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Research Article

IMPACTS OF PETROCHEMICAL-CONTAMINATED WASTEWATER ON *LEMNA MINOR* L. AND ITS PHYTOREMEDIATION EFFICIENCY IN MAKORHI, KARAK, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

The increasing demand for energy has resulted in a significant rise in the exploration of petrochemical resources around the globe. In Pakistan, district Karak has several sites dedicated to petrochemical exploration. Effluents from these sites pose serious threats to the aquatic environment. This investigation aimed to evaluate the potential of *Lemna minor* for bio-assessment and phytoremediation of toxicants in the effluents from petrochemical sites. Various parameters, including biomass, frond number, biochemical endpoints, fresh and dry weight, and antioxidant indicators, were analyzed. Over seven days, *L. minor* cultures were exposed to wastewater at different concentrations (1, 5, 10, 25, 50, and 100 mg/L). Among the tested treatments, concentrated wastewater had a significant impact on the growth of biomass, fronds, and pigments, including total carotenoids, chlorophyll *a*, and chlorophyll *b*. Conversely, total soluble protein and sugar contents increased, likely due to the defense mechanisms of the plant against pollutants in the effluent. Furthermore, exposure of *L. minor* to wastewater resulted in substantial changes in several oxidative stress indicators. The findings indicate that toxicants in the wastewater induced oxidative stress in *L. minor*. Antioxidant enzymes, such as ascorbate peroxidase, lipid peroxidation, peroxidase, and catalase, showed a marked increase in activity in response to this stress. The bio-concentration factor for *L. minor* was calculated as 0.29 mg/L, highlighting its suitability for heavy metal bioaccumulation. In conclusion, the effluents from petrochemical sites negatively impact the aquatic environment, and *L. minor* proves to be an effective standard organism for wastewater bio-assessment and phytoremediation.

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INTRODUCTION

The introduction of contaminants into the natural environment, rendering soil, water, and air unsuitable for life, is referred to as pollution. Recently, the contamination of the natural environment has increased significantly, making it toxic and unfit for living

organisms. Pollutants can be classified as either organic or inorganic. Among these, hazardous metalloids and heavy metals are considered the most dangerous toxicants, posing significant risks to biological systems (Bassyouni et al., 2020). Unlike organic pollutants, which can biodegrade, inorganic pollutants such as heavy metals cannot be broken down into less harmful forms (Ahmad et al., 2024). Although heavy metals are essential for biological systems at low concentrations, excessive levels can severely impair these systems. Furthermore, certain heavy metals, such as mercury, cadmium, lead, and zinc, are highly toxic even at low concentrations (Puttaiah and Kiran, 2007).

Many agencies have been discharging organic and inorganic pollutants into water bodies for decades. Among inorganic contaminants, heavy metal levels in water bodies are rising. Unplanned industrial growth and rapid, unplanned urbanization are the two primary causes of water pollution in Pakistan. Water contamination from various sources adversely affects soil fertility, biomass, and overall crop productivity, leading to the bioaccumulation of heavy metals in the food chain (Rajkumar et al., 2009).

Industrial effluents containing hazardous metals, when released into the environment, pose significant health risks to biota (Waisberg et al., 2003). Common contaminants released from petrochemical factories include heavy metals such as cadmium, lead, chromium, zinc, nickel, copper, and arsenic.

The Makori oil and gas field was discovered in 2005 by MOL Company Pakistan during the drilling of an exploration well in the Makori area of Banda Daud Shah district, Karak. This field, which commenced production in 2006, produces 520 metric tons of liquefied petroleum gas, 25,000 barrels of condensate oil, and 305 million metric standard cubic feet of natural gas daily. Unfortunately, the Banda Daud Shah brook has been continuously exposed to untreated effluents from the plant.

Recently, wells Makori-2 and Makori-3 were drilled. Although Makori-2 has dried up, Makori-3 has been operational with an early production facility since 2010. The Makori field was officially granted the status of a Development and Production Lease in April 2012, following the government's approval of its development plan (Pakistan Petroleum Limited, 2019).

The physicochemical properties of water bodies, such as salinity, total soluble solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), and biological oxygen demand (BOD), are negatively impacted by wastewater effluents contaminated with various toxic pollutants. This ultimately leads to a decline in water quality (Miretzky et al., 2006). Such contamination affects human health both directly and indirectly, in addition to disrupting natural habitats (Afshan et al., 2014).

Wastewater has diverse applications, with its frequent use in agriculture. Residential wastewater, in particular, contains nutrients beneficial to plants and is used in some countries to enhance crop yields. However, the use of wastewater carries the risk of introducing hazardous contaminants, particularly heavy metals and pesticides, which can bioaccumulate and enter the food chain (Teisseire and Guy, 2000).

Phytoremediation is a plant-based system and microbiological process employed to eliminate contaminants from the environment. It leverages the natural ability of plants to accumulate pollutants, facilitating the cleanup of water, soil, and air. The potential of aquatic plants to mitigate toxicants in aquatic ecosystems is well documented (Roongtanakiat et al., 2007). Phytoremediation is an environmentally friendly, cost-effective, and aesthetically pleasing approach for removing pollutants (Pivetz, 2001).

Recently, duckweeds have been utilized as model organisms in phytoremediation technologies for treating polluted water, making this an increasingly significant and intriguing field of research (Dar et al., 2011; Kulkarni et al., 2007). The phytoremediation of soil and water contaminated with petroleum is a non-destructive and economical technology that uses plants to clean up pollutants. With rising fuel demands, developing countries like Pakistan are working to establish oil and gas industries. However, effluents from these industries pose serious environmental hazards (Hall Jr. and Burton, 2005).

Consistent monitoring and effective management are essential to mitigate the environmental impacts of oil and gas industry effluents. Such monitoring should aim to determine the levels of toxicants released by industries and ensure compliance with pollution control guidelines established by regulatory agencies (Uyigue, 2002).

Duckweed (*Lemna minor*) is widely used among higher plants for the bio-assessment of toxicants in water bodies (Wang, 1990; Hou et al., 2007). Numerous species of algae, including *Selenastrum raphidocelis*, *Laminaria*, and *Chlorella*, are also employed in bioassays to assess pollutants in marine and freshwater environments (Anton et al., 1993; Azizullah et al., 2011). However,

duckweed is considered a superior organism for evaluating industrial effluents and water pollution (Lewis, 1995). This is largely due to its simplicity, short assay duration, and ability to monitor multiple generations (Danilov and Ekelund, 2001). Using duckweed as a model organism for the bioassessment of water pollutants can significantly reduce the reliance on animal testing, addressing both ethical and economic concerns (Ladeiro et al., 2013).

These bioassays have gained popularity due to their simplicity, widespread availability, and rapid growth. Duckweed is often regarded as an effective indicator of environmental pollutants (Laliberté et al., 1994; Ma et al., 2002; Roongtanakiat et al., 2007). Among aquatic macrophytes, *Lemna* spp. have been shown to be particularly beneficial for phytoremediation (Newete and Byrne, 2016). Duckweed is commonly used to recover nutrients such as nitrogen and phosphorus from domestic and agricultural wastewater and to remove hazardous metals (Mohedano et al., 2012; Zhang et al., 2014). Moreover, it can track the movement of heavy metals across varying concentrations, from less to more tropical regions (Mkandawire and Dudel, 2005).

Duckweed has been found to accumulate high levels of toxic heavy metals and metalloids, including zinc, manganese, cadmium, copper, uranium, nickel, boron, and arsenic (Böcük et al., 2013). Certain microorganisms in the rhizosphere of duckweed can stimulate the degradation of petroleum-contaminated materials. Furthermore, other biological systems such as lichens, fungi, microalgae, macroalgae, and aquatic plants are utilized in various countries as biofilters to purify wastewater and soil.

The current study examined the effects of effluents from the Makorhi Karak oil and gas plant on duckweed and evaluated its potential for phytoremediation of wastewater.

MATERIAL AND METHODS

Water sample collection and Lemna growth

In Makorhi, Banda Daud Shah, district Karak, Khyber Pakhtunkhwa, Pakistan, located at approximately 33°16'0" N latitude and 71°11'0" E longitude, wastewater samples were collected from the effluent of an oil and gas plant. The physicochemical analysis of these samples was conducted at the Pakistan Council for Scientific and Industrial Research (PCSIR) in Peshawar, Khyber Pakhtunkhwa.

Duckweed (*Lemna minor* L.) samples were obtained from ponds near Kohat city. Each sample, containing 40-50 fronds, was collected in one-liter beakers from various locations. The collected duckweed samples were transferred to Steinberg solution to establish laboratory cultures.

Experimental design

To evaluate the bioassessment of wastewater and the phytoremediation potential of duckweed, experiments were conducted using 300 ml plastic pots. Steinberg solution was prepared by mixing the collected wastewater samples with the required concentrations. To simulate natural conditions and ensure consistent lighting, the trials were conducted in a controlled growth room with randomly arranged replicates.

Each pot contained duckweed samples with 40-50 fronds, which were grown in Steinberg solution supplemented with varying concentrations of wastewater. Distilled water served as the control, while experimental concentrations included 1%, 5%, 10%, 25%, 50%, and 100% wastewater, all diluted with Steinberg solution. Each treatment was independently replicated three times.

Determination of frond number, frond size, and fresh and dry weight

After seven days of growth, fresh duckweed samples were prepared for analysis. The samples were first wrapped in filter paper to remove surface water, ensuring accurate weight measurements without excess moisture. The fresh biomass of the samples was then recorded using an electronic digital scale. For determination of dry weight, the samples were placed in paper bags and dried overnight in an oven set at 60°C. This process removed all moisture, leaving only the dry matter of the plants. The total dry biomass of the samples was divided by the number of fronds counted in each replication to calculate the average dry biomass per frond. This method provided a standardized measure for comparing plant growth across different treatments or conditions.

Determination of light-harvesting pigments

To quantify photosynthetic pigments, including total carotenoids and chlorophyll a and b, the protocol described by Lichtenthaler and Wellburn (1983) was followed. To neutralize acids in the plant samples and prevent pheophytin synthesis in the extracts, 25 mg of dried plant material was combined with 25 mg of magnesium oxide. This mixture was ground into a fine powder, after which 5 ml of methanol was added. The

extraction mixture was homogenized using a shaker for 2 h and then centrifuged at 4000 rpm for 5 m. The supernatant from each sample was transferred to a cuvette with a 1 cm path length for absorbance measurements at 666, 653, and 470 nm. The UV-2600 spectrophotometer was calibrated using methanol as a blank. The pigment contents, including total carotenoids, chlorophyll a, and chlorophyll b, were calculated using the following formulas:

Chl a = 15.65 OD666–7.340 OD653.

Chl b = 27.05 OD653–11.21 OD666.

$$\text{Total carotenoids} = \frac{(1000\ OD470 - 2.860\ \text{chl a} - 129.2\ \text{chl b})}{245}$$

Calculation of total soluble protein and sugar

The total amount of soluble sugar was calculated using the procedure of Dubois et al. (1956). This involved homogenizing and grinding 50 mg of fresh culture under liquid nitrogen, followed by the addition of phosphate buffer. After adding 3 ml of 90% ethanol, the temperature was maintained between 60 and 70°C for one hour. A 25 ml volumetric flask was used for the extraction of each sample, and an additional 25 ml of 90% ethanol was added. Then, 1 ml of the extract, 1 ml of 5% phenol, and 5 ml of sulfuric acid were mixed. The mixture was completed by adding 10 ml of distilled water. The combination was left for 30 min to reach room temperature. The glucose solution standard curve was used, and absorbance at a wavelength of 485 nm was measured using a cuvette containing 2 ml of the supernatant. The results were expressed in mg g^{-1} of FW.

For the total soluble protein (TSP) content, the procedure of Bradford (1976) was followed. The Bradford reagent, which consists of 1.8 ml of glacial acetic acid and 13.3 g of Cu-acetate, was prepared by adding water to create a 200 ml solution. Plant material weighing 100 mg was homogenized in 1 ml of pH 7.0 buffer using a mortar and pestle. The crude extract was centrifuged for 15 min at 4000 rpm. After centrifugation, 2 ml of purified water, 20 µl of the protein extract, and 0.5 ml of the Bradford reagent were added to the solution mixture. A spectrophotometer (UV-2600) was used to measure the absorbance at 595 nm. Bovine serum albumin (BSA) was used to create the standard curve, with distilled water serving as the standard blank.

Determination of oxidative stress markers, antioxidant enzymes, and proline

Using fresh material from the samples and a spectrophotometer, the H_2O_2 level was determined as a typical reactive oxygen species (ROS). The optical density (OD) of the reaction mixtures, measured at 390 nm, indicated that 1 ml of potassium phosphate buffer (10 mM) was extracted, followed by the addition of 2 ml of 1M KI.

Lipid peroxidation was assessed using the TBARS bioassay method (Yagi, 1982). To analyze the plant material, 100 mg of fresh *L. minor* was ground using a pestle and mortar. The ground material was mixed with 3 ml of a solution containing 0.25% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA). The mixture was heated to 95°C for 30 min to allow the reaction to occur, after which it was quickly cooled in an ice bath to stop the reaction. The cooled homogenate was centrifuged at 10,000 × g for 10 min to separate the solid debris from the liquid. The supernatant (the clear liquid above the sediment) was collected, and its optical density was measured at 532 nm to determine the presence of specific compounds, such as malondialdehyde (a marker of lipid peroxidation). Absorbance at 600 nm was also measured to correct for background interference, and this value was subtracted from the 532 nm reading.

Peroxidase activity in each sample was determined by using an enzyme extract (100 µl), guaiacol (100 µl), and H_2O_2 (100 µl) in a 3 ml reaction mixture with 300 mM molarity, along with 2.7 ml of potassium phosphate buffer (pH 7.0), and 25 mM EDTA (Zhou and Leul, 1998). The increase in optical density due to guaiacol oxidation was measured at 470 nm.

For catalase determination, 250 mg of fresh sample was used. After homogenization, the material was placed in a 10 ml extraction buffer containing 1% PVP, 3 mM EDTA, pH 7.3, 0.5 M Na-phosphate, 1% Triton X-100, and 2.8 ml of 50 mM phosphate buffer. The mixture was centrifuged at 10,000 rpm for 20 min at 4°C. The catalase level was determined by monitoring H_2O_2 reduction at 240 nm, with an extinction coefficient of 39.4 mM/cm for 30 seconds.

Ascorbate peroxidase (APX) activity was assessed by preparing a reaction mixture containing 2 mM EDTA (0.029 g dissolved in 50 ml of solution), 2.7 ml of 25 mM potassium phosphate buffer (pH 7.0), 100 µl of 300 mM hydrogen peroxide (H_2O_2), and 100 µl of enzyme extract. The reaction was initiated by mixing the components, and absorbance was measured at 290 nm over a one-minute period to monitor changes in absorbance. The decrease in absorbance at 290 nm indicated the

enzymatic activity of APX, which catalyzed the conversion of ascorbate in the presence of H_2O_2. This method provided a quantitative measure of APX activity in the sample.

To determine the proline content of the experimental plant, a spectrophotometer was used, and L-proline was used as a reference based on the bioassay of Dubois et al. (1956). Duckweed samples (0.5 g) were extracted by adding 3% w/v sulphosalicylic acid, homogenizing the mixture using a mortar and pestle, and then centrifuging the blend at 4000 rpm for 30 min. A solution of glacial acetic acid (1 ml) and acid ninhydrin (1.25 g in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) was combined with 1 ml of the supernatant. The reaction mixture was incubated at 100°C for 1 h. After incubation, the mixture was combined with 2 ml of toluene, and absorbance at 520 nm was measured using the upper colored layer.

Determination of phytoremediation potential

The phytoremediation potential of duckweed against probable oil and gas plant effluent was assessed by comparing growth differences before and after the experiment, following the approach of Pillai et al. (2000). The phytoremediation ability was evaluated using the bio-concentration factor (BCF), and the concentration of various toxicants was determined by calculating the pollutants' concentration within the growing medium and duckweed cells.

The bioconcentration factor was calculated using the method described by Abel (1989) as follows:

$$BCF = \frac{Cc}{Cw}$$

Where:

Cc is the concentration of the heavy metal in the cells (expressed in mg/L),

Cw is the concentration of the heavy metal in the water (expressed in mg/L of dry weight).

Statistical analysis

The average mean and standard deviation of the replicates were calculated using Microsoft Office Excel. One-way ANOVA was used to assess the significance of differences between treated and untreated samples. If the p-value was 0.05 or less, the least significant differences (LSD) were considered statistically significant.

RESULTS

Physicochemical characteristics of wastewater

Among the physicochemical characteristics of the petrochemical effluent is the remarkably high concentration of metals, which may be responsible for the developmental obstacles observed in *L. minor* (Table 1). The pH of wastewater ranged from 7.2 to 8.5, indicating that it was mildly basic. An electrical conductivity measurement of 10.59 µS/cm was recorded. Na had the highest concentration of all the metals tested, at 1900.33 mg/L, followed by Ca, Mg, and K, with concentrations of 157.67 mg/L, 78.40 mg/L, and 15 mg/L, respectively.

Table 1._Physical-chemical properties of effluent from petrochemical processes

Parameter	Mean and standard deviation
PH	7.2 to 8.5
EC (µΩ/CM)	10.59
Fe (mg/L)	1.75±0.026458
Co (mg /L)	0.23±0.032146
Cu (mg /L)	Bdl
Cd (mg /L)	0.02±0.01
Pb (mg /L)	0.10±0.005774
Cr (mg /L)	Bdl
K (mg /L)	15.00±2
Na (mg /L)	1900.33±14.84363
Ca (mg /L)	157.67±4.50925
Mg (mg /L)	78.40±2.179449

EC = Electrical conductivity, Fe = Iron, Co = Cobalt, Cd = Cadmium, Cu = Copper, Pb = Lead, Cr = Chromium, Na =Sodium, Mg = Magnesium, K = Potassium, Ca = Calcium, bdl = Below detection limit, mg/L = milligram per liter.

Effect on *L. minor* growth

Figure 1 illustrates the effect of wastewater on the number of *L. minor* fronds. In the untreated concentration, the highest number of fronds (110) was observed. When comparing the 100% treated wastewater to the untreated control, a significant reduction in frond growth was noted. Figure 2 (A, B, C, and D) shows the fresh and dry weight toxicity of wastewater on duckweed. The sample treated with 25% diluted wastewater exhibited the highest fresh weight of *L. minor*, indicating optimal growth at this concentration. In contrast, exposure to higher concentrations, including 100% wastewater, resulted in significant inhibition of fresh weight, suggesting toxic effects at these levels. The effect of wastewater on the fresh weight of *L. minor* per frond is detailed in Figure 2B, which highlights a dose-dependent

response. It demonstrated a substantial increase at the 25% concentration compared to the control, and considerable inhibition at the 100% concentration (Figure 2B). Comparing the sample treated with concentrated wastewater (i.e., 100%) to the untreated control, a substantial decrease in the dry weight of *Lemna* was also noted. The samples treated with 5% and 25% wastewater showed the highest dry weight. Figure 2D illustrates the impact of wastewater on the dry weight of each frond of *L. minor*. Compared to the control, a considerable increase in dry weight was observed at 25% and 5% concentrations, respectively, while significant inhibition was seen at the 100% concentration (Figure 2D). A lower wastewater content increased plant biomass, while higher concentrations were found to decrease fresh weight.

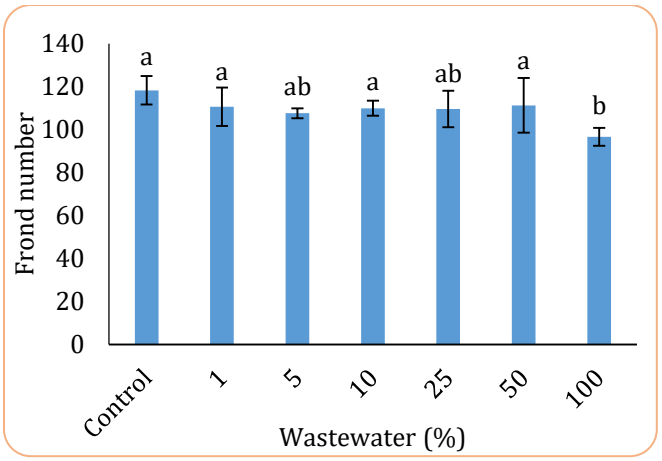

Figure 1. Impact of varying wastewater concentrations on the number of *L. minor* fronds. Means with the same alphabetic characters do not differ significantly.

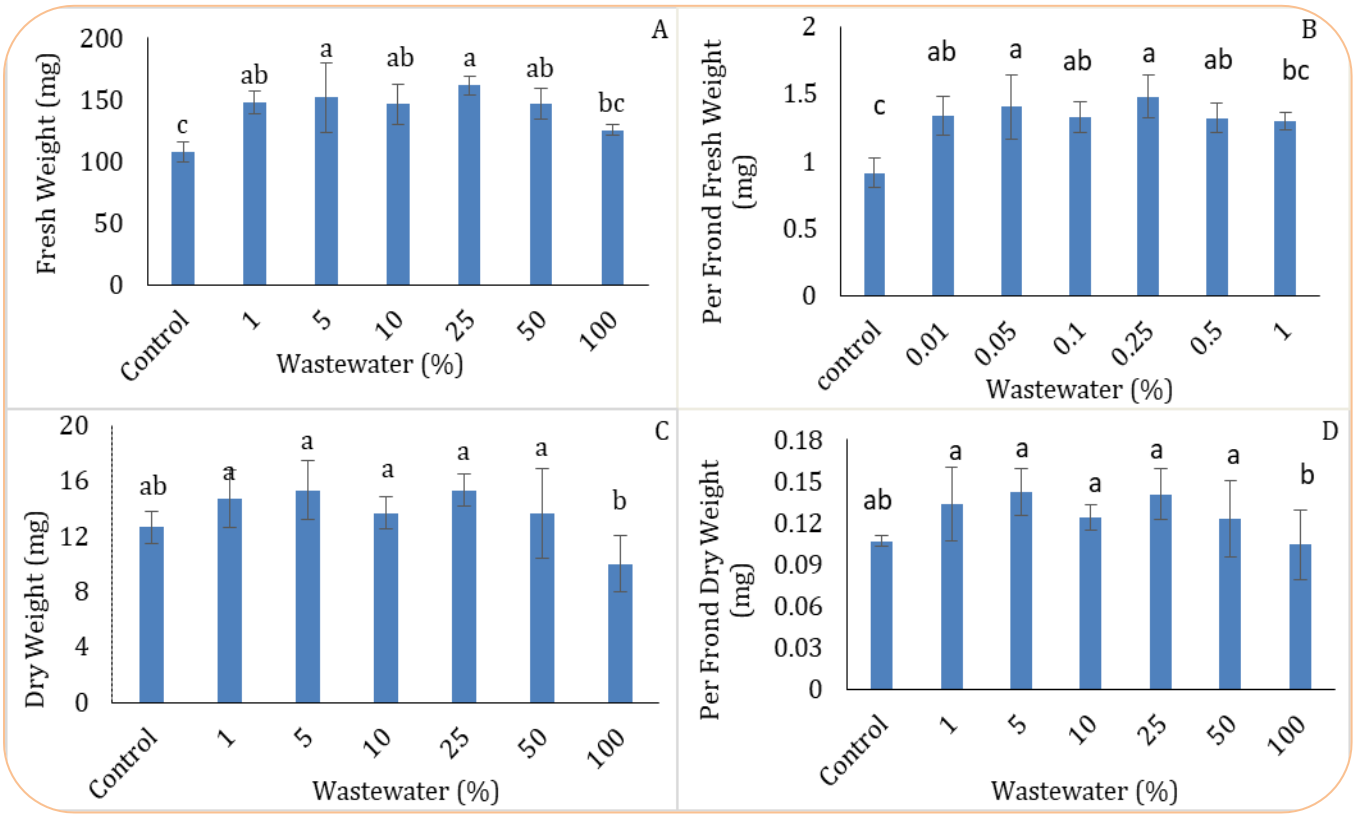

Figure 2. The impact of varying wastewater concentrations on (A) fresh weight, (B) fresh weight per frond, (C) dry weight, and (D) L. minor per-frond dry weight. There is no statistical significance between means with similar alphabetic letters.

Impact of wastewater on *L. minor* frond length and area

Figure 3A depicts the effect of wastewater on the frond area of *L. minor*. No significant inhibition was observed at any concentration, with the largest frond area (0.26 cm^2) recorded at a 10% concentration. In contrast, Figure 3B illustrates the impact of wastewater on frond length, showing a significant increase at a 5% concentration (1.75 cm) compared to the control. However, higher concentrations caused notable inhibition of frond length.

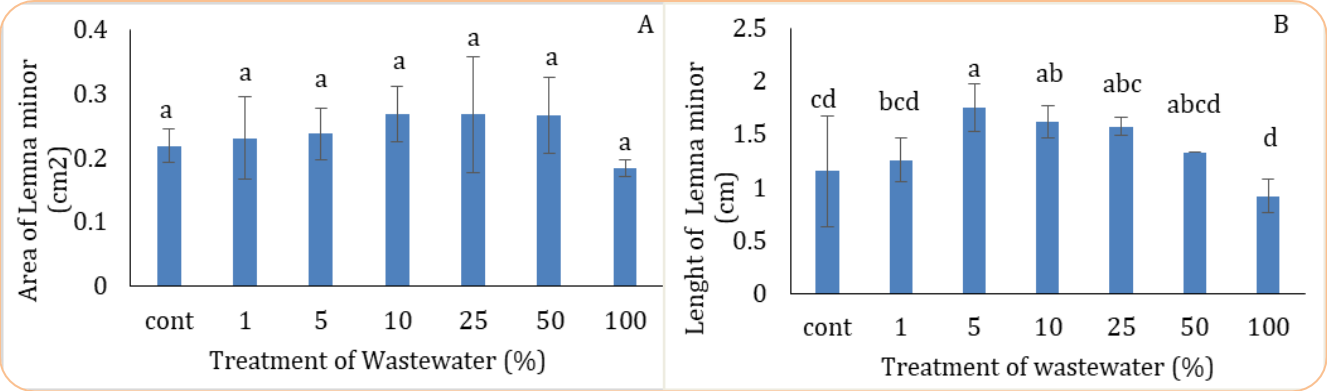

Figure 3. Effects of varying wastewater concentrations on the length and area of *L. minor.* Means with the same alphabetical letters are not statistically significant.

Effect on photosynthetic pigments

The experiments revealed an inverse correlation between wastewater concentration and the levels of photosynthetic pigments. Chlorophyll a, chlorophyll b, and total carotenoid content decreased progressively as the intensity of wastewater treatment increased. However, no significant decrease in chlorophyll b content was observed in samples treated with varying effluent concentrations, except at 50% and 100%, where a marked reduction in total carotenoid content was noted compared to the control. Interestingly, the chlorophyll concentration showed a dramatic reduction in most samples, except for those treated with 25% wastewater, as illustrated in Figure 4.

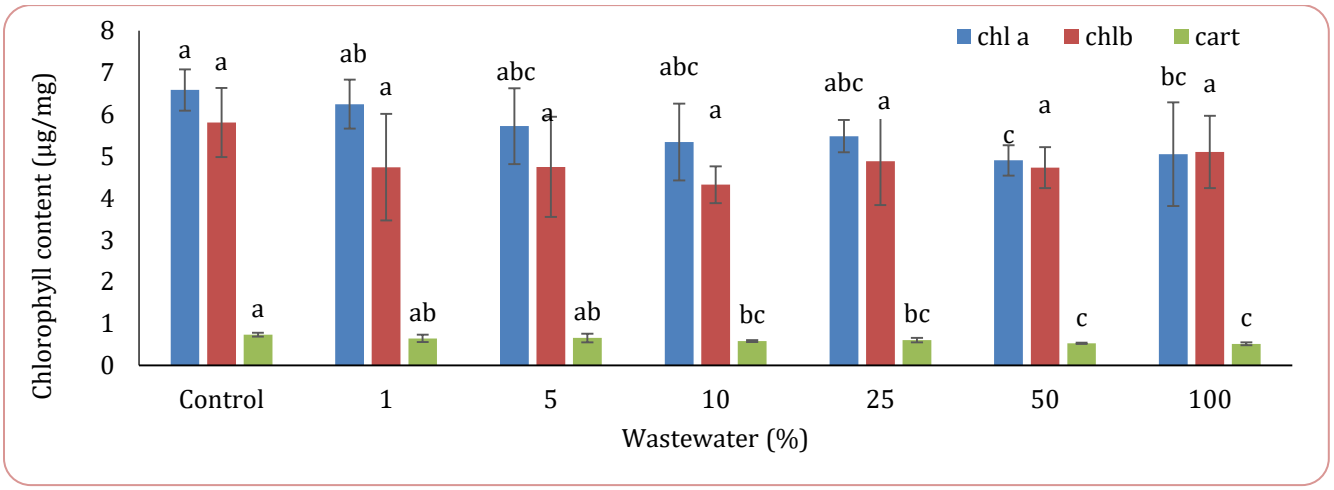

Figure 4. Impact of varying wastewater concentrations on the photosynthetic pigments of *L. minor*. Differences in means with identical alphabetical letters are statistically insignificant.

Effect on sugars and proteins

The results demonstrated a significant reduction in total soluble protein content as wastewater concentration increased. In Figure 5A, the total soluble protein content of *L. minor* exhibited an inverse relationship with rising wastewater levels. This reduction may be attributed to acute oxidative stress induced by toxicants in the wastewater, which likely hinders protein synthesis following exposure. Figure 5B illustrates a dose-dependent response of *L. minor* to the effluent, with no observable effect at lower concentrations and a marked reduction in total soluble sugar content at higher concentrations.

Effect on oxidative stress-related markers and lipid peroxidation in *L. minor* exposed to wastewater

Exposure of *L. minor* to wastewater led to the production of ROS, with TBARS and H_2O_2 levels serving as characteristic indicators of oxidative stress. The H_2O_2 concentration was measured and is presented in Figure 6A, showing that *L. minor* produced more H_2O_2 at higher

wastewater concentrations. Among the tested concentrations, a slight increase in H_2O_2 was observed at 10%; however, this increase was not statistically significant compared to the control group.

Lipid peroxidation, indicated by malondialdehyde (MDA) levels measured as TBARS, is another marker of oxidative stress-induced cellular damage. As wastewater concentration increased, lipid peroxidation also gradually rose, with a significant increase observed at 10% concentration relative to the control, as shown in Figure 6B.

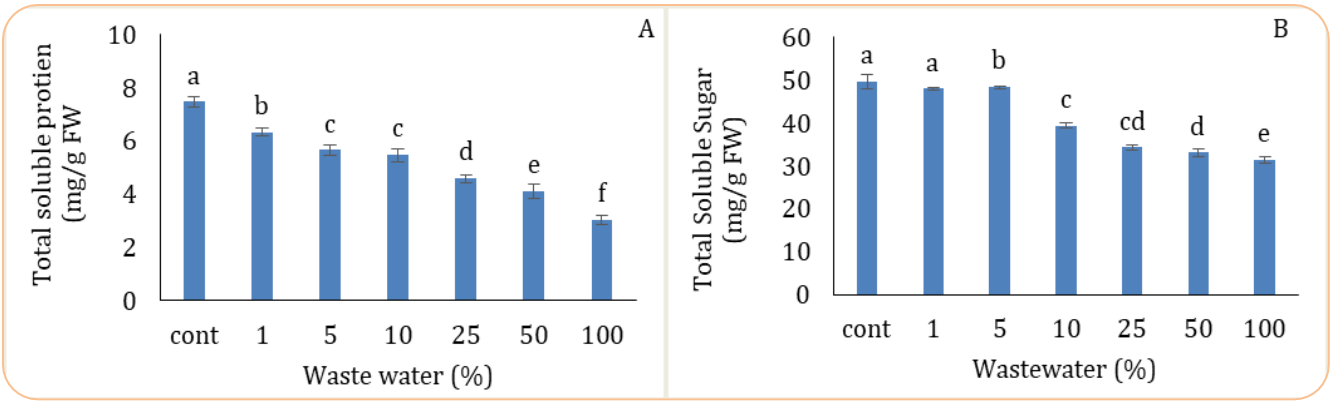

Figure 5. Effects of varying quantities of wastewater on the concentrations of (A) total soluble protein and (B) total sugar content in L. minor after seven days of treatment.

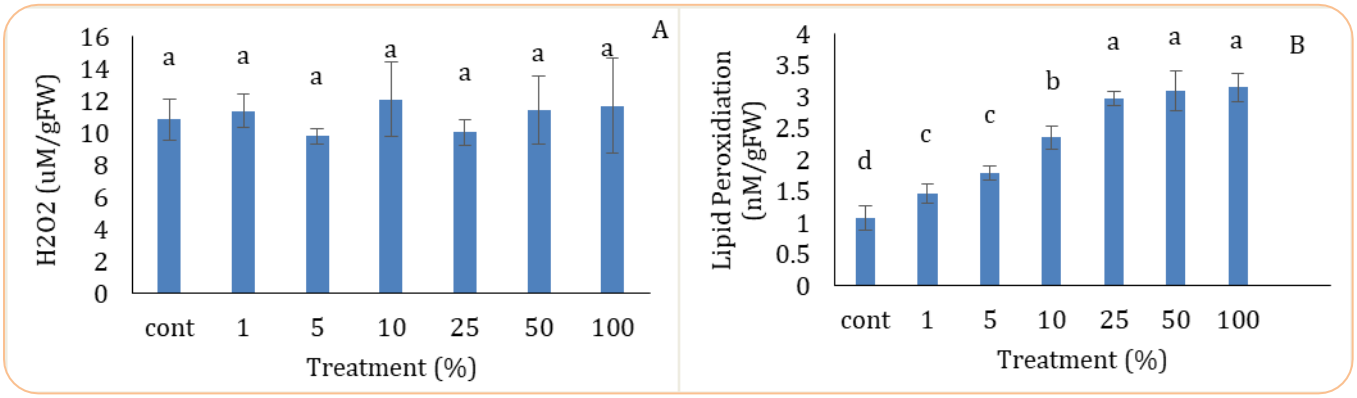

Figure 6. ROS and TBARS levels in *L. minor* exposed to varying wastewater concentrations for seven days. Different letters indicate significant differences ($p < 0.05$).

Effect of wastewater on antioxidant enzymes and proline

Figure 7A, B, C, and D illustrate the impact of petrochemical effluent exposure on the activities of antioxidant enzymes (POD, APX, and CAT) and proline content in *L. minor*. The results indicate that exposure to wastewater significantly enhanced antioxidant enzyme activity, with APX activity increasing progressively as wastewater concentration rose compared to the control. Figure 7B demonstrates the effect of wastewater on POD activity. At 1% wastewater concentration, peroxidase activity remained unchanged. However, as the wastewater concentration increased beyond 1%, a significant rise in POD activity was observed compared to the control. Catalase activity in *L. minor* treated with wastewater showed a substantial increase at higher wastewater concentrations, highlighting its role in oxidative stress response.

Proline content in *L. minor* also exhibited significant changes in response to varying wastewater concentrations. At lower wastewater levels (1%), proline levels (99.25 µM/g FW) were comparable to the control. However, at higher concentrations, 5%, 10%, and 100%, proline accumulation increased significantly to 135.8, 147.1, and 187.8 µM/g FW, respectively, as shown in Figure 7D.

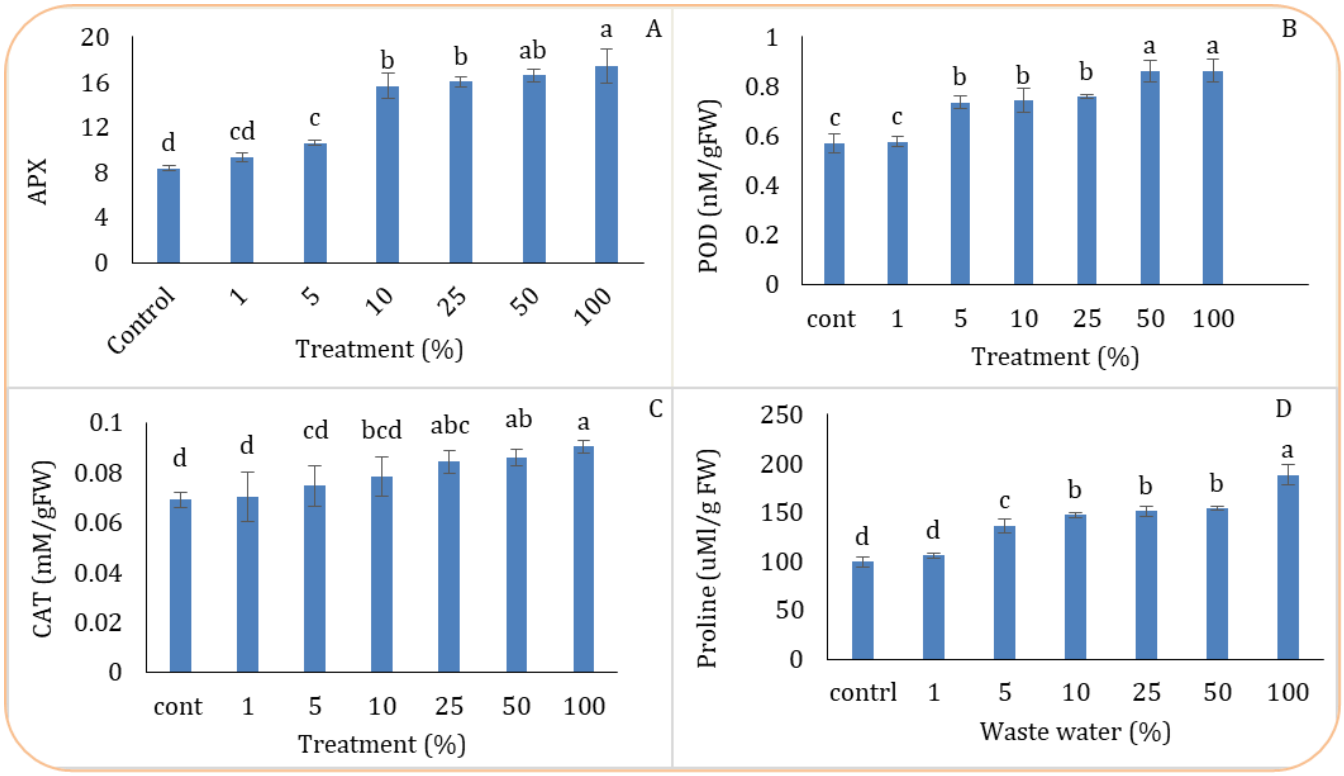

Figure 7. Effect of varying wastewater concentrations treated for seven days on the levels of (A) APX, (B) peroxidase, (C) catalase, and (D) proline in *L. minor*.

Heavy metals removal by *Lemna*

Lemna was grown for 7 days in a medium containing 100% wastewater. A separate medium with the same wastewater, but without *Lemna*, was maintained as a control. After 7 days, the wastewater from both setups was tested for heavy metal concentrations. In the medium containing *L. minor*, the control group had 1.75 mg/L of Fe, 0.22 mg/L of Co, and 0.10 mg/L of Pb. As indicated in Figure 8, the concentrations of Fe, Co, and Pb were 0.72 mg/L, 0.17 mg/L, and 0.07 mg/L, respectively. Lead and cobalt were absent from the *L. minor* pellet that had been acid-digested, although 0.21 mg/L of Fe was present. In the current study, the bioconcentration factor o *L. minor*, which measures the likelihood of accumulating metals or metalloids, was calculated to be 0.25. This indicates that *L. minor* is a promising option for heavy metal removal from wastewater.

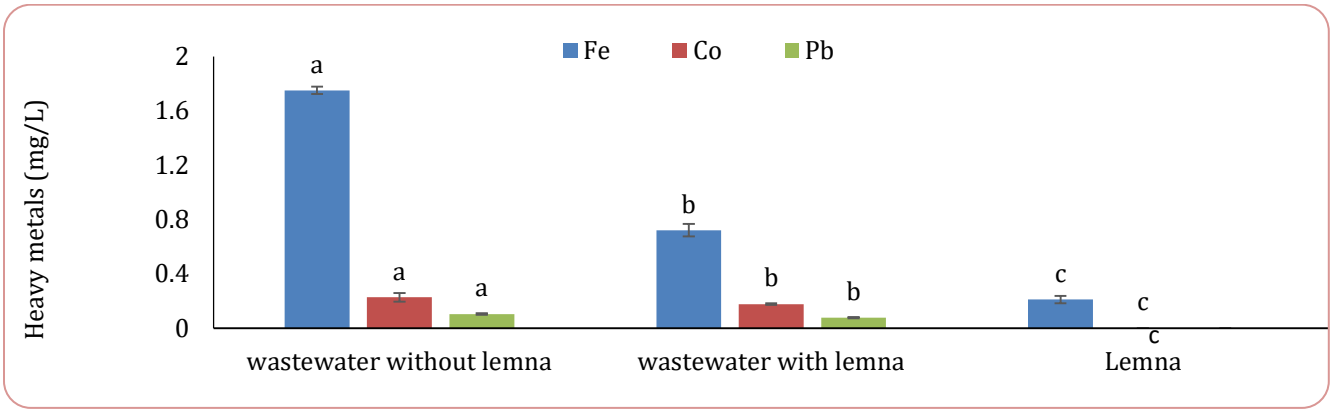

Figure 8. Concentration of heavy metals in wastewater treated with and without Lemna, as well as in Lemna after a 7-day exposure.

DISCUSSION

The current investigation showed significant inhibition in the growth of *L. minor* fronds exposed to concentrated (100%) wastewater compared to the control. Merkl et al. (2004) found similar results when examining the negative impacts on plant growth exposed to crude oil. The findings of Al-Baldawi et al. (2015) also demonstrated similar inhibitory effects on *L. minor* plants when subjected to wastewater. In this study, exposure to higher concentrations of wastewater decreased plant growth, particularly in terms of frond numbers, in a dose-dependent manner. Similarly, low molecular weight hydrocarbons present in petrochemical wastewater had harmful effects on the growth of frond numbers in *L. minor* (Zand et al., 2010).

This study also demonstrated a significant increase in biomass at high concentrations and a decrease at low concentrations of wastewater. Our results are consistent with those of Radić et al. (2010), who reported that exposure of *L. minor* to wastewater resulted in reduced plant growth. Singh and Singh (2006) observed similar effects of wastewater on *L. minor* and recommended that prolonged exposure of *Lemna* to wastewater affects biomass yield, as measured by both fresh and dry weight.

The findings of this investigation are consistent with those of Al-Baldawi et al. (2015), who discovered that wastewater inhibits *L. minor* growth in a dose-dependent manner. Our results, using test organisms, are consistent with those of Radić et al. (2010), who found that *L. minor* grows less in terms of biomass and frond count when exposed to wastewater. Zand et al. (2010) suggested that hydrocarbons with low molecular weight toxicity could be the reason behind the detrimental effects of petrochemicals on Lemna plant growth.

The level of photosynthetic pigments was analyzed, and the effects of wastewater treatment were found to be dose-dependent. Specifically, with an increase in wastewater concentrations, a gradual increase in the levels of total carotenoids, chlorophyll a, and chlorophyll b was observed. A similar study conducted by Singh and Singh (2006) also found remarkable effects of wastewater on the level of photosynthetic pigments in *L. minor*. The results of Radić et al. (2010) further support our current research by showing negative impacts of concentrated wastewater on the photosynthetic pigments in *L. minor*.

To analyze the stress imposed by petrochemical wastewater, various parameters were examined, including the activities of POD, catalase, and APX, which were found to be significantly increased. Furthermore, the activities of APX increased with higher wastewater concentrations. Similar trends were observed in catalase activity and proline contents. The present results align with those of Radić et al. (2010), demonstrating that wastewater treatments can increase antioxidant activities in *L. minor*. As wastewater content increased, a steady rise in ROS and lipid peroxidation was also observed. Concluding remarks along these lines were made by Radić et al. (2010), who found that *L. minor* exposed to wastewater had higher ROS and TBARS levels.

The observed results demonstrated that the total soluble protein content significantly decreased as the wastewater concentration increased. Similarly, the response of *L. minor* to effluent water in terms of total soluble sugar was dose-dependent; higher concentrations of effluent resulted in a reduction of soluble sugar. Singh et al. (2008) further discovered that modest exposure of *L. minor* to industrial effluent can lower sucrose and total soluble protein levels. The bioconcentration factor of *L. minor* in the current study, which measures the accumulation of metals or metalloids, suggests that this species can be used to remove hazardous heavy metals from wastewater. Megateli et al. (2009) also demonstrated that Lemna is a bio-accumulator of heavy metals in phytoremediation. Gupta and Prakash (2013) recommended *L. minor* as the most effective species for the bioremediation of heavy metals.

CONCLUSION

Present findings conclude that exposure of *Lemna minor* to petrochemical wastewater resulted in a drastic change in biomass and frond growth, as well as a decrease in photosynthetic pigments. Among the pigments, chlorophyll a and b showed greater sensitivity than carotenoids. Furthermore, oxidative biomarkers suggest that toxicants in the wastewater may cause oxidative stress, which in turn elevates ROS levels. The toxicological analysis also concluded that **L. minor** has the ability to act as a phytoremediation agent, ultimately remediating the toxicity of heavy metals.

RECOMMENDATION

Based on the current findings, it is advised that wastewater effluents from petrochemical plants contain toxicants that negatively impact aquatic life. Therefore, wastewater should undergo proper treatment before

being discharged into the environment. To mitigate risks, the government should impose strict regulations on such operations and launch awareness programs. The effective and purposeful use of *L. minor* for phytoremediation of polluted soil to convert it into valuable agricultural land is strongly recommended. A concrete policy should be framed in this regard.

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AUTHORS' CONTRIBUTIONS

FS, FMS, GMS, MTG, and KUR designed the research work; FS and NU collected the data; FS, SI, and NU contributed to the data analysis; FS and KUR contributed to the write-up; FS, NU, and all authors reviewed the final version of the manuscript and gave their approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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