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Quantitative Estimation of Total Nitrogen and Phosphorus in *Lemna sp*. Used for The Removal of Congo Red Dye from Contaminated Water

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ABSTRACT: This study focuses on the quantitative estimation of total nitrogen (N) and phosphorus (P) in the aquatic plant *Lemna sp.* (duckweed) and its role in removing Congo red dye from contaminated water. Water pollution is considered one of the most critical environmental issues caused by industrial and agricultural activities. The accumulation of nitrogen and phosphorus in aquatic systems leads to eutrophication, excessive algal blooms, oxygen depletion, and overall water quality deterioration. Biological treatment using aquatic plants has been recognized as a low-cost, eco-friendly, and effective alternative compared to physical and chemical methods. Samples of *Lemna sp*. were collected from Shatt al-Kufa during four seasons and cultivated in experimental basins with different concentrations of Congo red dye. Results revealed that duckweed exhibited a high ability to absorb and accumulate nitrogen and phosphorus in its tissues. Nitrogen removal efficiency reached about 65% in winter and summer, while it decreased to 28% in spring. Phosphorus, on the other hand, was accumulated in large amounts inside plant tissues, highlighting the necessity of periodic biomass harvesting to achieve effective removal from the aquatic environment. The presence of Congo red dye did not impair the plant's ability to absorb nutrients but showed seasonal variations in uptake efficiency. The findings confirm that aquatic plants such as Lemna minor represent a promising phytoremediation approach for treating industrial wastewater contaminated with dyes and nutrients, due to their combined capacity for direct uptake and stimulation of microbial activity in the rhizosphere.

KEYWORDS: *Lemna sp*., Phytoremediation, Nitrogen, Phosphorus, Congo red dye

INTRODUCTION

The world's largest and most pervasive issue as a result of industrial, agricultural, and technological advancements is pollution. Since water contamination affects our daily life (1), it is one of the most significant environmental challenges. Without any substantial treatment, thousands of chemical compounds are released either directly or indirectly into the water, causing organic and chemical pollution (2).

radioactive pollution and thermal pollution. The presence of some toxic inorganic and organic compounds, as well as changes in the quantities of some of the fundamental elements found in the environment relative to their natural levels (3) , results in pollution. The aquatic environment is severely influenced by the large-scale discharges of nitrogen and phosphorus caused by indoor pollution, crops, and animal production (4). Several modalities of therapy include chemical, biological, and physical approaches. Experts in water treatment have attested to the power of biological treatment and its benefits over chemical and physical approaches because of its low cost(5) , ease of application of technical equipment, and safety and simplicity (Vikas and Sandip, 2013 (6)).Aquatic plants are believed to be the most efficient method of improving water quality and purification since they are highly effective at eradicating or minimizing many different kinds of harmful substances (7) , are relatively cheap, easy to maintain, and don't need specialized knowledge or particular regions (Sivasubramanian et al., 2012) (8) . Phytoremediation is a means of employing plants to treat the contamination of the environment. Because it absorbs heavy metals and chemicals trapped in the roots, stems, or leaves, it is both environmentally beneficial and effective at eradicating many kinds of pollutants, including dangerous chemicals (9). Proteins, enzymes, nucleic acids, denitrifying and nitrifying bacteria, and ammonium bacteria all require nitrogen (N)(10). Energy transfer, fertilizer synthesis, photosynthesis, and plant growth all depend on it. On the other hand, aquatic ecosystems may suffer by too much nitrogen (11).

Increased algal blooms and excessive phytoplankton proliferation are thought to be predominantly induced by phosphorus (P) (12). Controlling phosphorus levels by individuals, however, has no major impact on eutrophication, according to certain results from analyses.

An additional significant aspect of eutrophication has been revealed to be nitrogen (N). Water nutrient levels can be decreased by lowering phosphorus and nitrogen levels. Aquatic plants enhance microbial activity in the root zone by consuming and synthesizing nutrients, Although the symbiosis between snails and submerged plants can lower nitrogen and phosphorus concentrations in the water (13), different plant species have varying capacities to eradicate excess nutrients. So, surrounding algae and phytoplankton, as well as large plants, are indicators of a nutrient-rich environment. Both phosphorus and nitrogen are crucial for plant growth. Recovery of nitrogen and phosphorus lessens the damages that pollution causes to the environment while also rendering it achievable to recycle them in agriculture (14). Due to their ability to absorb and store huge amounts of nitrogen and phosphorus, aquatic plants—like duckweed (Lemna spp.) and water hyacinth (Eichhornia crassipes)—have drawn more attention to phytoremediation. Indian grass (Chrysopogon zizanioides), water lettuce (Pistia stratiotes), and common reed (Phragmites australis) are frequently utilized to extract nutrients from river water or polluted wastewater. highlighted the extraordinary capacity of Pistia stratiotes to extract phosphorus and nitrogen from a range of dirty water types. Pistia stratiotes and Lemna sp. were used to treat pig discharge in order to remove total nitrogen (TN, 63.2%) and total phosphorus (TP, 36.2%), respectively (15).

The capability of 21 plant species to detoxify nitrogen and phosphorus varies depending on the type of pollutant, as stated by Iamchaturapatr et al. The majority of earlier studies have documented the effectiveness of employing a particular plant species to cleanse wastewater or contaminated rivers. Nevertheless (16), more thorough research is required to comprehend completely how various aquatic plant species work together to take out nitrogen from water and remediate the consequences of contaminated water. Because aquatic plants are able to absorb and accumulate nutrients, they are commonly used in ecology to regulate nitrogen and phosphorus levels and restore nutrient-rich lakes.

One of the biggest contaminants of water systems is industrial wastewater (17), utilized in related sectors like textile dyes. Many of these dyes are non-toxic or inert, while some, like benzidine, have extremely harmful effects on both people and the environment. Since it contains an N=N bond, Concord dye, one of the commercial azo dyes used in the textile industry, is a rich source of nitrogen and one that pollutes the environment (18).

Despite not being an essential element of the dye, additives added during the dyeing process frequently result in the presence of phosphorus in industrial wastewater (19). Recent studies, aquatic plants like *Lemna minor* and *Eichhornia crassipes* may remove both nitrogen and phosphorus from dye-contaminated water by increasing microbial activity in the rhizosphere and by directly taking in the vital nutrients (20), as well as root adsorption. According to earlier studies, these plants offer a promising biotechnology for the treatment of azo dye-containing industrial effluent. There are a number of techniques for eliminating dyes, but the most crucial ones are adsorption, chemical oxidation, ozone treatment, and biological techniques, multiple procedures including reverse osmosis. Numerous contaminants released by the textile industry, including dyes and heavy metals (21), have been treated by aquatic plants. The bulk of current research has been on treating dyes emitted from the textile industry because of their harmful, mutagenic, and cancer-causing capabilities. In order to lessen the detrimental effects of industrial pollutants on the ecosystem by lowering the concentrations of these pollutants in the aquatic environment, a variety of aquatic plants, including Phragmites australis, Typha aphyl la, *Ceratophyllum demersum*, Lemna sp., *Hydrilla verticillate*, and *Sporangium emresum*, have been employed (22).

MATERIALS AND METHODS

Collecting of sample

Samples were collected in a bucket of ice from three locations in Shat al-Kufa in the winter, spring, summer, and fall in order to get them to the lab as quickly as possible. Following a thorough water rinse to eliminate algae while maintaining the plant's root hairs, the samples were taken from freshly grown water lentils. Until they arrived at the lab, they were kept in polyethylene bags, and each plant was given 50 g of fresh weight. The ponds were filled with chlorine-free distilled water and left in the sun for 24 hours. For two weeks, samples were gathered and growth was tracked. For a duration of two weeks, fifteen liters of tap water were placed in seventeen thirty-liter plastic basins lined with aluminum foil. They followed by employed to develop plants, and an additional three copies of Azo dys (Gongo red) dye were added in three different doses (0.01, 0.04, and 0.07). To facilitate comparison, two groups of control basins were used: one group had plants devoid of dye, and the other group simply had dye concentrations devoid of plants. There were 21 days in the experiment. *Lemna minor* is an aquatic plant that was employed in this investigation. It was cleaned and given two weeks to get used to tap water (23).

solutions Regents

Phosphor in plants

98 % Sulfuric acid (H_2SO_4), B. 50% Perchloric acid ($HClO_4$), C. 2.5% ammonium molybdate (w/v) in dH2O, D. 10% ascorbic acid (w/v) in dH2O, E. Reagent: Solution C, D, and dH_2O are mixed in a ratio of 1:1:8, F. Standard solution 10 mM KH2PO4 with H_2SO_4 and $HClO_4$ (24).

Samples digestion

Each sample was put to a Pyrex test tube comprising 3 milliliters of 98% sulfuric acid (H2SO4) at a dry weight of 0.2 grams. They were then moved toward a heater inside a fume hood set at 2400C, and after 7–10 minutes, 1.5 ml of 50% perchloric acid (HClO4) was carefully added. After exposing the samples to the heater for two hours until they turned clear, the heater was turned off to let the samples cool. Then, each sample was put into a plastic beaker through filter paper, and 50 milliliters of dH_2O was then included to finish the volume (25).

Total Phosphor

The ascorbic acid and digestion spectrophotometric methods were used to measure it in accordance with Laboratory Manual ID: 1.23 Version 1. fifty milliliters of the mixed sample, one drop of the phenolphthalein indicator solution, one milliliter of 10 N H_2SO_4, and either 0.4 or 0.5 grams of $(NH_4)_2S_2O_8$ or $K_2S_2O_8$. After slowly boiling the combination for 30 to 40 minutes on a hot plate that had been warmed, it was diluted with 30 milliliters of distilled water. Adding up to 100 milliliters of distilled water is required after neutralizing one drop of the phenolphthalein indicator solution with NaOH until it becomes a light pink. Put 50 milliliters of the digested material into a 125 milliliter conical flask and add one drop of phenolphthalein as a marker. Reagent was mixed and combined. After waiting ten minutes, measure each sample's absorbance at 880 nm. Reagent blank for reference purposes (26,33).

Determination of Nitrogen in Plant Samples

The total nitrogen content in plant tissues is commonly determined using the (Kjeldahl method). Dried and ground plant samples are digested in concentrated sulfuric acid in the presence of a catalyst to convert organic nitrogen into ammonium sulfate. After digestion, the mixture is neutralized with sodium hydroxide, and the released ammonia is distilled into a boric acid solution. The collected ammonia is then titrated with a standardized acid solution to quantify the nitrogen concentration. The final nitrogen percentage is calculated based on sample weight, and the results are usually expressed as percent nitrogen per dry weight of plant material(26).

solutions indicators (N)

indicator dyes

After 0.066 grams of gongo red were dissolved in alcohol, 1 liter of water was added to finish the volume. One L of the 2% boric acid solution was mixed with 20 ml of the indicator mix. The finished indicator solution had to be kept in a dark container away from light because of the rapid photo-oxidation (27).

Removal ratio

The removal ratio was calculated after calibrating the dye and finding and determining the wavelength of Congo Red 499 λmax (Figure 1). The dye concentration in each tank was obtained through the standard curve as shown in Figure (2) Using the equation, the removal ratio was calculated (28) :

Removal ratio = (initial concentration - final concentration after treatment) / (initial concentration) × 100.

The removal ratio was extracted for all planted and control ponds, and then the removal ratio was subtracted from the control .

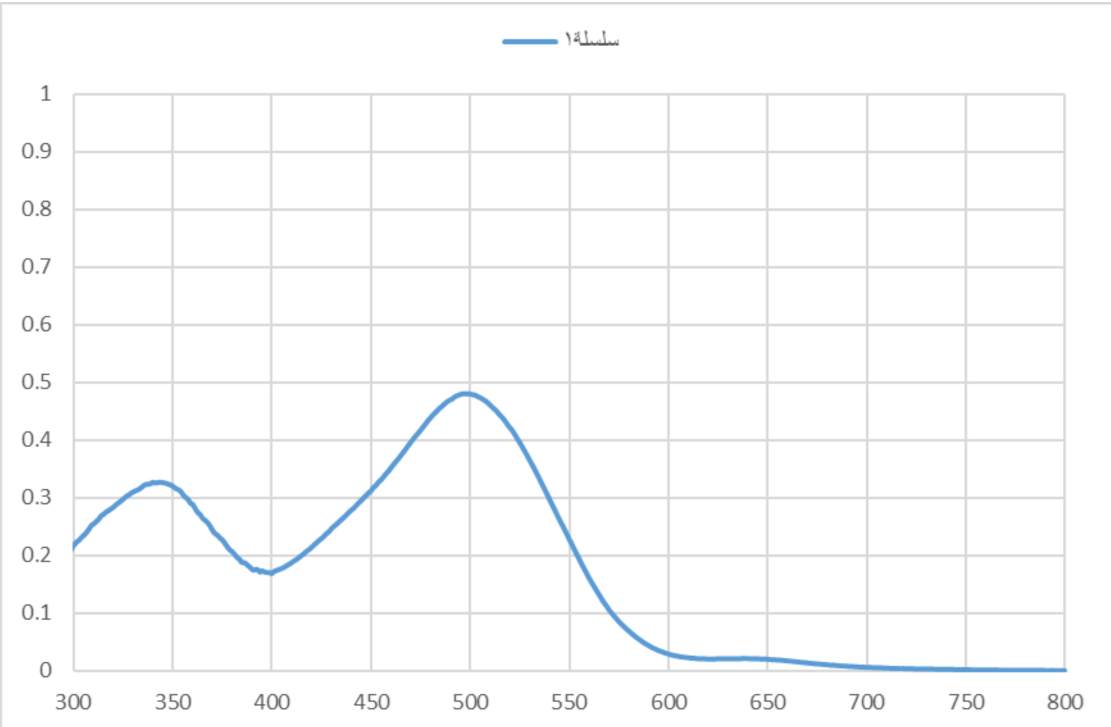


Figure (1) Calibration curve and determination of the wavelength of Congo Red dye λmax Congo Red 499 nanometers.

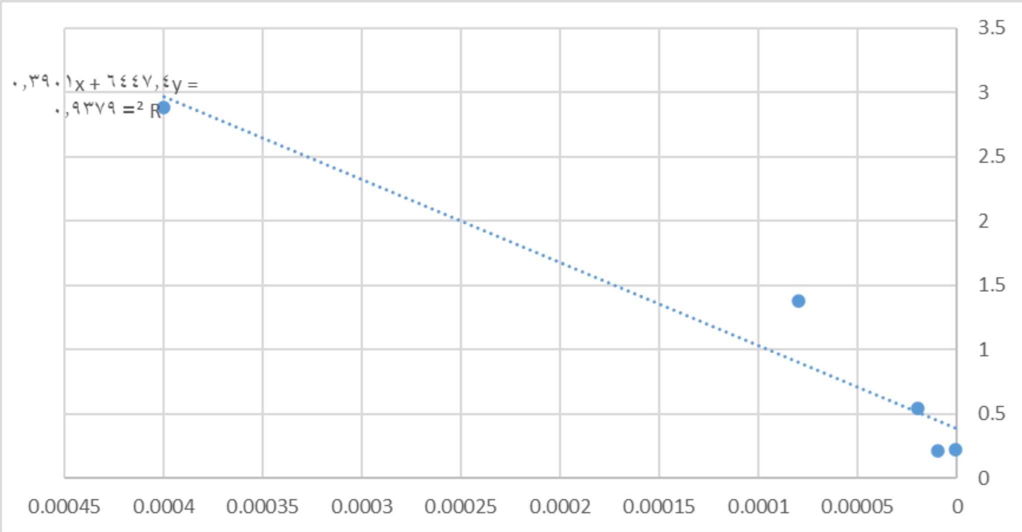


Figure (2) Standard curve for Congo Red dye

Statistical Analysis

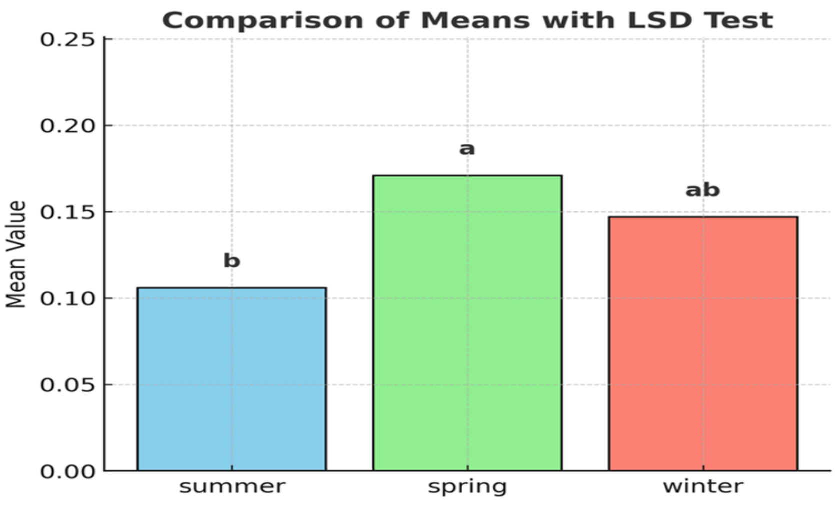
The results of the study were analyzed statistically using Analysis of Variance (ANOVA), Least Significant Difference (LSD) test, and Coefficient of Correlation (r) to find the type of relationship between the different factors in the plants under study.

RESULTS AND DISCUSSION

Ecologists are well aware of the use of aquatic plants to regulate the removal of nitrogen and phosphorus and replenish nutrient-rich lakes. Pistia stratiotes have a strong capacity to extract phosphorus and nitrogen from a variety of contaminated water types, according to (29). discovered that the effectiveness of aquatic plants differs by species; duckweed was more efficient at absorbing nitrogen, while water hyacinth (*Eichhornia crassipes*) shown a greater potential for phosphorus uptake. Phosphorus (TP, 36.2%) and nitrogen (TN, 63.2%) were eliminated by treating wastewater with Lemna sp. and Pistia stratiotes, respectively. The majority of earlier research has shown that cleaning wastewater or contaminated rivers with a single plant species is effective. Nevertheless, further research is required to fully understand the processes of treating contaminated water after removing nutrients from it utilizing combinations of several aquatic plant species. Beginning in the second month and concluding at the end of the sixth month, the laboratory cultivation experiment examined the aquatic plant life cycle. For both plants and sediments, the sampling rate was tripled. In each case, 50 grams of sediment samples and 10 grams of plant samples were gathered concurrently from each container. The entire set of submerged plant samples was examined (30).

First: Total nitrogen content in duckweed

The results of the statistical analysis (ANOVA) in Figure 3 showed a significant difference in the total nitrogen content within the tissues of duckweed, with a value of 3.16 at a probability level of 0.0496. At $p < 0.05$ and using the LSD test, it was found that the main difference was between the winter and spring seasons, while no statistically significant differences were recorded between the other seasons.



(P) = 0.0496 (< 0.05)

Figure (3) : shows the total nitrogen content in the tissues of duckweed plants during the three seasons.

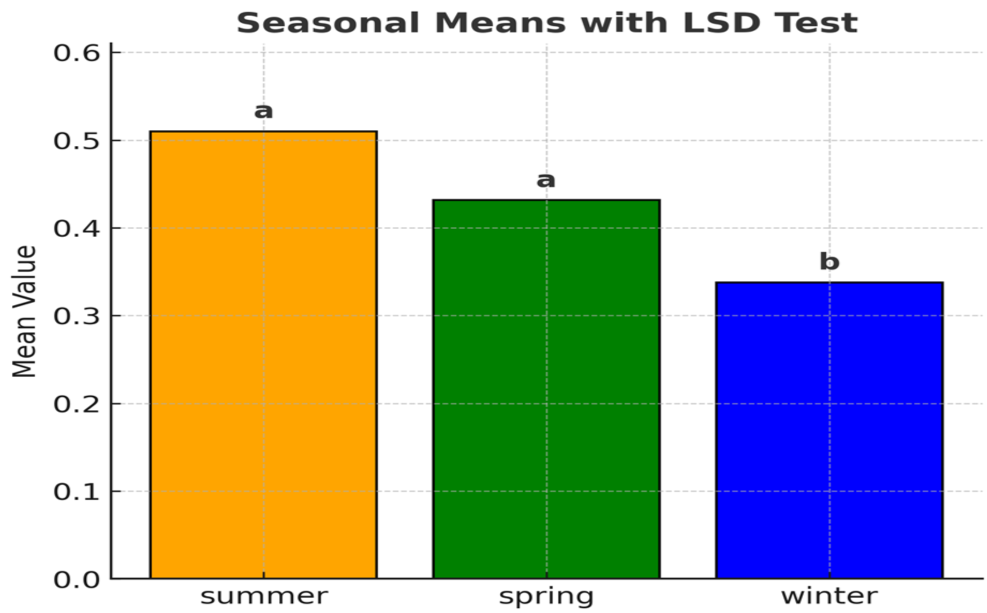
In Figure (3), the arithmetic mean values show variation in the plant's ability to absorb and store nitrogen depending on the prevailing environmental conditions in each season. We find that the lowest nitrogen content was recorded in winter (0.106), while the highest value was recorded in spring (0.171), with summer recording an average level (0.147). From an environmental perspective, the decrease in nitrogen concentration within plant tissues in winter can be explained by the slowdown in biological processes due to lower temperatures, which leads to reduced growth and metabolic rates and, consequently, lower nitrogen consumption. In spring, when temperatures gradually rise, plant biological activity accelerates significantly, leading to increased growth requirements and higher rates of nitrogen absorption from the aquatic environment and internal storage. In summer, due to the significant rise in temperature, which may cause heat stress to the plant, the abundance of light and the associated increase in microbial activity in the environment may lead to higher rates of biodegradation of pollutants and make more nitrogen available to the plant. This explains the average levels recorded, which is consistent with the study by (31) that *Lemna minor* was able to remove high levels of nitrogen and phosphorus from industrial water contaminated with dyes. Table (1) shows that the correlation coefficient for nitrogen in plants and water was negative and weak (r = -0.18), indicating an inverse relationship, i.e., as the concentration of nitrogen in water decreases, its concentration in plants increases. This relationship is attributed to the fact that duckweed efficiently absorbs nitrogen from the environment, thereby reducing its level in the water while accumulating more internally.

Table (1): shows the correlation coefficient for nitrogen in water and plants.

	Plant	Water
Plant	1.000	-0.180
Water	-0.180	1.000

Second: Total phosphorus content in water lentils

The results of the statistical analysis of phosphorus in Figure 4 showed significant differences in phosphorus accumulation within plant tissues between different seasons, with a value of 3.65 at a probability level of 0.0319, indicating that the seasonal effect in this aspect is clearer than that of nitrogen.



P< 0.05 = 0.0319

The average values showed positive concentrations for total phosphorus content, reflecting the accumulation of phosphorus within the tissues of duckweed. The highest value was recorded in summer (0.510) and the lowest in winter (0.338). The increase in values is due to higher temperatures and light intensity, which stimulate plant growth and metabolic processes, thereby increasing the plant's ability to absorb and store phosphorus. When analyzing the relationship between phosphorus concentration in water and plants, a relatively strong positive correlation (r = 0.618) was found, indicating that higher phosphorus levels in the aquatic environment directly translate into increased accumulation within plants. This positive correlation can be explained by the fact that phosphorus is one of the elements that determine the growth of aquatic plants, and therefore, the higher its concentration in water, the greater its absorption and storage.

Removal percentage for plants :

1. Nitrogen removal: The results showed that nitrogen was removed with high efficiency in winter and summer (about 65% in each season), while efficiency decreased in spring (28%), as shown in Table 2. Rapid plant growth in spring requires high nitrogen consumption for tissue building, leading to its accumulation within the plant. However, at the same time, high overall growth may lead to internal nutrient recycling and no significant difference in water concentration. In winter, despite low biological activity, the lack of external demand from other organisms and the absence of competition make plant uptake evident at the expense of water concentration. The higher removal rate in summer is attributed to increased microbial and physicochemical interactions that make nitrogen more absorbable.

Table (2): shows the nitrogen removal rates in different seasons.

Season	Plant	Water	Removal %
Winter	0.106	0.301	64.78 %
Spring	0.171	0.239	28.45 %
Summer	0.147	0.413	64.39 %

2. **Phosphorus removal**:

The removal rate showed negative values (from -262% to -499%) in the different seasons in Table 3. This does not indicate a failure of removal, but rather reflects the calculation method used, which is based on a comparison between the concentration of phosphorus in water and its content in plants. Since the concentration of phosphorus in plant tissue was much higher than its concentration in water, the equation showed negative percentages. Plants absorb phosphorus and accumulate it with high efficiency, and converting these accumulations into "actual removal" depends on collecting the plant's biomass and harvesting it from the environment.

This result confirms the importance of periodic harvesting of aquatic plants used in bioremediation, as nutrient accumulation within tissues is considered a preliminary step, but actual removal from the ecosystem is only achieved by removing biomass from the environment. Thus, duckweed can be considered an effective means of treating excess phosphorus if its use is combined with an appropriate harvesting plan.

Table (3): Phosphorus removal rate in the three seasons

Season	Plant	Water	Removal %
Winter	0.338	0.095	-255.79 %
Spring	0.431	0.072	-498.61 %
Summer	0.510	0.141	-261.70 %

The relationship between duckweed and Congo red dye

Congo red is a complex industrial pollutant that can affect the balance of the aquatic environment, either through light absorption (which reduces the intensity of radiation available to plants) or through chemical reactions with dissolved nutrients. The results showed that duckweed retained its ability to absorb nitrogen and phosphorus and even showed considerable efficiency, especially in phosphorus accumulation and nitrogen removal. This indicates that the presence of the dye did not impair the plant's ability to perform its biological role, but may have had a seasonal effect on absorption rates (20) .

The impact of nitrogen and phosphorus on the environment The accumulation of nitrogen and phosphorus in water bodies contributes to eutrophication, accompanied by excessive algae growth, depletion of dissolved oxygen, and deterioration of water quality (28). In addition, the accumulation of azo dyes increases water turbidity and hinders photosynthesis, as well as decomposing into toxic aromatic amines through microbial processes confirmed that aquatic plants, in conjunction with microbial activity, provide an effective mechanism for dealing with azo dye pollutants (32).

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