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Effects of Salt Stress on the Biochemical Characteristics of the Aquatic Plant *Lemna minor* L. (1753)

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Abstract - One of the most essential abiotic elements that has an important influence on plant development, growth, and yield is salinity. The current study revealed how the biochemical characteristics of *Lemna minor* L. plant were impacted by different levels of sodium chloride (0, 50, 100, 125, 150, 175, and 200 mM). The experiment was carried out in March and April of 2024. The measured amount of chlorophyll an at 50 mM was certainly greater when compared with that of the control treatment, achieving 1.0464 mg/g. At the same time, the percentages of chlorophyll a, b, and total at the other concentrations were lesser than that of the control. The results showed a rise in proline levels that related to elevated salt concentrations, with the peak proline level measured at 0.573 mg/g at a concentration of 200 mM, reflecting a 309 percent increase relative to the control treatment. The analysis of minerals revealed changes in nutrient levels. The largest rise in zinc level, 0.8993 mg/g, was measured at 125 mM, which implies a 23.2% increase. Boron levels were elevated at 200 mM, reaching 0.8550 mg/g. Manganese levels lowered considerably at raised salt concentrations, with the highest concentration, 0.1987 mg/g detected at 50 mM. Copper levels showed considerable fluctuations, reaching a high of 0.0194 mg/g at 200 mM and a low of 0.0121 mg/g at 175 mM. Iron levels increased at a concentration of 125 millimolar, reaching 1.4966 mg/g, a 339% increase compared to the control treatment. Calcium levels also increased with increasing salinity concentrations, with the highest level recorded at 1.6815 mg/g at a concentration of 150 mM. Magnesium levels showed an increase with reducing salinity concentrations, with the highest concentration at 50 mM reaching 1.0032 mg/g. The results above indicate that *Limna minor* L. promotes osmotic adaptation and selective mineral management to cope with salt stress. The study aims to assess the ability of duckweed (*Limna minor* L.) to grow and reproduce under different levels of salinity, in order to determine the extent to which it can be utilized as an economical feed source for animals in salinity-affected environments.

تأثير التغيرات الملحية على الخصائص البيوكيميائية لنبات *Lemna minor* L.

احمد يوسف لفته هزاع ومحمود شاكر هاشم وعقيل عبد الصاحب عبد الحسين الوائلي وطارق حطاب المالكي
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المستخلص – تُعدّ الملوحة من أهم العناصر غير الحيوية التي تؤثر بشكل كبير على نمو النبات وتطوره وإنتاجيته. كشفت هذه الدراسة عن تأثير مستويات مختلفة من كلوريد الصوديوم (0، 50، 100، 125، 150، 175، 200 ملي مولار) على الخصائص البيوكيميائية لنبات عدس الماء (*Lemna minor* L.). أُجريت التجربة في شهري مارس وأبريل من عام 2024. كانت كمية الكلوروفيل أ المقاسة عند تركيز 50 ملي مولار أعلى بكثير من تلك

الموجودة في معاملة السيطرة، حيث بلغت 1.0464 ملغم/غم. في الوقت نفسه، كانت نسب الكلوروفيل أ، ب، والكلوروفيل الكلي عند التركيزات الأخرى أقل من تلك الموجودة في معاملة السيطرة. أظهرت النتائج ارتفاعاً في مستويات البرولين مع زيادة تركيز الملح، حيث بلغ مستوى البرولين ذروته 0.573 ملغم/غم عند تركيز 200 ملي مولار، مما يعكس زيادة بنسبة 309% مقارنة بمعاملة السيطرة. كشف تحليل المعادن عن تغيرات في مستويات العناصر الغذائية. سجل أعلى ارتفاع في مستوى الزنك، 0.8993 ملغم/غم، عند تركيز 125 ملي مولار، ما يشير إلى زيادة بنسبة 23.2%. وارتفعت مستويات البورون عند تركيز 200 ملي مولار، لتصل إلى 0.8550 ملغم/غم. وانخفضت مستويات المنجنيز بشكل ملحوظ مع ارتفاع تركيزات الملح، حيث سجل أعلى تركيز له بلغ 0.1987 ملغم/غم عند تركيز 50 ملي مولار. وأظهرت مستويات النحاس تقلبات كبيرة، حيث بلغت ذروتها 0.0194 ملغم/غم عند تركيز 200 ملي مولار، وادنى قيمة بلغت 0.0121 ملغم/غم عند تركيز 175 ملي مولار. وارتفعت مستويات الحديد عند تركيز 125 ملي مولار، لتصل إلى 1.4966 ملغم/غم، أي بزيادة قدرها 339% مقارنة بمعاملة السيطرة. ارتفعت مستويات الكالسيوم أيضاً مع زيادة تركيزات الملوحة، حيث سجل أعلى مستوى وبلغ 1.6815 ملغم/غم عند تركيز 150 ملي مولار. كما أظهرت مستويات المغنيسيوم زيادة مع انخفاض تركيزات الملوحة، حيث بلغ أعلى مستوى 1.0032 ملغم/غم عند تركيز 50 ملي مولار. تشير النتائج المذكورة أعلاه إلى أن نبات العدس المائي (*Limna minor* L.) يعزز التكيف الأسموزي والإدارة الانتقائية للمعادن لمواجهة الإجهاد الملحي. تهدف هذه الدراسة إلى تقييم قدرة عدس الماء (*Limna minor* L.) على النمو والتكاثر في ظل مستويات مختلفة من الملوحة، وذلك لتحديد مدى إمكانية استخدامه كمصدر علف اقتصادي للحوانات في البيئات المتأثرة بالملوحة.

كلمات مفتاحية: *Limna minor* L.؛ الإجهاد الملحي؛ الكلوروفيل؛ البرولين؛ العناصر الغذائية المعدنية.

Introduction

River estuaries are places where rivers meet together with the ocean. Because of the ocean's impact, saltiness, or salinity, is an expected feature of those places. This saltiness impacts the types of plants and animals that can flourish in different parts of the estuary. (Gibson *et al.*, 2002). Changes in salinity can happen because of climate changes or other occurrences. For instance, heavy rain can push freshwater farther down the river than normal, while dry spells can raise salt levels further up the river. These changes in the weather might affect the plants, animals, and other living things in estuaries. (Wetz and Yoskowitz, 2013).

Plants that grow in water are drastically effected by changes in water salinity, particularly those that survive in freshwater (Al-Saad *et al.*, 2010). One instance of this is duckweed, a species of monocot botanical plant that is part of the Lemnaceae family. These plants flourish in places shielded from strong winds and currents, generally developing dense populations that float on the surface of watercourses full of organic nutrients (Landolt and Kandeler, 1992).

The aquatic duckweed plant has a wide range of applications, like use in a feed for animals in homes (Leng *et al.*, 1995) as well as a pollution remedy. (Ziegler *et al.*, 2016). The plants that produce duckweed can contain a high protein content, with percentages reaching 40-43% of its dry weight, and also large quantities of phosphate, potassium, minor minerals, and amino acids, added to different plant based sources (Stadtlander *et al.*, 2019). Furthermore, it is characterized by a low fiber content, measuring to only 5% in dry matter. Five distinct genera of Duckweed (*Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*) that contain nearly 36 species found around different areas (Bog *et al.*, 2019). Within Iraq, three species of *Lemna* have been identified (*Lemna trisulca*, *Lemna gibba*, and *Lemna minor*). Both of the first two are predominant in the northern regions, and the third is generally found in the middle and southern regions. (Haraib, 2013; Al-Joboury and Al-Dabbas, 2023).

Duckweed is considered one of the most important plants for promoting agricultural sustainability, especially in light of climate change. This plant is characterized by its abundance of nutrients, chemically active compounds, enzymes, and antioxidants, which greatly enhances its ability to resist biotic and abiotic stresses (Del Bono *et al.*, 2021). Further, the plant is believed to be one of the greatest yielding aquatic plants for bioremediation and can be seen an excellent option for producing biofuel (Kuznetsova *et al.*, 2019; Ali *et al.*, 2020). Two of the most important elements of the environment impacting the growth of aquatic plants are water scarcity

and salt. Certain aquatic plants show resistance to salinity, while others are unable to endure NaCl concentrations over 50–100 mM due to their higher susceptibility to such conditions. (Kaijser *et al.*, 2019). Salinity has been described as an abiotic stressor that exerts osmotic and phytotoxic effects on plants. with aquatic plants, saltwater impacts their water potential, lowering the absorption of adequate water necessary for physiological functions. (Shahzad *et al.*, 2019). In another experiment, duckweed was placed in different levels of NaCl, ranging from 2 to 12 g/L, over a 20-day duration to demonstrate the effects of salinity on the estimated chemical makeup of the plant. The results discovered that the protein content decreased with high salinity levels, with the lowest readings being recorded at 12 g/L from NaCl. Moreover, it was found that the accumulation of major and minor nutrients was greater when the salinity levels decreased. (Ullah *et al.*, 2021).

A study was conducted in Saudi Arabia, on how different levels of duckweed cloned strains respond to different levels of salt . The study tested these cloned strains under six concentrations : a control group, a two-week control group and five salt levels 50 , 100 , 100 , 150 , 200 and 250 mM sodium chloride. The conclusions indicated that Medina-2 (*spirodela polyriaza*) and Kassem (*landoltia pancata*) improved cloning of all other genotypes in both morphological and physical testing when exposed to salt stress(Al-Dakhil *et al.*,2023). In another study a demographic approach was taken to study whether duckweed that had contact with ancestral stress could cope with immediate salt stress compared to those without a background. Three generations of other clones were placed in a saline environment 2 g/L of NaCl, (a non-lethal level of salt stress) and a control group (NaCl). After that, they were grown for another 0 to 3 generations in a controlled environment to demonstrate the effect of how and when the salt was stressed. The research results proved that sudden stress caused a decreased level of activity due to decreased reproduction. The effect of previous stress, combined with its relation with present stress, was more complex. Recent stress has accelerated plant reproduction, probably leading to lowered offspring quality. (Chmilar *et al.*, 2023).

This study sought to find out the impact of different levels of salinity on the biochemical characteristics of duckweed (*Lemna minor* L.). The aim was to determine the optimal salt concentrations for its growth while preserving its biomass. Studies in science provide limited information regarding the ability of waterweed to adapt to saltwater environments in southern Iraq.

Materials and Methods:

Fresh biomass of plants came from natural ecosystems in the watery regions of Abu Al-Khaseb, Basra, Iraq. The tests began in March and April of 2024 at the Aquatic Plants Laboratory, in the Marine Biology Department of the Marine Science Center at the University of Basrah. Once gathered, the plants were rinsed with water and placed in normal growth conditions (25°C, 12:12 h light/dark cycle) to promote their proliferation.

To assess the effect of salt stress, lentil plants (at a density of 10 g/L) were exposed to graded levels of sodium chloride (0, 50, 100, 125, 150, 175, and 200 mM) in 5-liter plastic pots, with each treatment applied in three repetitions. At the end of the two-week incubation period, plantlets were taken in and assessed based on the variables that follow, according to the protocols offered for each measurement.

Estimation of chlorophyll pigment content :

The amount of chlorophyll pigment was measured by taking 0.5 g of fresh leaves and extracting the chlorophyll using 80% acetone as the solvent (Goodwin, 1976). Following its extraction, the sample's optical density was measured with a spectrophotometer at wavelengths of 663 and 645 nm. In the end, the total chlorophyll content (mg/g) has been determined using annuity formula:

-Chlorophyll a (mg g^{-1}) = $12.7 D(663) - 2.69D(645)$.

-Chlorophyll b (mg g^{-1}) = $22.9 D(645) - 4.68D(663)$.

-Total Chlorophyll (mg g^{-1}) = $20.2D(645) + 8.02D(663)$.

D(663) = Absorbance at a wavelength of 663 nm

D(645) = Absorbance at a wavelength of 645 nm

The proline content

The volume of free proline was measured using the method mentioned by Bates *et al.*, (1973). A weight of 0.5 g was taken from each sample. These samples were crushed in a mortar after the addition of 2 ml of 3% sulfosalicylic acid solution then treating the filtrate with a mixture of ninhydrin and glacial acetic acid and heating it at 100°C, then it was quickly cooled by putting the pipes in an ice bath. After allowing the mixture to sit at room temperature (25 ° C), the proline concentration was measured using a spectrophotometer set for a wavelength of 520 nm.

Estimating of mineral constituents:

To estimate the concentration of minerals, 1 g of powdered samples was taken and digested with a 1:1 acidic solution of ($\text{HNO}_3\text{:HCL}$) in a volume of 3 ml in 25 ml Pyrex tubes. After being sealed with glass stoppers, the tubes were placed under vacuum for a full day. The tubes were then transferred to a water bath for 1 hour to complete the digestion. Three milliliters of distilled water were added to each sample and placed on a heating plate at 70°C until the volume reached 2 ml. Finally, the resulting extract was diluted with appropriate amounts of distilled water for analysis (Rice *et al.*, 2012). Mineral element concentrations were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP™ PRO, Thermo Fisher Scientific).

Statistical Analysis

The results of all experiments has been displayed as the mean \pm standard error of the mean (SEM) calculated from three separate replications. Data was analyzed using Genestat version 19 with a one-way analysis of variance (ANOVA), by using the LSD test at 0.05 percentage level (Steel and Torrie, 1981).

Results:

Chlorophyll Content

For an assessment of the ability of duckweed to grow in different salt stress levels, it was examined for its chlorophyll content (A, B, and total) (Figure 1 A-C). As the NaCl concentration increased, we noticed that the chlorophyll content (A, B, and total) decreased for most

concentrations, except for the treatment with 50 mM, which had a significantly higher chlorophyll a content of (1.0464) mg/g compared to the others. All other treatments showed a significant decrease. The treatments 175 mM and 200 mM had the least amount of chlorophyll, showing more than a 60% drop compared to 50 Mm. In treatment control, the chlorophyll b content was the highest, way better than the other treatments. The other treatments dropped a lot, between 65% and 84% less than treatment control. The total amount of chlorophyll was highest in treatment control, which had a value of 3.0607 mg/g, and this was much more than the other treatments. After treatment control, there was a steady drop in chlorophyll levels starting from 50 mM. Treatments 100 Mm to 200 mM were similar to each other, but all had lower levels than treatment control and 50 mM.

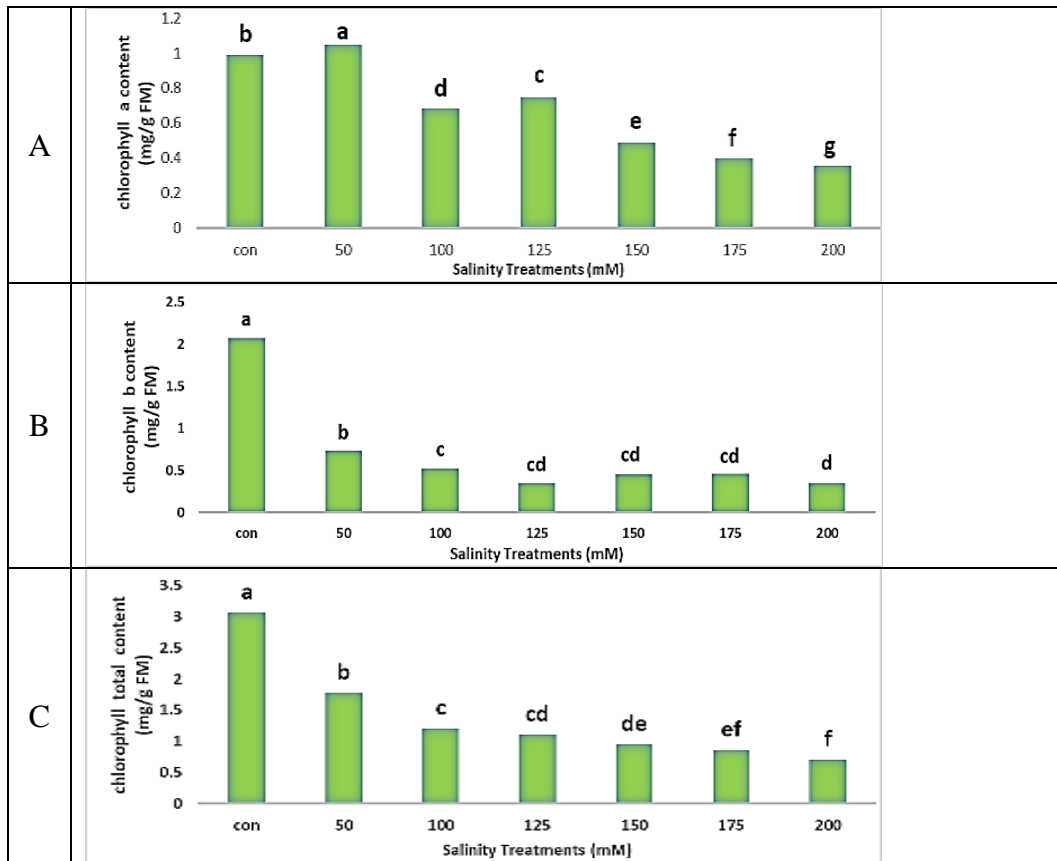


Figure 1. Impact of different levels of salt stress the amounts of chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C). The values shown represent the average standard deviation from three replicates per for each concentration. Different letters indicate a statistically significant difference at the 0.05 level.

Proline Content:

The findings showed a good connection between proline levels and higher amounts of NaCl. Proline acid levels went up a lot, from 0.140 mg/g in the control to 0.573 mg/g in the 200 mM , which had the highest proline level and was pretty similar to the 175 mM .The other concentrations had lower values and were not much different from one another. There was a

309% increase in proline content for the 200 mM treatment and a 219% increase for the 175 mM treatment when compared to the control .

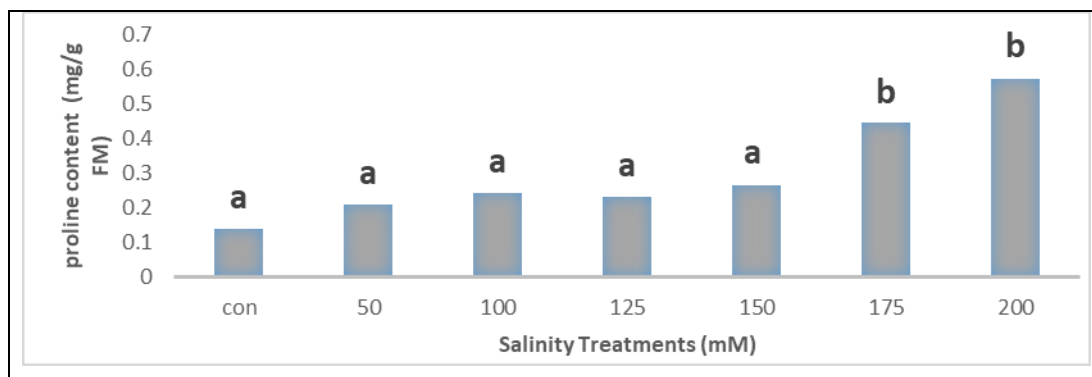


Figure 2. Impact of varying NaCl concentrations on the proline levels in duckweed.

In the common water lentil plant (*Lemna minor*), proline is an essential amino acid that plays many roles, including acting as an osmotic regulator and a defense mechanism against stress. In this context, proline levels can change in response to various environmental factors and stresses, such as exposure to salt stress or others. (Bassi and Sharma, 1993).

Estimation of Mineral Elements:

Excessive absorption of sodium chloride disturbs the fragile osmotic and ionic balance in plant cells, leading to major issues in the uptake, movement, and allocation of vital mineral elements in plant tissues. Results obtained from treating lentil plants with different levels of NaCl (Table 1) this effect was clearly demonstrated, as it was noticeable in the various essential nutrients required for plant growth.

Zinc: the results indicated that the highest average concentration was reached 0.8993 mg/g at 125 mM, showing a significant increase of 23.2%. Other treatments as well revealed significant increases when compared to the control: 50 mM (7.8%), 100 mM (2.1%), 175 mM (19.0%), and 200 mM (5.6%). yet, the 150 mM treatment failed to show a significant difference from the control treatment.

Boron: Results of the boron data proved that the lowest mean concentration appeared at 200 mM, achieving 0.8550 mg/g, with a substantial climb of (+26.9%). The treatments at 100 mM (+5.9%) and 175 mM (+15.8%) additionally revealed significant increases, yet major decreases were observed in the treatments at 50 mM (-1.5%), 125 mM (-2.8%), and 150 mM (-20.8%).

Manganese: research results proposed a notable rise of (+14.6%) in the 50 mM treatment, achieving the highest average of 0.1987 mg/g. In contrast, the other treatments showed significant reductions when compared to the control treatment, with 100 mM (-17.0%), 125 mM (-52.2%), 150 mM (-73.8%), 175 mM (-66.4%), and 200 mM (-63.9%).

Copper: The results indicate an initial significant reduction copper in the treatment with doses of (50, 100, 175) mM, revealing decreases of 10.4%, 15.3%, and 16.0%, respectively. Following this, there was a major rise in copper concentrations in the treatments of (125, 150, and 200) mM, with increased rates of (+20.8%, +14.6%, and +34.7%, respectively, as compared to the control therapy.

Salinity did not lower the levels of Fe in duckweed; instead, it raised them by a lot at every level. The most significant change occurred at 125 mM NaCl, where iron increased by +339% relative to the control. This is likely due to a concentration effect, the presence of chelated Fe, and a response to salt stress that helps the body. But this doesn't mean that salt is good; a higher iron level could mean less growth instead of better nutrition.

Calcium: Are all saline treatments above 50 and 200 mM indicated a significant rise in calcium levels as compared to the control, this increase varied between (+22.2% to +28.9%). The mineral magnesium: The results indicate an intense and significant rise at low salinity (50–100 mM), with Mg raising substantially (70.2% and 44.2%), respectively. It then levels off near the control at from salinity (125–150 mM), after which it a marked decline at high salinity (175–200 mM) of (5.0% and 6.2%), respectively, when compared to the control treatment.

Table 1. Nutrient content in duckweed (*L. minor*) following treatment with various NaCl concentrations.

Treatment (NaCl) mM	Nutrient concentration (mg/g DW)						
	Zn	B	Mn	Cu	Fe	Ca	Mg
0	0.7299	0.6735	0.1734	0.0144	0.3405	1.3049	0.5893
50	0.7871	0.6633	0.1987	0.0129	0.4176	1.5943	1.0032
100	0.7455	0.7132	0.1440	0.0122	0.4123	1.6483	0.8497
125	0.8993	0.5547	0.0829	0.0174	1.4966	1.6549	0.6244
150	0.7293	0.5336	0.0454	0.0165	0.3674	1.6815	0.6267
175	0.8683	0.7796	0.0583	0.0121	0.6189	1.6448	0.5597
200	0.7708	0.8550	0.0626	0.0194	0.3826	1.6327	0.5526
LSD	0.0041	0.0058	0.0003	0.0001	0.0038	0.0151	0.0155

Discussion:

Chlorophyll levels in plant leaves vary greatly depending on the plant species and the surrounding environmental conditions. The study results showed that chlorophyll a levels showed a significant increase at a concentration of 50 mM NaCl compared to the control group and other concentrations (Figure 1). This increase represents a temporary adaptive mechanism that contributes to protecting the photosynthetic system from light and oxidative stress by increasing pigment production, a behavioral pattern observed in some plant species with high salt tolerance. (Munns and Tester, 2008; Van Zelm *et al.*, 2020).

Meanwhile, chlorophyll a levels experienced a gradual and sharp decline, reaching their lowest levels at 175–200 mM NaCl, where the decrease was approximately 60% compared to the 50 mM treatment. This decline, associated with acute physiological disturbances, is caused by damage to the chlorophyll synthesis pathway, which is attributed to ionic imbalance and the accumulation of reactive oxygen species (ROS). Alternatively, the cause may be inhibition of vital enzymes responsible for chlorophyll synthesis, such as magnesium chelatase and protochlorophyllide oxidoreductase, which leads to the cessation of synthesis and the exacerbation of the degradation of chlorophyll molecules already present in the chloroplasts. (Shabala and Cuin, 2008 ; Vineeth *et al.*, 2023).

The main function of the secondary pigment chlorophyll b, is to absorb light at wavelengths greater than the ones used by chlorophyll a, and then transfer that energy to chlorophyll a. Regarding the results, the control treatment possessed the highest level (2.08 mg/g), yet to salt concentrations rose, chlorophyll b levels increased substantially (65–84%) compared to the control treatment, especially at concentrations of 100 mM and above. These results indicate that even low salinity might have a major effect on pigment durability. This is in accordance with past research that revealed that salt stress reduces the expression of genes related to chlorophyll synthesis. (Turan and Tripathy, 2015; Hameed *et al.*, 2021).

Further, under oxidative stress conditions, chlorophyll b is far more susceptible to breakdown and decomposition than chlorophyll a due to it is less stable and more exposed to photodamage. (Li *et al.*, 2024). Therefore, at wavelengths where chlorophyll b is less effective, its absorption efficiency reduces. At this happens, a lower amount of energy gets sent to chlorophyll a, that lessens photosynthesis' efficiency. (Mao *et al.*, 2023).

A crucial indicator of photosynthetic capacity is the total amount of chlorophyll concentration, which involves all chlorophyll a and b. the concentration (3.0607 mg/g) was detected for the control treatment, reflecting optimal potential under perfectly conditions. The reduction was observed starting from 50 mM, where the chlorophyll content, although still relatively high, was much lower than in the control group, indicating that even slight salinity can cause stress. Beyond this threshold (100–200 mM NaCl), total chlorophyll levels dropped dramatically, revealing a point at which compensatory mechanisms fail. At 200 mM, the levels were over 77% lower than the control, underscoring the severe impact of elevated salinity on pigment production and stability. This decrease is associated with impaired carbon fixation, reduced growth, and increased mortality rates in freshwater macrophytes exposed to saline conditions (Petjukevics and Skute, 2022 ; Balasubramaniam *et al.*, 2023).

Proline is one of the most important amino acids that builds up in plants as a sign of problems caused by non-living stress factors. Its accumulation is a key biochemical indicator used to show how much plants are affected by salt stress (Martinez *et al.*, 1996). The study results showed that higher salt concentrations encourage plants to increase the levels of proline acid in their tissues (Figure 2). These findings matched several studies conducted on the effects of salt stress in water lentils. Our study's data aligned with the results of a study that examined six different salt concentrations of sodium chloride (0-250 mM), which indicated a direct relationship between proline acid levels and salt concentrations (Al-Dakhil *et al.*, 2023). The buildup of proline acid in

growing plants under salt stress might be due to an increase in its production from the amino acid glutamate, or it could be partly because of a decrease in its oxidation due to lower activity of the enzyme dehydrogenase. (Cheng *et al.*, 2013).

Salt stress on *Limna minor* L. led to significant changes in mineral uptake, leading to nutrient imbalances. For example, at medium and high sodium chloride concentrations, zinc levels increased significantly, which may be related to the concentration effect and the role of specialized transporters (Zrt-/Irt-like protein) in managing zinc uptake under salt stress (Balasubramaniam *et al.*, 2023). Due to changes in the aqueous medium's uptake kinetics and the activation of BOR1/BOR4 channels, boron levels also decreased as salinity increased. (Qu *et al.*, 2024). On the other hand, manganese levels greatly decreased at most salinity levels, apparently due to its difficult to dissolve and disruption of its uptake transporters (NRAMPs), which got a negative impact on photosynthetic efficiency. (Kanwal *et al.*, 2024).

Copper's level declined at low salinity and then rose from there at high salinity, based to the results, showing an independent response. This may be explained by changing membrane transport capacity and increased cell permeability under stress. (Chain *et al.*, 2022). Even though iron levels grew substantially in all treatments, in particular at 125 mM, the incorporation of chelating agents, that bind regulate iron and retain cellular function, mitigated salt stress. (Cheng *et al.*, 2024). The increase in calcium levels with increasing salinity concentrations is consistent with its role in transmitting the integrity of membranes signals and activating the salt-sensitive SOS mechanism. (Liu *et al.*, 2024). Magnesium, on the other hand, surged significantly at low salt levels until slowly dropping at high salinity levels. This might represent a clue of the plant's early sensitivity to stress. (Brulo *et al.*, 2024).

Accordingly, the findings indicated that salinity leads to a change in the plant's ionic composition through the interaction of concentration effects, ion availability, and changes in gene expression of carriers. This is consistent with recent research on mineral imbalance disruption under the influence of acute salt stress.

Conclusions:

The study results showed that *Lemna minor* L. responds to salinity stress: low salinity (50 mM NaCl) enhanced positive effects on chlorophyll and specific nutrients, while high salinity levels caused significant physiological damage. The results also showed elevated proline levels, and specific mineral changes point to effective stress-relieving strategies. The investigation suggests increasing applied studies on its use in feed production and wastewater treatment in degraded environments, long with conducting a field assessment of its effectiveness to ensure its suitability for sustainable farming systems.

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