demersum* ranged from 2,500 to 8,070 g fresh weight. All experimental setups obtained a total biomass yield of 59.18% to 68.89% compared to the initial level. The weight differences between experimental groups were statistically significant ($p < 0.05$). Regarding the final weight, the groups were ranked as $T5 > T4 > T3 > T2 > T1$, and concerning total biomass, the groups were ranked as $T5 > T4 > T3 > T2 > T1$. The specific growth rate (SGR) was ranked as $T1 > T2 > T5 > T4 > T3$, and the relative growth rate (RGR) was ranked as $T5 > T4 > T3 > T1 > T2$. As for crude protein content, the groups were ranked as $T5 > T4 > T3 > T2 > T1$ (Table 3).

THE EFFICIENCY OF *C. DEMERSUM* IN WASTEWATER NUTRIENT REMOVAL

The present study investigated the potential of *C. demersum* to remove organic and inorganic substances from wastewater. The pH and $NH_4^+ - N$, $NO_2^- - N$, $NO_3^- - N$ and $o - PO_4 - 3$ concentrations. Were determined. Based on the results, the pH values of the different experimental groups ranged from 7.79 to 8.94. The $NH_4^+ - N$ concentrations varied between 18.03 and 476.2 mg/L; a notable removal rate of 84.86% was observed in the T4 group. Regarding $NO_2^- - N$ concentrations, they were measured between 1.49 and 7.06 mg/L. The range for $NO_3^- - N$ concentrations was 5.0 to 33.98 mg/L, while for $PO_4 - 3$, the concentrations ranged from 27.99 to 145.4 mg/L (Table 4).

The removal percentage values of nutrients eliminated from solid waste leachate through the trial established with the *C. demersum* plant are given in Figure 1, followed by a graphic abstract in Figure 2 of the procedures.

DISCUSSION

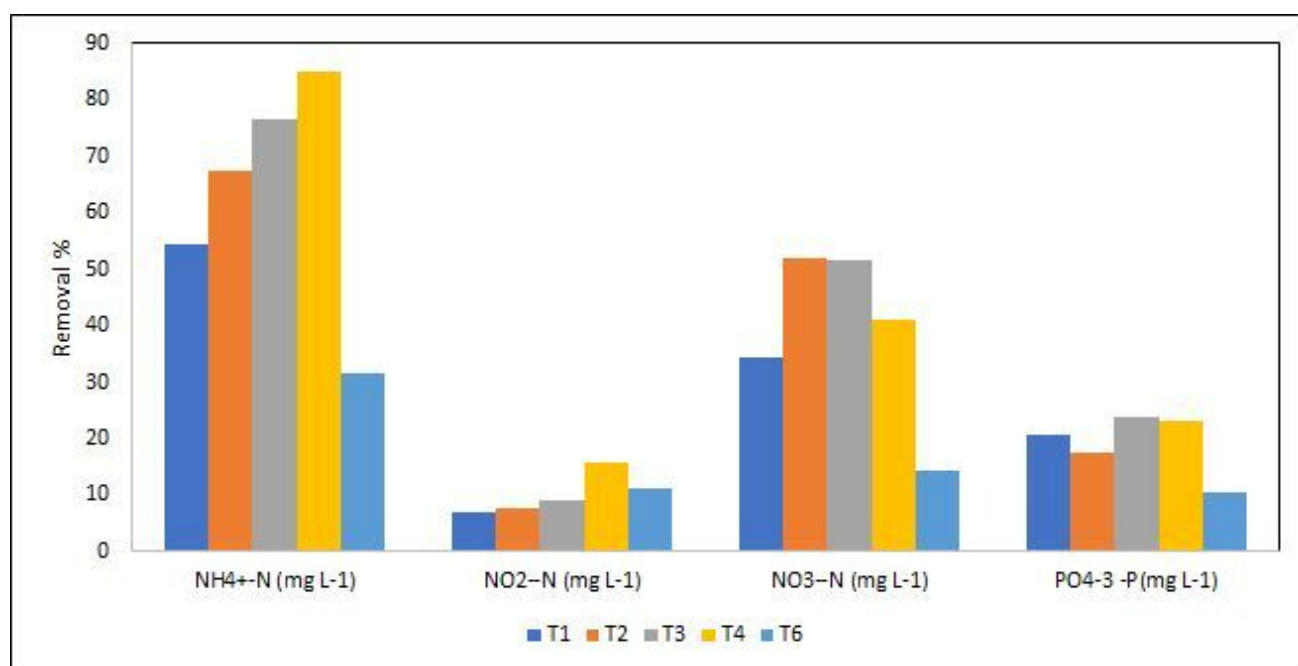
The present study determined biomass values ranging from 59.178% to 68.887% and protein values ranging from 14.20% to 22.28% for different dilution ratios. Relative growth values in five different dilution groups were between 103.19 ± 5.72 and 117.75 ± 7.64 , and the SGR values ranged from 0.037 ± 0.002 to 0.034 ± 0.001 . Aquatic plants with high growth rate and biomass accumulation possess advantages for water treatment due to their ability to live in water and efficiently absorb pollutants from the water.²⁵ However, production biomass in leachate water can be limited and lead to partial plant losses.²⁶ It has been reported that, under $400 \mu\text{mol}/(\text{m}^2\text{s})$ Ammonium ($NH_4^+ - N$) stress, *C. demersum* limited its growth, however it showed increased biomass compared to the control when the stress was relieved (an increase from 1.5 g to 2 g in 14 days after stress relief).²⁶ Patel and Kanungo¹⁶ a biomass increase of $1.58 \text{ g m}^{-2} \text{ day}^{-1}$ in treating domestic wastewater with *C. demersum*. Another study with leachate water and *Lemna minor* reported similar properties, a biomass increase rang-

Table 3. *C. demersum* growth in different wastewaters (mean $\pm$ SD, n = 3)

Treatment	Initial Weight	Final Weight	Total Biomass	SGR ^A (g/gd)	Total Biomass %	RGR ^B (g/m ³ d)	Crude Protein (%)
T ₁	2.503 $\pm$ 0.015 ^a	6.347 $\pm$ 0.371 ^b	0.085 $\pm$ 0.011 ^b	0.037 $\pm$ 0.002 ^a	59.178 $\pm$ 2.384 ^b	103.199 $\pm$ 5.721 ^c	14.207 $\pm$ 5.236 ^b
T ₂	2.500 $\pm$ 0.010 ^a	6.587 $\pm$ 0.402 ^b	0.091 $\pm$ 0.009 ^b	0.036 $\pm$ 0.001 ^a	61.947 $\pm$ 2.386 ^b	105.635 $\pm$ 4.100 ^c	17.747 $\pm$ 6.314 ^a
T ₃	2.517 $\pm$ 0.031 ^a	7.633 $\pm$ 0.929 ^{bc}	0.113 $\pm$ 0.021 ^{ab}	0.033 $\pm$ 0.002 ^d	66.702 $\pm$ 4.057 ^{ab}	114.020 $\pm$ 6.730 ^b	19.306 $\pm$ 5.170 ^a
T ₄	2.510 $\pm$ 0.017 ^a	7.830 $\pm$ 0.759 ^c	0.118 $\pm$ 0.759 ^a	0.033 $\pm$ 0.001 ^d	67.763 $\pm$ 2.806 ^a	116.027 $\pm$ 5.195 ^a	21.382 $\pm$ 1.746 ^a
T _{5C}	2.503 $\pm$ 0.006 ^a	8.070 $\pm$ 0.530 ^c	0.124 $\pm$ 0.012 ^a	0.034 $\pm$ 0.001 ^c	68.887 $\pm$ 2.113 ^c	117.751 $\pm$ 7.643 ^a	22.283 $\pm$ 2.150 ^a

Table 4. Physico-chemical characteristics of domestic wastewater before and after phytoremediation with “*Ceratophyllum demersum* L.”

Parameters	T_1	T_2	T_3	T_4	T_6
pH					
Initial	8.94	8.84	8.72	8.66	8.94
Final	8.23	8.32	8.00	7.79	8.56
EC (ms/cm)					
Initial	40.4	38.4	34.55	20.25	40.4
Final	25.8	30.15	19.62	17.83	26.84
NH₄⁺-N (mg L⁻¹)					
Initial	476.2	357.5	238.1	119.5	476.2
Final	216.3	115.9	55.4	18.03	326.1
Removal (%)	54.57	67.58	77.73	84.86	31.52
NO₂⁻-N (mg L⁻¹)					
Initial	7.06	5.30	3.53	1.77	7.06
Final	6.58	4.90	3.21	1.49	6.26
Removal (%)	6.80	7.55	9.07	15.82	11.33
NO₃⁻-N (mg L⁻¹)					
Initial	33.98	25.49	16.99	8.5	33.68
Final	22.31	12.27	8.20	5.0	28.87
Removal (%)	34.34	51.86	51.74	41.18	14.28
PO₄⁻³-P (mg L⁻¹)					
Initial	145.4	109.05	72.7	36.35	145.4
Final	115.43	90.09	55.38	27.99	130.03
Removal (%)	20.61	17.39	23.82	22.99	10.57

**Figure 1. Removal percentile values of the nutrients**

ing from 43.20% to 60%, with the highest increase in the 50% dilution group.²⁷

In this study, the protein yield (CP prot) in the experimental groups at the end of the 45-day study was as follows: T1 = 20.90%, T2 = 36.68 %, T3 = 41.78%, T4 = 47.43% and T5c = 49.56%. Al-Nabhan and Abbawy [20], in their study conducted for 21 days with 1:1 and 1:3- dilutions, reported protein values of 20.68%±0.25, 25.70%±0.06, and 30.37%±0.05, proving its effectiveness in improving waste-

water quality in the facility. Therefore, the researchers emphasized that it is an important candidate for phytoremediation.

The highest protein and biomass values were determined in the T4 group following the Hoagland medium treatment. The Initial protein was 11,24. In terms of protein content, the groups are ranked as follows: T5 > T4 > T3 > T2 > T1 (Table 3).

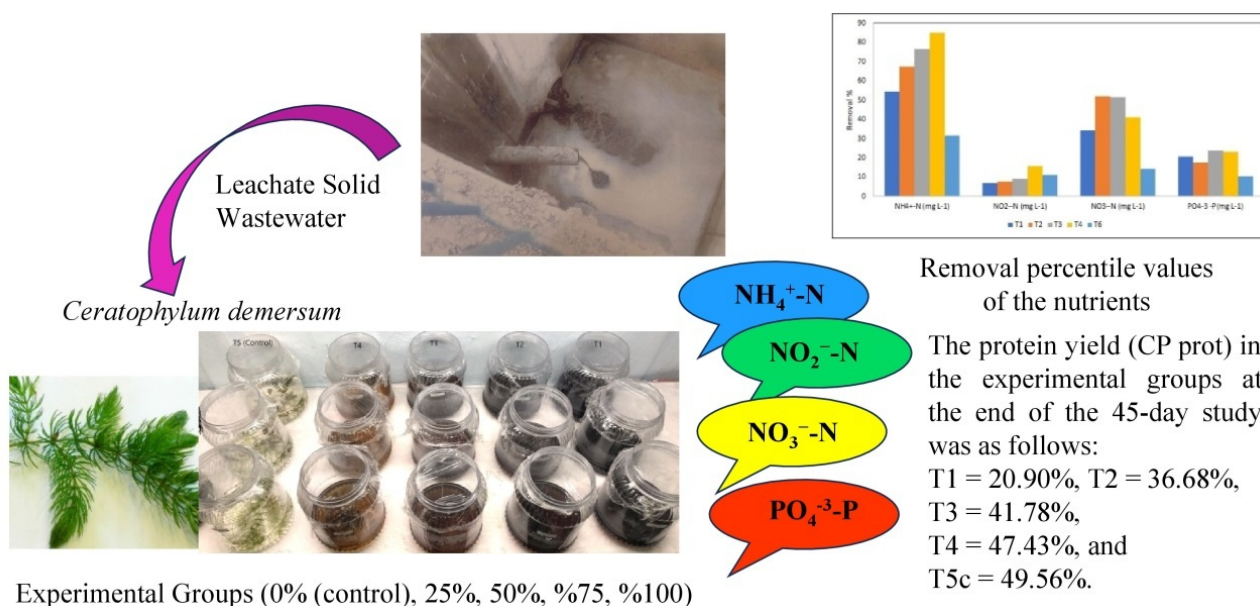


Figure 2. Graphical abstract

The present study measured the wastewater's electrical conductivity (EC) value as 40.40 ms/cm, which decreased to 17.83 ms/cm after treatment with the *C. demersum* plant. In a study by Patel and Kanungo,¹⁶ they reported the electrical conductivity (EC) value as 926.50 μ mhos/cm before phytoremediation with *C. demersum*, and this value decreased to 529.20 μ mhos/cm for domestic wastewater after treatment. Similar to our findings, a decrease in EC value was observed after wastewater treatment. Electrical conductivity is an essential parameter for assessing the quality of drinking and irrigation waters related to the concentration of charged particles in the water.¹⁸

The NH_4^+-N value in our study decreased from 476.2 mg/L to 18.03 mg/L. Foroughi¹⁷ reported that the NH_4^+-N concentration in wastewater decreased from 60 mg/L to 13.33 mg/L within approximately six days, and Foroughi et al.¹⁵ found a reduction in NH_4^+-N concentration from 135 mg/L to 29.16 mg/L. All these studies observed a decrease in NH_4^+-N concentration after treatment. Tracy et al.²⁸ stated that *C. demersum* is a nitrophilic plant capable of tolerating high nitrogen concentrations and significantly removing nitrogen in the water column.

The NO_2^--N concentration decreased from 7.06 mg/L to 1.49 mg/L. Patel and Kanungo¹⁶ decreased NO_2^--N concentration from 0.376 mg/L to 0.196 mg/L, representing a 47.27% reduction after a seven-day trial using the same species. Tekoğlu²⁷ found that *Lemna minor* reduced the NO_2^--N concentration from 1.62 mg/L to 1.39 mg/L. Similar NO_2^--N concentration decreases were observed in all studies after treatment.

The NO_3^--N concentration decreased from 33.98 mg/L to 5 mg/L. Patel and Kanungo¹⁶ decreased NO_3^--N concentration from 52.05 mg/L to 27.45 mg/L, representing a 47.27% reduction after treatment. Foroughi¹⁷ observed a decrease in NO_3^--N concentration from 90 mg/L to 26.66 mg/L in 18 days, and Foroughi et al.¹⁵ found a decrease in NO_3^--N concentration from 60 mg/L to 27.5 mg/L. A NO_3^--N

concentration decrease was observed after treatment in the present study and all other studies.

The $\text{PO}_4^{3-}-\text{P}$ concentration decreased from 145.4 mg/L to 27.99 mg/L in our study. Patel and Kanungo¹⁶ found a substantial reduction in phosphate, lowering the concentration from 18.47-59.73 mg/L to 1.47-3.40 mg/L through oxidation. Kulasekaran et al.¹⁹ observed phosphate concentrations of 15.6-22.8 mg/L in raw sewage and 0.3-1.4 mg/L in treated sewage. Phosphate concentration significantly decreased with treatment. Phosphate is an essential factor causing eutrophication²⁹ and is a significant indicator of water pollution.³⁰ Therefore, *C. demersum* is an important phytoremediator for removing all nutrients, including $\text{PO}_4^{3-}-\text{P}$, from solid waste leachate. Several studies³¹⁻³⁴ have demonstrated that significant amounts of dissolved organic matter, including soluble nutrients, are continuously released by aquatic vascular plants.

Freshwater constitutes 2.5% of the total water in the world and the need for freshwater is increasing every year in parallel with the rapid increase in the world population. For this reason, the sustainable use of our water resources has become more important in recent years and studies are carried out in many different methods. It is known that these studies, in which biochemical mixtures and products are used, both increase the cost and may adversely affect human health in the future. For this reason, natural treatment studies with aquatic plants such as *C. demersum* have become even more important in recent years as they eliminate all these negativities.

The present study has conclusively demonstrated the significant role of the aquatic plant *C. demersum* in enhancing environmental conditions through the absorption of elements in polluted environments. Accordingly, this aquatic plant holds promise as a valuable phytoremediator. Compared to terrestrial plants, macrophytes in aquatic ecosystems are renowned for their accelerated growth and higher biomass production, as well as their greater capacity for re-

taining pollutants and providing enhanced purification effects owing to their direct interaction with contaminated water. These inherent characteristics render them particularly well-suited for wastewater treatment, as corroborated by the results of the present study.

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AUTHOR'S CONTRIBUTION PER CREDIT

Conceptualization: Hatice Tekoğul (Lead). Methodology: Hatice Tekoğul (Lead). Formal Analysis: Hatice Tekoğul



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