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Physiological responses in two aquatic plants (*Ceratophyllum demersum* and *Lemna minor*) as indicator to phytoremediation of wastewater polluted by some nitrogen compound

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Abstract:

The current study included evaluating the efficiency of some aquatic plants, such as " *Lemna minor* and *Ceratophyllum demersum* ", in improving the quality of the wastewater that was taken after the final treatment of the water treatment plant in Al-Almamierh through the biological treatment of total dissolved nitrogen (TDN), total inorganic nitrogen and dissolved organic nitrogen in wastewater. The plants were cultivated separately in that water and in natural environmental conditions with conducting some chemical tests for the water by by collecting water samples every three days throughout the course of 24 days. The present research showed that the effectiveness of the *L. minor* and *C. demersum* plants in lowering the levels of total dissolved nitrogen. Where the "*L. minor* and *C. demersum*" plants exhibited the greatest decrease rate of about (24.12 % and 29.94%) respectively .Also current plants showed the efficiency in reducing inorganic nitrogen compounds, where both *L. minor* and *C. demersum* showed the greatest decrease rate of total inorganic nitrogen, which was about (76.25% and 73.96%) respectively, and recorded the highest percentage of dissolved organic nitrogen reduction which amounted to about (30.14% and 26.33%) respectively. Regarding the physiological state of the current plants, it is noted that both plants recorded an increase in the content of carbohydrates and proline content compared to the control, while the protein content decreased in *L. minor*, but increased in *C. demersum* plant.

Keywords: *Lemna minor*; *Ceratophyllum demersum* ; phytoremediation; wastewater

Introduction

Nitrogen (N) is found in many different organic substances, including chlorophyll, coenzymes, nucleic acids, proteins, and amino acids [1]. It is generally known that a lack of N affects several biochemical and physiological processes, slowing down cell division and perturbation in process of photosynthesis [2]. Aquatic plants, especially large underwater plants, contribute significantly to the process of restoring the ecosystems of polluted shallow lakes. [3]. Because floating aquatic plants grow more quickly than other plants, it has been determined that using them to treat wastewater is preferable [4].Furthermore, the biomass that has been gathered can be used for a variety of things, such animal feed or as a feedstock for the creation of renewable energy [5]. *Ceratophyllum demersum* is useful as an oxygenator or for use in a closed equilibrated biological aquatic system[6]. At the root and stem, carbon and nutrients are absorbed [7].However, due to eutrophication, with eutrophication rising, submerged macrophytes decrease continuously in many eutrophic lakes throughout the world. [8] [9]. For lake plants, phosphorus (P) is frequently the most important growth constraint [10]. But recent years have seen an increase in interest in how nitrogen (N) affects the decline of submerged macrophytes. [11]. The purpose of this study was to show the potential of several aquatic plants, including C. demersum and L. minor, to reduce the levels of dissolved nitrogen compounds in municipal wastewater while also examining the physiological responses of the plants following treatment, Because the presence of these substances in high concentrations can have detrimental effects on the levels of dissolved oxygen concentration (O2), nutritional status and ultimately the health of animals and plants in water[12]

Material and Methods

Sample collection and identification

Sample collection

Aquatic plants were gathered from several locations in Hilla River and thoroughly cleaned with tap water and then with warm water. Then placed in plastic basins with dimensions of (70 x 30 x 35)with tap water for two weeks for the purpose of acclimatization and discarding the contaminants attached to them from their original origin. After which the plants were placed at a rate of 10 g / liter (13) with the addition of 20 liters of municipal wastewater that collected by polyethylene containers of 20 liters ,with leaving Basin that contain only untreated wastewater which represented the control .A certain amount of water was taken every three days in order to measure certain nitrogen compounds concentrations .

Chemical analysis

TDN was measured with the standard per-sulfate digestion method [14] and modified by [15]. The total inorganic nitrogen was estimated by summing the concentrations of ammonia, nitrite and nitrate that dissolved in the sample and the results were expressed as mg/l , The difference between TDN and the total of the inorganic nitrogen species was used to determine dissolved organic nitrogen (DON)[16] [17]. For the physiological markers, the Biuret technique was used to assess total protein content [18]. The amount of carbohydrates was calculated using the method of [19] . The amount of Proline was estimated according to the method of [20].

Results and Discussion

Before phytoremediation, the table below displays some of the chemical characteristics of municipal wastewater.

Table 1: Some chemical properties of municipal wastewater before phytoremediation

Measured factors	"Values Mean± SD"
Total Dissolved Nitrogen mg/l	131±10.66
Total Inorganic Nitrogen mg/l	46.742±6.42
Dissolved Organic Nitrogen mg/l	84.3±3.11

The numerous types of N_2 that can exist in water include dissolved and particulate organic nitrogen, nitrite, ammonium, and nitrate. Within the nitrogen cycle, these diverse forms can convert and act as sources or end products for one another [21]. The values of the concentrations and the percentage of removal of total dissolved nitrogen for wastewater treated by plants are shown in Figure (1)(A, B) which shows the "*L. minor* and *C. demersum*" plants recorded the largest rate of decline of about (24.12% and 29.94%) respectively, compared to the control, which showed the largest rate of decline approximately (26.44%) . The removal of large amounts of nitrogen may be due to the processes of ammonia volatilization, nitrification, denitrification , microbial assimilation as well as the uptake of plants such as duckweed [22]. The ability to remove nitrogen is due to the fact that aquatic plants can use nitrogen for their growth and offer bacteria a favourable environment to facilitate nitrification and de-nitrification which should result in higher nitrogen removal efficiency [23]. When nitrate or ammonium are taken up by plants as vital plant nutrients, nitrogen can be stored in organic form in wetland vegetation, in addition, through the settling of N-containing particle debris in the wetland input, significant nitrogen removal may occur [24].

Figure(2)(A,B)shows the values of concentrations and removal percentage of total inorganic nitrogen for wastewater treated with plants which showed the efficiency of the current plants in reducing inorganic nitrogen compounds, where both *L. minor* and *C. demersum* recorded the highest reduction rate of total inorganic nitrogen which was about (76.25% and 73.96%) respectively, in comparison to the control which recorded the highest reduction rate of about (64.61%) . In fact, the nitrogen losses in the constructed wetland systems are related manly to many removal mechanisms such as ammonia volatilization, ammonification (mineralization), anaerobic ammonium oxidation (anammox), di-ssimilatory reduction, autotrophic nitrification and heterotrophic de-nitrification, plant and microbial assimilation and re-mineralization during decomposition, sedimentation, filtration, adsorption and microbial assimilation [25]. In constructed wetlands, microbial processes are the main mechanisms for removal of nitrogen from wastewater [26]. As for the superiority of the "*C. demersum*" plant in removal, this is due to the plant's ability to withdraw large quantities of inorganic nitrogen forms where [27] explained that "*C. demersum*" plant can tolerate high nitrogen concentrations and has a good removal effect on nitrogen in the water .

DON one of the main nutrients that cause low dissolved oxygen conditions. with discharges from wastewater treatment plants being one of the major contributors [28].Figure (3) shows the values of the concentrations and the percentage of removal of total dissolved organic nitrogen for wastewater treated with plants where the "*L. minor* and *C. demersum*" recorded the highest percentage of organic nitrogen reduction of about (30.14 % and 26.33%) respectively, compared to the control which about (36.99%). In the current study, aquatic plants did not show a high efficiency in reducing the values of dissolved organic nitrogen compared to the control,this may be due to the low penetration of light into the treatment basins that contain both submersible and floating plants where light play a significant part in converting

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dissolved organic nitrogen (DON) into compounds with lower molecular weights, which makes DON accessible to aquatic organisms like algae, bacteria, and phytoplankton [29]. also possible effluent Under specific environmental conditions, DON can biodegrade to a number of substances, including nucleic acids, free amino acids, urea, and other uncharacterized labile chemicals, and eventually ammonia. A portion of DON that bacteria can ammonify is known as BDON [30].

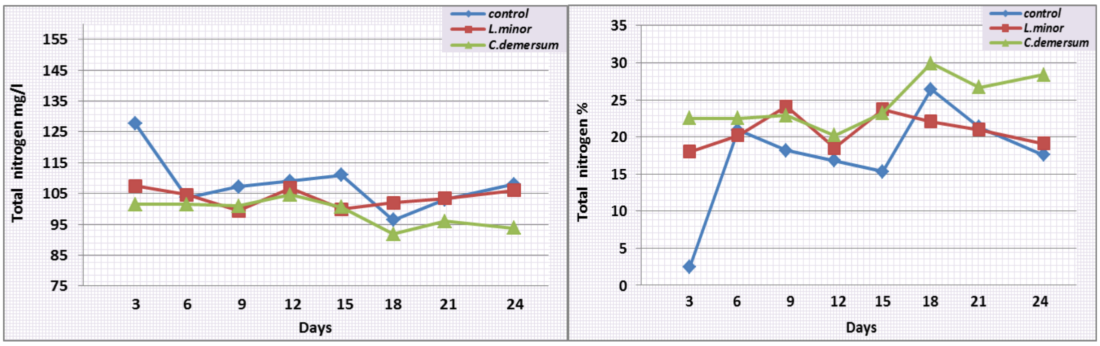


Figure (1): Concentrations and percent bio removal of Total dissolved nitrogen mg/l

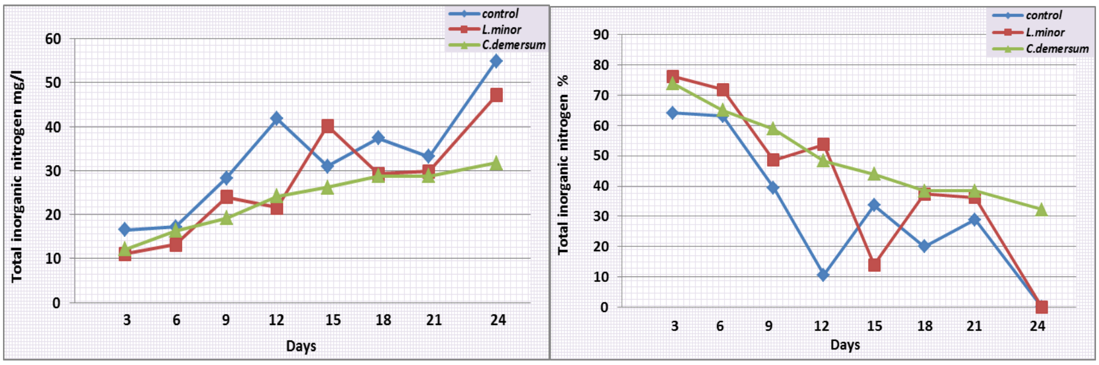


Figure (2): Concentrations and percent bio removal of total inorganic nitrogen mg/l

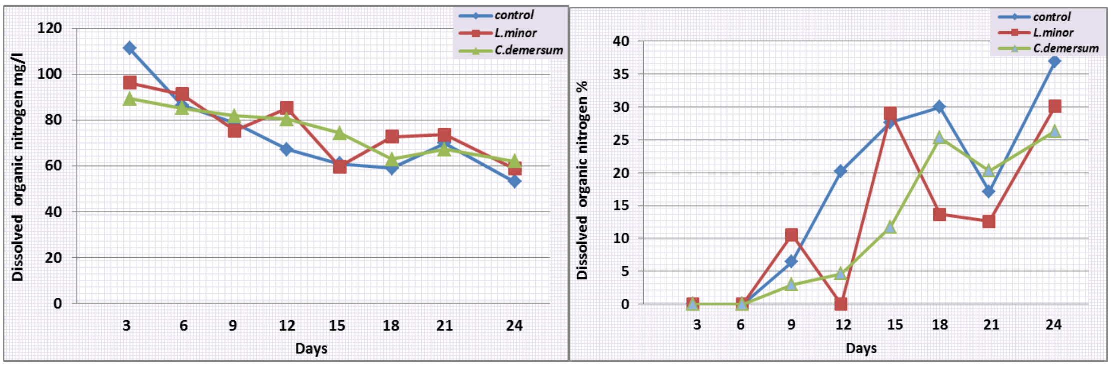


Figure (3): Concentrations and percent bio removal of Total organic nitrogen mg/l

Some physiological indicators in aquatic plant

In **Table 2**, a few biochemical indicators' values for the aquatic plants used in the current study are shown. As for physiological marker results ,the dissolved protein content is an important indicator of the physiological state of plant [31]. The statistical results of the current study indicated that there was a significant difference ($p \leq 0.05$) in protein.

Table. 2 Aquatic plants' physiological and biochemical markers' "mean ± standard deviation"

"Parameters"	Plants	Control	Treated	"P value"
		"Mean ±S.D"		
Protein content (mg/g)	***L. minor***	**23.94±4.90**	**15.13±1.1**	**0.039***
	C.demersum	**5.44±0.05**	**14.47±1.3**	**0.005****
Carbohydrate content (mg/g)	***L. minor***	**54.03±7.3**	**88.62±4.5**	**0.042***
	C.demersum	**125.42±6.3**	**152.36±3.4**	**0.03***
Proline content µ mole /g	***L. minor***	**15.41±0.68**	**24.68±5.1**	**0.036***
	C.demersum	**12.49±1.4**	**17.64±3.15**	**0.048***

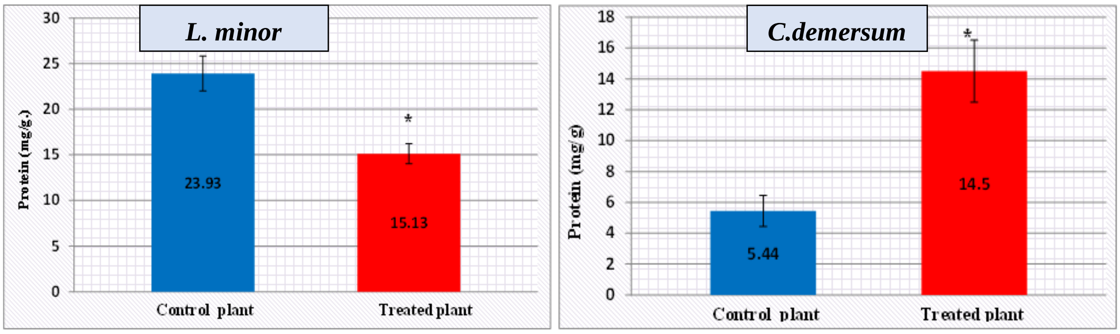
Values in both plants, where the results showed that the "*L. minor*" plant recorded a clear decrease in protein values after municipal wastewater treatment, which reached (15.13 mg/g) compared to the control plant, which recorded (23.94 mg/g) . The opposite happened with "*C. demersum*" plant where the recorded value in plant that representing the control (5.44 mg/g), but it increased after wastewater treatment to (14.47 mg/g) figure (4) (A,B). Protein breakdown under stress may be the source of the decrease in the amount of soluble proteins. [32]., or by protein fragmentation brought on by reactive oxygen species' toxicity [33]. Reactive oxygen species are oxygen-containing chemical reaction molecules like hydrogen peroxide, superoxide anion (O_2), and hydroxyl radical (OH^-), which cause oxidative stress and produce these compounds as byproducts during metabolism. This stress affects plant cells and causes them to die as well as the breakdown of protein. [34].

Also may be due to the toxic effects of salinity on the physiologically active parts of the tissues where high salinity influence the cell content of amino acids and reduce RNA and DNA in the plant [35]. Redundant ROS damage the nucleic acids, proteins and lipids in ammonia stressed cells [36]. Decline level of protein may be due to decrease in nitrate reductase activity because this type of enzyme is believed to be rate limiting in the overall assimilation of nitrate [37]..From other hand ,the increase protein content in "*C. demersum*" plant may be as a result of absorption of nitrogen compounds such as ammonia , nitrite and nitrate where NO_3 is reduced to NO_2 and the last is transformed in to ammonium which is assimilated in to amino acids and other organic compounds [38]. Amino acids are the primary building block for protein synthesis in cells and can help regulate a plant's carbon metabolism, becoming part of the basic process of the plant's life activities [39]. In plants, soluble carbohydrates are significant metabolites in metabolism and play a critical role in both the detoxification of foreign substances and the defense mechanism against stress. [40]. External stress is capable of changing the amount of soluble carbohydrates in plants [41].

The concentration of carbohydrate in current study illustrated in the figure (5) (A, B), which increased after treatment in the in both plants compared with the control plant where the value of carbohydrate that recorded in the "*L. minor*" plant increase to (88.62 mg/g) after treatment compared to control plant that recorded (54.03 mg/g), at same time the concentration of carbohydrate in "*C. demersum*" plant increased after treatment and reached (152.36 mg/g) compared to the control plant that recorded (125.42 mg/g). The increase in carbohydrate values may be due to the increase in carbohydrate values may be due to the utilization of soluble carbohydrates as energy sources to power intricate detoxification processes, such as the removal of nitrate -N from plant cells and assimilation to free amino acids [42]. When photosynthesis is reduced, soluble carbohydrates can fuel carbon for energetic metabolism, provide osmotic adjustment, protect macromolecules (like proteins) and membranes, and play crucial roles as signaling molecules and the regulating biosynthesis and sensing plant hormones. [43]. Nitrogen supply has an impact on how carbohydrates are distributed inside plants, which in turn has an impact on how carbon is assimilated, allocation and partitioned. [44]. [45] showed that increase in sugars content occur with increasing level of N nitrogen supply.

Proline is an amino acid which maintains the plant cells vitality under drought and salinity conditions because it prevents or reduces the proteins breakdown in the cell [46]. The results of the current study indicate an increase in the concentration of proline in both plants compared with the control plant for each of them, this was shown through the statistical analysis that recorded a significant difference ($p \le 0.05$) in proline values, where the value of proline that recorded in the "*L. minor*" plant was (15.41 µ mole /g) in the control plant, but it increased after treatment to (24.68 µ mole /g). The same is the case for the "*C. demersum*" plant, where the proline concentration increased after treatment and reached (17.64 µ mole /g) compared to the control plant that recorded (12.49 µ mole /g) figure (6) (A, B).

The increase in proline content may be due to fact that when ammonia levels in aquatic areas rise, the Proline buildup can dramatically increase. [47]. Previous research showed that too much ammonia-N in plant tissues decreased calcium and potassium uptake, which led to a water imbalance in the cellular and entire plant body. [48]. It is well known that the large amounts of free ammonium in plant tissues cause ammonium [49]. Generally, excess ammonium decrease Ca^{+2} and K uptake leading to imbalance in plants, and it thus inhibits water uptake [49].Thus , By maintaining membrane integrity, preventing protein denaturation, and preserving cell turgor, proline buildup may limit water loss [50]. Where proline accumulation is associated with osmo-protection functions [51], osmotic adjustment [52], and antioxidant activity [53]. Proline known works to protect plants from the influence of ROS, as it plays an important role in osmoregulation and protection of enzymes [54], stabilization of protein synthesis [55] and as scavenging of free radicals [56].



Figure(4): Protein content (mg/g) in *L. minor* and *C. demersum*

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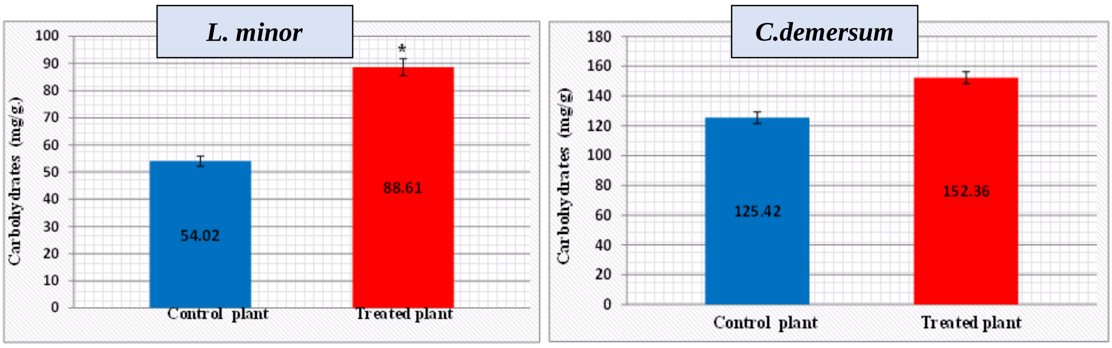
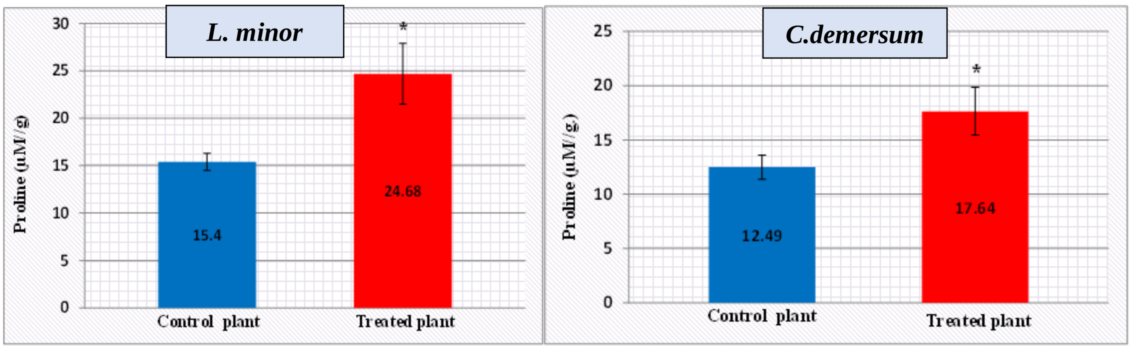


Figure (5): Carbohydrate content (mg/g) in *L. minor* and *C. demersum*



Figure(6) : Proline content µ mole /g in "*L. minor* and *C. demersum*"

Conclusions

According to the research presented in this study, aquatic plants were not very efficient at lowering the values of dissolved organic nitrogen, but both "*C. demersum* and L. minor" plant were effective at removing total dissolved nitrogen and total inorganic nitrogen from waste water, when compared to the control. A significant increase was show in carbohydrates and proline content for each plants and the protein for "C. demersum" plant, but protein content for "*L. minor*" plant decreased after treatment. The increase and decrease of biomarkers indicates that both plants were subjected to oxidative stress during treatment. Nevertheless, both plants continued to remove pollutants until the end of the experiment.

Author Contributions Statement

All research stage: laboratory work, collection and analysis of data, research write were done by the author.

Declaration of competing interest

The author confirms that there was no competing interest with others.

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