CATRINA (2025), 35 (1):67-82

Integrating Seasonal Dynamics and Wastewater Treatment with Biomass Production of *Lemna gibba* L. for Sustainable Water Remediation

Nasser A.M. Barakat*, Shery A. Habib, Walid W. Nazir
Botany and Microbiology Dept, Faculty of Science, Minia University, Minia, Egypt

Received:\August 9, 2025; Accepted: November 1, 2025

ABSTRACT

Duckweed (*Lemna gibba* L.) is an eco-friendly aquatic macrophyte with significant potential for wastewater treatment and biomass valorization. This study evaluated its nutrient removal efficiency and growth performance under controlled greenhouse conditions using agricultural (AG) and domestic sewage (SG) wastewater at five dilution levels across three seasons. Growth and biochemical responses were monitored over 180 days. Results demonstrated that *L. gibba* effectively removed anions (NO_3^-, PO_4^{3-}, Cl^-, SO_4^{2-}) and cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), with higher removal efficiencies in AG wastewater than in SG, except for Cl^- and SO_4^{2-}, which were more efficiently removed from SG. Biomass production peaked at 75% wastewater concentration (T3), with fresh weight increases of 133% (AG) and 146% (SG), while dry weight gains were comparable (AG 187%, SG 185%). Relative growth rate and crop growth rate were significantly affected by seasonality, with the second season (S2) showing the highest productivity. Biochemical analyses revealed higher protein yields in SG and slightly greater carbohydrate yields compared to AG. Generally, *L. gibba* exhibited strong phytoremediation capacity and produced nutrient-enriched biomass suitable for aquaculture or bioresource applications. These results support its use as a cost-effective and sustainable approach for reorganized wastewater treatment and resource recovery.

Keywords: Agricultural wastewater; Duckweed; *Lemna gibba* L.; Phytoremediation; Wastewater treatment.

INTRODUCTION

The current global population is estimated at 7.6 billion and is projected to reach 8.6 billion by 2030, 9.8 billion by 2050, and 11.2 billion by 2100 (Chen *et al.*, 2018). This rapid growth places increasing pressure on freshwater resources. Water scarcity remains a life-threatening issue, nearly two-thirds of the world's population faces water scarcity for at least one month each year, and about 2 billion people lack consistent access to clean drinking water (UN, 2007; UN, 2024; WHO, 2025; UNESCO, 2022). The uneven distribution and scarcity of freshwater highlight the urgent need for innovative and sustainable solutions to ensure adequate water availability. However, over 80% of wastewater generated is discharged into ecosystems without proper treatment, contributing to environmental degradation and health risks (World Water Assessment Programme, WWAP, 2017). Wastewater comes from various sources, including agricultural runoff, sewage, animal farms, domestic effluents, and small-scale industries, often containing both organic and inorganic pollutants (Gavrilescu *et al.*, 2015; Harish *et al.*, 2025). These untreated releases degrade water quality, promote eutrophication, and disrupt aquatic ecosystems.

According to the 2023 UN-Habitat Global report on sanitation and wastewater management, wastewater treatment issue remains critically inadequate in many developing countries, particularly in rapidly urbanizing and peri-urban areas in which population growth exerts significant pressure on existing sanitation infrastructure. The report emphasizes the need for context-specific, low-cost, and accessible wastewater treatment solutions to reduce environmental contamination and defend public health. It promoters the integrated approaches based on the Citywide Inclusive Sanitation (CWIS) framework, which emphasizes comprehensive resource management, strengthened institutional governance, data-driven sanitation planning, climate resilience, and the adoption of innovative technologies to enhance waste-water control, treatment, and service delivery at scale. This framework provides a strategic blueprint for local and national authorities to improve service quality, regulatory supervision, and investment in sanitation infrastructure, particularly in underserved communities.

Agricultural drainage water, a byproduct of land irrigation and farming practices, contains a complex mixture of fertilizers, pesticides, and other agrochemicals. The uncontrolled discharge of this water leads to contamination of adjacent water bodies, adversely affecting aquatic ecosystems and biodiversity, which presents significant environmental challenges. The slow progress in improving water and sanitation infrastructure underscores the urgent need for collaborative solutions through partnerships and cooperation, especially as global water demand is projected to rise sharply (El-Shafei *et al.*, 2024). Meanwhile, sewage, or domestic wastewater, origin-nates from residential, commercial, institutional, and public facilities within a given area (El-Ezaby *et al.*, 2023). It primarily consists of two subtypes: greywater,

* *Corresponding author e-mail: nasserbarakat2003@mu.edu.eg*

generated from sinks, bathtubs, showers, dishwashers, and washing machines, and blackwater, originating mainly from toilets. The treatment of domestic wastewater is crucial for mitigating environmental pollution and enabling the safe reuse of treated effluents for agricultural irrigation and recreational purposes (Kumar *et al.*, 2021). Therefore, green remediation technologies, grounded in ecological principles, are increasingly recognized as urgent and promising approaches for sustainable wastewater treatment. These techniques use natural biological processes to treat wastewater, offering advantages such as low energy requirements, reduced costs, minimal chemical use, and generation of high-quality biomass (Almuktar *et al.*, 2018; Wang *et al.*, 2025). Such systems are particularly valuable in developing countries where financial and technical constraints limit the implementation of advanced treatment plants.

Among nature-based solutions, aquatic plants have been increasingly recognized for their substantial capacity to remove nutrients and pollutants from wastewater, positioning them as effective biological agents for tertiary treatment (Vymazal, 2011; Li *et al.*, 2018). Recent studies provide further evidence of their high efficiency in eliminating contaminant minerals such as nitrogen, phosphorus, and heavy metals, achieving removal rates approaching 100% in some cases (Mishra *et al.*, 2023; Phewnil *et al.*, 2023). Additionally, these plants offer potential as sustainable feedstock for biomass valorization, integrating wastewater treatment with resource recovery strategies.

Duckweed species (family Lemnaceae) have attarcted particular interest over the past three decades as efficient phytoremeditators of wastewater (Papadopoulos and Tsihrintzis, 2011); (Mohedano *et al.*, 2012) and (Gaur and Suthar, 2017). Lemnaceae are the fastest-growing angiosperms, with reported doubling times of less than 48 hours under optimal conditions (Ziegler *et al.*, 2015). Their simple morphology, consisting of small fronds and reduced root systems, allows for rapid nutrient absorption directly from the water column (Eng and Siau, 2025). As they grow, duckweeds remove nitrogen, phosphorus, and other elements from wastewater while simultaneously producing protein-rich biomass suitable for use as animal feed or bioenergy feedstock. This dual role positions duckweed systems as models of circular economy approaches, enabling resource recovery alongside pollution control. Among duckweed species, *Lemna gibba* L. (common duckweed) is widely distributed in nutrient-rich ponds, lakes, ditches, and canals (Foroughi *et al.*, 2013). It thrives under varied aquatic conditions, exhibits rapid biomass accumulation, and efficiently assimilates nitrogen and phosphorus, making it particularly suited for wastewater treatment. In Egypt, outdoor production rates of duckweed have been reported at 7-22 dry weight (DW) ha^{-1} yr^{-1} (Merah *et al.*, 2025), and its biomass has long been used as a protein source for fish and livestock (Fourounjian *et al.*, 2020).

Despite extensive research, several knowledge gaps remain. Most previous studies have examined duckweed performance in either agricultural runoff or domestic sewage, often under single-season or single-concentration conditions (Kubota *et al.*, 2024), raw and diluted domestic sewage (Al-Ameen & Al-Taee, 2022). Far fewer studies have investigated the combined effects of wastewater type, dilution strength, and seasonal variation on nutrient removal efficiency and biomass productivity. Yet, these factors are critical for scaling duckweed systems under real-world conditions, where water quality and climatic seasonality can vary widely. Therefore, this study investigates the capacity of *Lemna gibba* to remediate nutrients and produce biomass under dual wastewater sources including agricultural drainage and sewage effluent, across different dilutions and growing seasons. Specifically, we evaluate the removal efficiency of major anions (Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) and cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), alongside plant growth parameters (fresh and dry biomass, relative growth rate, crop growth rate, harvest index) and metabolic yields (carbohydrates, proteins and amino acids). By integrating wastewater type, concentration, and seasonality, this research provides novel insights into the ecophysiological adaptability of *L. gibba* and its potential role in sustainable wastewater management within arid and semi-arid regions such as Egypt.

MATERIALS AND METHODS

Experimental designs

Lemna gibba: Selection and Collection

Lemna gibba L. was selected for this study based on its rapid growth rate, which allows biomass renewal approximately twice per week, surpassing growth rates observed in other species within the Lemnaceae family (Iqbal *et al.*, 2019; Harun *et al.*, 2013). This free-floating aquatic macrophyte is well-known for its high nutritional content and widespread distribution in freshwater ecosystems such as ponds, lakes, ditches, and slow-moving rivers enriched with elevated nutrient levels (Zeshan *et al.*, 2016; Takács *et al.*, 2025). Its ability to efficiently assimilate key nutrients, notably nitrogen and phosphorus, from surrounding water qualifies it as a potent biological agent for nutrient removal and wastewater treatment (Choo *et al.*, 2023). *Lemna gibba*'s adaptability across variable aquatic conditions and its high nutrient uptake capacity underscore its efficacy as a sustainable, nature-based remediation tool (Foroughi *et al.*, 2013). Owing to these characters, *L. gibba* has been extensively cultivated commercially, primarily within the private sector, and is a significant protein source for various fish species and small animals raised by numerous small-scale farmers (Fourounjian *et al.*, 2020).

Specimens for the present study were collected from an enriched freshwater lake proximate to the Minia University campus during the spring season (April to May 2022) at coordinates Latitude 28°13'539" N, Longitude 30°70'264" E. Collected plants were transported to the greenhouse facilities of the Botany Department, Faculty of Science, Minia University, Egypt. Morphological identification as *Lemna gibba* L. was identified following established taxonomic keys of Täckholm (1974) and Boulos (2005).

In the green greenhouse, the collected duckweed was washed several times with running tap water in a large circular plastic sink to avoid contamination and remove debris. The plants were grown on tap water for 2 days for establishment and stabilization, and acclimatization. Healthy individuals were first immersed in 1.5% sodium hypochlorite (NaClO) for 3-4 minutes for sterilization and then rinsed with distilled water and taken for subsequent experiments.

Wastewater types and sources

Samples of both wastewater types were collected using sampling bottles at a depth of 50 cm from water surface. The first sample was collected from an agricultural canal (AG) nearby the campus, and the second from the inlet unit of the sewage wastewater treatment plant (SG) at Minia University campus. The approximate geographic coordinates for Minia University, Minia, Egypt, where the wastewater treatment plant and agricultural canal sampling sites are located, are: Latitude: 28.1099° N and Longitude: 30.7503° E. Collection was performed into six pre-cleaned, large-sized polyethylene containers (each with a capacity of 20 liters), totaling 60 liters of each wastewater type. The samples were immediately transported to the laboratory. Prior to physicochemical analysis, the water samples were passed through glass fiber filters with a pore size of 10 µm to remove suspended solids.

Cultivation and treatment groups

Both wastewater types (agricultural wastewater, AG) and untreated sewage wastewater, SG) were manually diluted with tap water using a pre-sterilized glass rod prior to cultivation. Each container was planted with 4 g fresh weight of *Lemna gibba*. Five experimental treatment groups were established separately for each wastewater type, as following:

T0 (Control): 0:1 (100% tap water + 4 g fresh weight *L. gibba*); T1 (Diluted 1:3): 25% wastewater of each type + 75% tap water; T2 (Medium Dilution 1:1): 50% wastewater of each type + 50% tap water; T3 (Diluted 3:1): 75% wastewater of each type + 25% tap water and T4 (Undiluted): 100% wastewater of each type, no tap water

The experiment was conducted in triplicate (n=3). Each experimental container (pot) had dimensions of 18 cm radius and 13.5 cm depth. Prior to use, all pots were disinfected by washing with 1.5% sodium hypochlorite (NaClO) solution for 3-5 minutes. They were then filled with wetland soil collected from a local pond to a height of approximately 5 cm. Each pot was subsequently filled with exactly 2 liters of the designated wastewater treatment and 4 grams of fresh weight *L. gibba*. The experimental setup was maintained under shade within a greenhouse, with a green plastic net suspended above the pots to provide approximately 50% shading from natural sunlight where *L. gibba* exhibited better growth under reduced light conditions rather than direct sunlight.

A completely randomized design (CRD) with three replications was used. All pots were inoculated with 4 g (fresh weight basis) of *L. gibba*. In total, 90 pots were used across the designed treatments (5 dilution water levels × 3 replicates × 3 seasons × 2 wastewater types). The study covered an entire growing season of 180 days (April to September), with evaluations conducted at two-month intervals. The experiment was to assess the capacity of *L. gibba* to improve water quality and to monitor changes in its morphological and physiological properties in response to different pollutant levels.

Physicochemical analysis of wastewater used

Physicochemical parameters of the surface wastewater were analyzed at two-month intervals in each experimental cascade. Major ion concentrations were determined and expressed in mg/L. Values measured before phytoremediation were considered initial values, while those recorded afterward were designated as final values. Sodium and potassium concentrations were quantified using the flame photometry method (Williams and Twine, 1960). Calcium and magnesium levels were determined by versing titration methods (Schwarzenbach and Biedermann, 1948, Noaman *et al*., 2025). Phosphorus was measured calorimetrically as described by (Woods & Mellon, 1958). Chloride and nitrates were determined according to (Jackson, 1960). At the end of each experiment period (interval), the harvesting of duckweed is performed using a scoop net. The labelled plastic bags were used to transfer L. gibba L. from the specific wastewater concentration and season to the laboratory, washed several times with tap water, and distilled water to remove debris

Biomass measurement and biochemical analysis of *Lemna gibba*

The inoculated thalli were harvested from each labeled pot using a plastic strainer. The samples were then subjected to centrifugation twice for 30 seconds each in a salad spinner to remove excess water, followed by spreading on absorbent tissue paper for 10 minutes. The biomass was quantified by measuring both fresh weight (FW) and dry weight (DW) at the end of each experimental period. Fresh weight was determined immediately using a high-precision scale. Subsequently, samples were dried at 60°C until reaching a constant weight, then weighed again to determine dry weight in grams. Dried duckweed samples were ground to a fine powder using an electrical household coffee grinder and sieved through a 0.5 mm mesh for frond extraction. A 0.5 g aliquot of dried tissue was boiled in 25 mL of distilled water for 2 hours at 100°C using a water bath.

Biochemical parameters were assessed as follows: reducing sugars (R.S.) were measured according to the Dubois *et al.* (1956) method; soluble proteins (Prot.) were determined using the Lowry *et al.* (1951) method; and amino acids (A.A.) were quantified following Moore and Stein (1948). These analyses were performed on samples from all pots to evaluate the impact of wastewater treatment on the biochemical productivity of the experimental setups. However, the growth rate (RGR) was calculated following the methodology described by Tavares *et al.* (2010), while crop growth rate and harvest index were determined based on the procedures outlined by Mohedano *et al.* (2012).

Statistical Analysis

All data are expressed as the mean of three replicates per experimental condition and were analyzed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA) at a 95% confidence level. A multivariate analysis of variance (MANOVA) was performed to evaluate the effects of different wastewater dilution levels (none, low, medium, high, and raw) on various water quality parameters and the growth rates of each system. This analysis aimed to determine whether significant differences existed among treatments across the measured characteristics.

RESULTS

Wastewater treatment efficacy of *Lemna gibba* across water types and seasonal changes

The data obtained and their multivariate analysis that examining water types, dilutions, seasonality, and their interactions revealed significant effects ($p \leq 0.05$) on all measured anions (Cl^{1-}, NO_3^{1-}, PO_4^{3}-, and SO_4^{2-}), as summarized in Tables (1 and 2). *L. gibba* demonstrated the capacity for growth and nutrient/ion remediation across wastewater of varying strengths in two water bodies. Remarkably, chloride (Cl^{1-}) concentrations generally increased with rising water strength in all studied seasons and water types. The mean percentage reduction of Cl^{1-} relative to the initial control value (100%) was slightly higher in sewage wastewater (mean = 67.47%) compared to agricultural runoff (mean = 65.69%), with reductions ranging between 53.21%, 65.96%, and 78.81% across seasons 1, 2, and 3, respectively.

In sewage water, the mean Cl^{1-} removal percentages were 61.13%, 71.26%, and 70.07% for seasonal treatments: S1, S2, and S3, respectively. Season 2 (S2) exhibited the highest chloride removal in both water types. Overall, chloride removal increased with water strength across all seasons and water types, except in sewage water during the first season, where this trend was not observed (Table 2).

The performance of nitrate (NO_3^-) removal exhibited a trend similar to that of chloride (Cl^-), with minimal variation across seasons. The percentage of NO_3^- removal was higher in activated sludge (AG) wastewater (78.17%) compared to sewage (SG) wastewater (71.57%). Seasonally, S3 was identified as the most active period for nitrate removal across all water types, following the order: T4 > T3 > T2 > T1, except during the first season in SG. In AG water, nitrate removal percentages slightly varied with season, ranging from 74.28% at S1 to 78.76% at S2 and 81.48% at S3, respectively (g/Fresh weight/L). On the other hand, SG wastewater demonstrated a lower mean nitrate removal at S1 (59.26%), with an increase up to 80.30% during S2 and S3, indicating higher removal efficiency in these seasons**.** However, for phosphate (PO_4^{3-}), the mean removal percentage was higher in AG wastewater (77.33%) than in SG (67.72%). In AG, PO_4^{3-} removal remained relatively consistent across seasons, with mean values of 75.13%, 78.91%, and 77.95% in S1, S2, and S3, respectively. In SG, the mean removal was 64.36% at S1, 74.16% at S2, and 64.65% at S3, with S2 representing the peak removal season. An inverse relationship was observed between water dilution and PO_4^{3-} removal efficiency; as dilution decreased, removal percentages increased in both water types across the seasons, except during S1 in SG.

Regarding to sulfate (SO_4^{2-}), removal percentages were higher in AG wastewater (78.93%) compared to SG (ranging from 85.55% in S1 to 73.52% in S3). In AG, the seasonal removal rates were 68.26% at S2 and S3. Notably, in SG, S1 was the most effective season for sulfate removal, with a removal rate of 85.55%, which decreased in subsequent seasons. As observed with other parameters, sulfate removal improved as water dilution decreased in both water types and across seasons, except during the first season in sewage treatment.

Generally, the removal efficiencies indicate that AG wastewater exhibited higher removal rates for NO_3^- and PO_4^{3-}, whereas SG wastewater achieved greater removal of Cl^- and SO_4^{2-}. Seasonally, S1 demonstrated the highest removal for SO_4^{2-} across all seasons, while S2 was most effective for Cl^- and PO_4^{3-}, and S3 for Cl^- and NO_3^-. A positive correlation was observed, with increased removal rates corresponding to higher water strength, except in S1 under SG treatment. (Refer to Table (2) for kinetic parameters related to Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}.).

Evaluation of cation removal efficiency

Multivariate analysis of data obtained for wastewater treatment revealed that water type, dilution, seasonality, and their interactions significantly influenced the removal efficiency of all measured cations, calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and sodium (Na^+), with the exception of the effects of water type on Ca^{2+} and Na^+, as well as the interactions between water type and seasonality on K^+ and Na^+, and water type and concentration on Mg^{2+} (Table 1). For calcium and magnesium removal, slight seasonal variations were detected. For Ca^{2+}, the highest removal was recorded in the high-calcium water (SG) at 74.07%, compared to 72.6% in the alternative water source (AG). The mean removal percentages across seasons were 76.68%, 65.15%, and 73.96% for Ca^{2+} in AG, and 73.96%, 73.59%, and 74.66% in SG for seasons S1, S2, and S3, respectively. Season S2 exhibited the lowest Ca^{2+} removal in both water types. A positive correlation was noted between water concentration and Ca^{2+} removal, with removal efficiencies increasing as concentration increased, across all seasons except AG during S1.

Table (1): Dynamic changes in water quality parameters across seasons and treatment groups through wastewater type.

Water type	Measured Parameter (mg $^{-1}$)	Different worked seasons														
		S1					S2					S3				
		Treatment groups														
		T0	T1	T2	T3	T4	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
AG	CL^-	16.67	58.82	52.38	48.00	31.52	46.67	45.00	37.93	31.43	22.84	46.67	29.41	25.00	20.00	10.30
	NO_3	44.00	35.00	28.17	22.50	17.20	37.14	35.00	21.54	17.14	11.25	33.33	30.00	19.23	13.33	11.43
	PO_4	20.00	29.27	28.26	25.45	19.40	44.44	26.83	21.15	20.63	15.71	33.33	28.57	25.00	20.00	14.59
	SO_4^{2-}	28.57	36.36	30.77	26.67	20.00	46.15	45.00	36.67	30.00	22.00	37.50	30.00	23.08	17.65	10.00
	Ca^{2+}	7.69	13.33	21.05	26.09	32.74	54.55	48.00	41.38	32.56	17.45	33.33	33.33	26.32	22.73	13.75
	Mg^{2+}	25.00	28.57	25.00	22.22	15.00	50.00	42.11	32.14	23.68	16.77	22.22	20.00	18.18	15.38	13.95
	K^+	46.11	37.14	26.53	21.57	19.81	46.11	28.57	26.23	18.57	15.13	46.11	28.57	26.23	12.31	8.34
	Na^+	33.33	26.67	17.00	12.61	10.58	33.33	30.00	23.01	19.86	18.46	50.00	47.50	35.83	29.46	13.88
SG	CL^-	17.00	22.2	27.3	45.5	60.5	45.5	40.0	30.8	24.2	20.3	44.4	40.9	31.0	28.6	19.1
	NO_3	18.20	28.6	37.8	46.6	50.0	42.9	31.7	26.9	21.5	19.0	42.9	25.5	22.6	17.3	13.3
	PO_4	20.00	21.6	25.0	37.0	58.9	40.0	34.0	29.3	24.6	18.7	47.1	46.6	40.3	37.0	17.4
	SO_4	7.70	10.0	13.2	13.1	21.4	38.5	29.3	24.6	20.8	14.2	38.5	31.6	28.1	26.9	19.6
	Ca^{2+}	16.70	18.2	18.5	29.0	38.4	41.7	34.8	29.0	21.1	20.7	41.7	34.8	29.0	21.1	16.4
	Mg^{2+}	17.90	20.0	38.5	42.9	48.7	54.5	46.2	31.6	22.1	16.8	54.5	46.2	31.6	29.2	18.8
	K^+	18.20	20.0	30.4	40.0	49.1	45.5	40.0	27.3	23.3	17.2	45.5	40.0	27.3	23.3	12.9
	Na^+	14.30	18.2	20.0	26.0	39.5	42.9	28.1	24.1	20.3	17.2	42.9	55.8	35.9	30.4	26.4

S1, S2 and S3, different season; T0, no treatment; T1, T2, T3 and T4, treatment with dilution of wasterwater used.

Table (2): Multivariate analysis of the effects of wastewater type, season, and dilution (concentration) and their interaction on water chemistry and biochemical parameters at significance levels ($p \leq 0.05$).

Factor	Measured minerals								Biomass production ‡		biochemical constituents		
	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	F.WT	D.WT	Carb. Content	Prot. content	A.A.†
Water type	0.027	0.000	0.000	0.000	0.274	0.000	0.001	0.084	0.000	0.000	0.033	0.000	0.000
Seasons	0.000	0.000	0.002	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
Dilution used	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Interaction among factors													
Water Type * Seasons	0.000	0.011	0.000	0.000	0.000	0.000	0.296*	0.868	0.044	0.003	0.000	0.000	0.000
Seasons * dilution used	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.002	0.000	0.000	0.000
Water type * dilution	0.000	0.000	0.000	0.000	0.010	0.197*	0.000	0.003	0.103	0.218*	0.000	0.021	0.000
Water type * Seasons * dilution used	0.000	0.000	0.000	0.003	0.024	0.000	0.000	0.001	0.007	0.012	0.010	0.000	0.000

‡ F.Wt, Fresh weight; D.Wt., Dry weight**.** †Car. content, Carbohydrate content; Prot content, Protein content; AA, Amino acid content. Values marked with an asterisk (*) as a superscript indicate non-significant differences.

For magnesium, the removal was higher in AG at 77.25%, compared to 67.3% in SG. Seasonally, AG showed the highest removal efficiency during S3, followed by S1, and the lowest during S2, whereas SG displayed the opposite trend, with the highest removal in S2 and the lowest in S1. The obtained data indicated that increased water strength generally enhanced Mg^{2+} removal in both water types under all seasons, except in SG during S1 (Table 2). Meanwhile, for potassium, the average removal efficiency was higher in AG (77.51%) than in SG (70.76%). Seasonally, S3 exhibited the highest removal rates, with S1 being the lowest, and S2 showing intermediate values. An increase in water concentration corresponded with increased K^+ removal across all seasons and water types, except in SG during S1.

For sodium, the removal efficiency was higher in AG (76.24%) than in SG (71.54%). Parallel to other cations, Na^+ removal increased with water strength in both water types across all seasons, except in SG during S1. Generally, for all cationic parameters, the removal rate (%) for (Ca^{2+}, Mg^{2+}; K^+ and Na^+), the removal rate (%) of these studied cations was higher in AG > SG except for Ca^{+2}. Seasonality showed an irregular pattern, whereas water strength Ca^{+2} increased under all water strengths in all seasons except for AG at S1. And vice versa for Mg^{+2}, K^+, and Na^+, which decreased under SG at S1. (Table 2).

Evaluation of duckweed growth parameters in different water types and dilution levels

Data obtained indicate that both water sources, AG wastewater and SG, possess comparable potential to support duckweed biomass accumulation, with nutrient composition significantly affecting growth rate and metabolic synthesis.

Biomass production

The data obtained for duckweed biomass demon-strates that both water types exhibit equal or higher potential for promoting vegetative growth (biomass) of *L. gibba*. The nutritive composition of each water type remarkably influences growth rate and metabolic activity within the treatment system. The recorded data indicate that both agricultural and sewage wastewater types support equivalent or enhanced vegetative growth (biomass) of *Lemna gibba*. The nutritive quality of these waters significantly influenced the growth rate and metabolic synthesis of the plant (Table 1, Fig. 1A-B).

Fresh Biomass

Data obtained and their multivariate analysis (MANOVA, Tables 1 and 2) revealed significant effects ($p \leq 0.05$) of treatments and their interactions on fresh and dry biomass, except for the interaction between water type and concentration.

Agricultural wastewater

Fresh biomass increased significantly with decr-easing dilution (increasing wastewater strength) in all three seasons. In season 1, fresh weight values were 123.81, 152.11, and 191.47 g at dilutions T1, T2, and T3, respectively, declining at the highest strength (T4: 111.93 g). Average fresh biomass increased by approximately 144.83% compared to control during the first season and showed a similar trend in season 2 with a 152.2% increase. In the third season, biomass showed only a slight increase of 102.3% relative to control (Figure 1A). Season 2 exhibited the highest growth enhancement.

Sewage Wastewater

Fresh biomass increased with increasing wastewater strength during the first season, peaking at 187.25% at T3 (75% strength) before declining below control at undiluted sewage (T4: 93.62%). The average across all dilutions was 136.9%. Season 2 followed a similar pattern but with higher increases (191.21%, 204.54%, and 185.9% at T1, T2, T3 respectively), and decline at undiluted sewage (88.73%). Season 3 showed a steady increase with increasing wastewater strength up to 141.34% at T4. On average, sewage wastewater treatments enhanced fresh biomass by 145.68%, more than agricultural effluents at 133.12%. Seasonality patterns were consistent across water types, with season 2 showing the highest growth (S2 > S1 > S3). Among dilutions, T3 had the most pronounced positive effect (Figure 1B).

Dry biomass

Agriculture wastewater

Figure (2A-B) presents the effects of different concentrations of AG on dry weight (g) across three seasons (S1, S2, S3). The dry weight values show a distinct trend, with the highest dry mass production observed predominantly at the 0.75 concentration during the first and second seasons, reaching up to approximately 350 g in S1. In contrast, the lowest dry weight was typically observed in the undiluted (1.0) treatment during all studied seasons, particularly in S3. Significant differences ($p \leq 0.05$) are among treatments within each season (Fig. 2A).

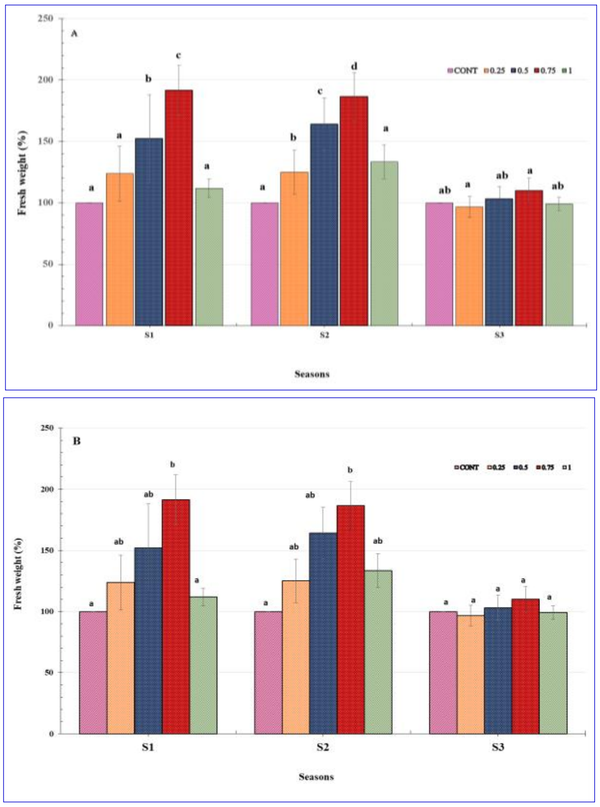

Figure (1): Effect of seasons on fresh weight of *Lima gibba* in both used wastewater type. A, Agricultural wastewater. B, Sewage wastewater.

Dry weight production was generally higher in the second season compared to the first and substantially lower in the third season across all treatments, indicating a pronounced seasonal effect and a possible decline in plant performance or response to AG application under the tested conditions. This pattern suggests both concentration-dependent and season-dependent variations in biomass accum-ulation, underlining the significance of optimizing both factors to maximize yield.

Sewage wastewater

For treatment of sewage wastewater, at various concentrations (0.25, 0.5, 0.75, and 1), the dry weight production across three successive seasons was highly influenced. In both S1 and S2, dry weight was significantly ($p \leq 0.05$) increased in response to higher concentrations of SG, with the maximum value observed at 0.75 in S1 and at 0.5 in S2. However, the application of the highest concentration (T1) resulted in a marked reduction in dry weight, particularly in S2, suggesting a potential inhibitory effect at this level. In contrast, Season 3 (S3) exhibited a markedly different response, with dry weight values across all designed treatments, including the control, converging to a narrower range and showing no significant differences (Fig. 2B). This pattern indicates a diminished impact of SG treatment during S3, likely reflecting a seasonal or environmental constraint on biomass accumulation that predominates the treatment effect.

In summary, a very minor difference was calculated because of water strength on dry biomass production across seasonality, where AG (186.9% compared to 185 % in SG). With positively enhanced dry weight production as a response to water dilution, where T3 is the most active concentration. Also concerning to seasonality of both water types were in the following order (S2>S1>S3) AG (249.7;188.99 and 121.9) whereas SG S3>S2>S1 (207.9; 188.83 and 158.12) and S2 is more active season in AG and S3 for SG and T3 is the highest effect among the dilution of both water types (Figure 2B).

Relative growth rate (RGR)

Data obtained (Fig. 3A-B) for RGR revealed that, at AG (292.64 %), and based on the effect of the mean average of water dilution on that parameter, it showed a great difference as a response to season, reaching up to 10 times (532.4 %) in the 3rd season compared to the 2nd season (53.24 %). Regarding the AG wastewater strength (292.64%), it recorded an increase as water strength increased, and T3 had the highest effect at that water type, as well as S3 had a much higher RGR production (Fig.3A-B). In contrast, the SG treatment showed an RGR of approximately 140%, displaying variable seasonal trends. In season 2, RGR decreased as water strength increased, whereas in season 3, RGR increased with water strength. The highest RGR values were observed at T3 (75%) across both water types, except under SG treatment during season 1, where the response differed. water dilutions, S3 consistently yielded the highest RGR values across both water types, and T3 was the most effective dilution in all cases, except for SG during season 1. These findings underscore the influence of water quality and seasonal variation on growth performance (Fig. 3B).

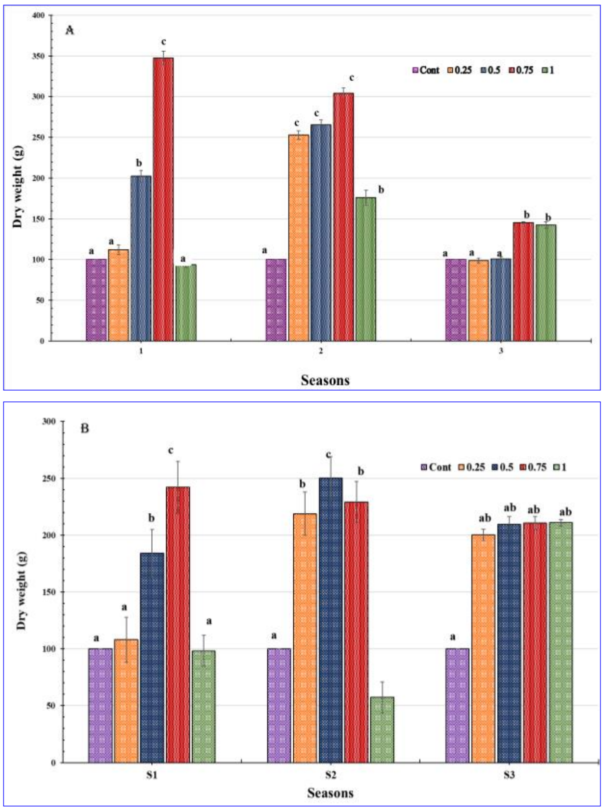

Figure (2): Effect of seasons on fresh weight of *Lima gibba* in both used wastewater type. A, Agricultural wastewater. B, Sewage wastewater.

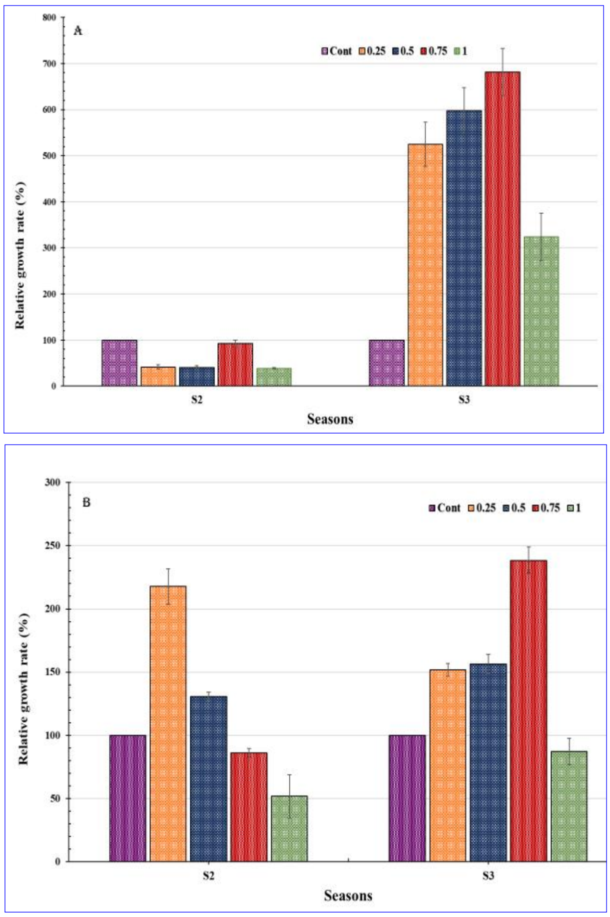

Figure (3): Effect of seasons on relative growth rate of *Lima gibba* in both used wastewater type. A, Agricultural wastewater. B, Sewage wastewater.

Duckweed Crop growth ratio (CGR g/m2/day)

Data in figure (4) illustrate the key trends of the CGR on base of water types was higher in the SG (9.42 g/m2/day or 328.57 %) as mean averages of water strength than AG (7.26 or 234.97 %) according to water dilutions in AG 25 % was the more effective concentrations among other dilution in both seasons except under SG at S3, and the 3rd season is more active in both water types. For SG, based on water dilution, it showed a varied crop growth rate, computed the highest CGR (8.12 g/m2/day or 199.08 %) in the 2nd season and (15.64 g/m2/day or 609.11 %) in the 3rd. But generally, on base of water types of SG>AG, regarding water dilution which has a negative effect (decrease in CGR as water strength increased) in both water types among all season except S3 and T1 is the more active dilution seasonality S3 registered the highest active season for seasonality SG was higher (328.57 %) than AG (234.97 %) in all water types. (Figure 4).

Harvest index (H-index) of *L. gibba* (dry weight/fresh weight)

Referring to the data present in Figure (5), we can say the mean average of AG wastewater concentration was 134.29 % which was higher than that of SG (128.87) %. Compared to the control and in all cases, it showed an increase in harvesting index with an increase in water strength up to the highest level (75 %) in the 1st and 2nd seasons of both water types, except in the last harvesting season, where it showed a different pattern.in the following order (AG: T1>T2>T3 <T4 and SG: T3>T2>T4>T1.) S2>S1>S3 under AG. Regarding to H index under SG treatment, the data showed a variable effect of water strength at S1, where T3 had the highest effect on the H index. Both T2 &T3 had the highest effect at S2, while T1 &T2 had the highest H index at S3. In general, S3>S1>S2.

The biochemical and nutritional value of *Lima gibba*

The chemical composition and nutrient content of duck weed, such as protein concentration, carbo-hydrate levels, and amino acid profiles, reflect the nutritional value during its growth under different type of wastewater.

Carbohydrate content

Data presented in Table (3) elucidate that a slight difference was calculated on the mean average of water types and their strength among different seasons registered (2.68 and 2.76 g/m2/day) for AG and SG, respectively. Referring to the seasonality regardless of water strength, data confirm the under-AG treatments S1>S2>S3, whereas under SG, it was in the following order S3>S2>S1, and T3 was the most effective concentration among all AG dilutions in all seasons. While in SG it varies among different dilutions and seasons, whereas under SG, T3 and T2 are more effective water strengths for S1 and S2 S3 respectively. All recorded data for treatments and their interactions had highly significant effects on carbohydrate content of *L.gibba* (Table 3).

Protein content

It was higher in SG (8.58 g/m2/day compared to 4.9 g/m2/day under AG treatments). It was noticed that the protein yield has a proportional increased proportionally as water strength increased up to 75 % (high concentration), then decreased under all treatments except the yield at 100 % (raw SG) during the 3rd growth period. It is interesting to find that protein yield under all SG treatments was roughly less than 2 times, the protein yield was in the following order: S1>S2>S3, and vice versa was calculated under SG treatment, where S3>S2 >S1, i e S2 was in the middle in both water types (Table 3).

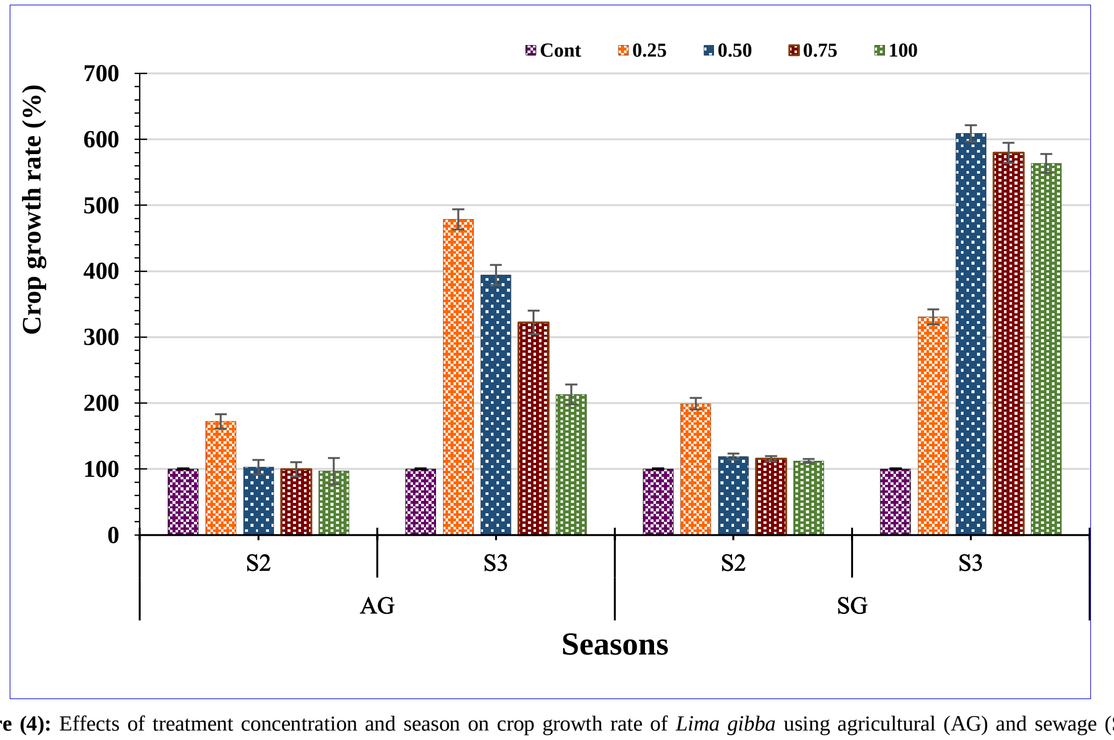

Figure (4): Effects of treatment concentration and season on crop growth rate of *Lima gibba* using agricultural (AG) and sewage (SG) wastewater.

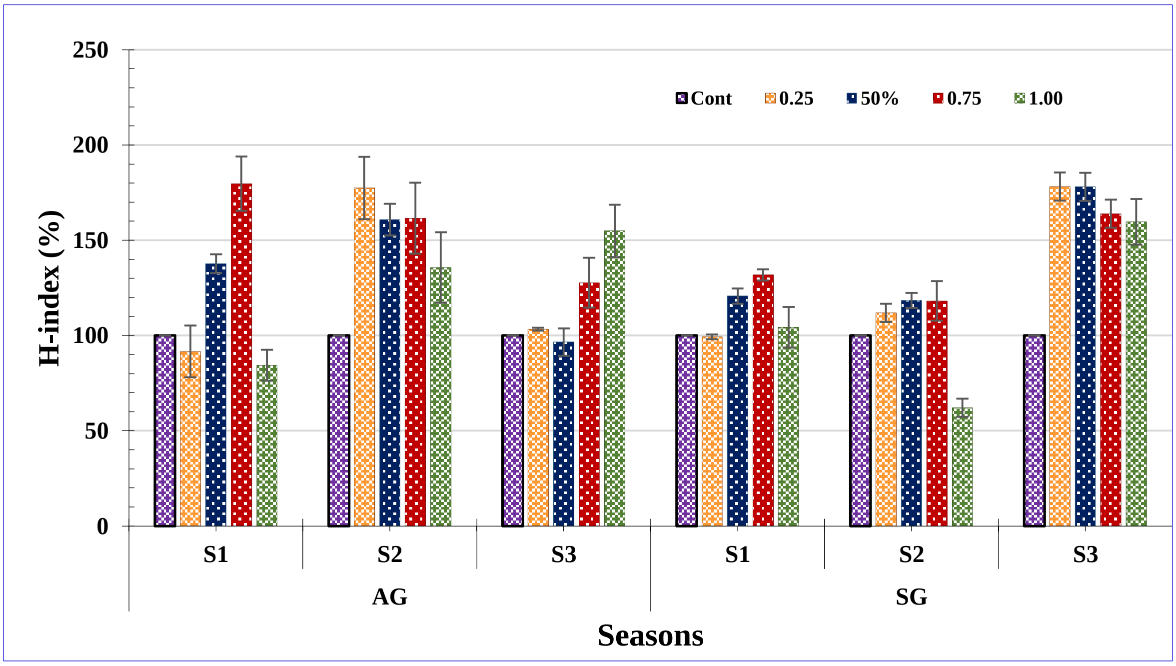

Figure (5): Effects of treatment concentration and season on harvest index (H-index), expressed as dry weight/fresh weight, of *Lima. gibba* using agricultural (AG) and sewage (SG) wastewater.

Amino acid content

Data here showed a slight difference in A.A. yield, which was (0.5 & 0.4 g/m2/day) under SG and AG treatments, respectively. It was interesting to notice that the Amino acids yield positively increased as water strength increased up to 75 % (high concentration), then decreased under all treatments except the yield at 100 % (raw AG) at the 3rd growth period. According to the seasonality, data of the amino acids yield was in the following manner: S1>S2>S3, and vice versa was calculated under SG treatment, where S3>S1 >S2 (Table 3).

DISCUSSION

Nutrient Absorption Capacity

Duckweed (*Lemna gibba* L.) demonstrated variable nutrient uptake efficiencies depending on the chemical and molecular structure of cations and anions. At low nutrient dosages, the removal percentage was generally low but increased with higher concentrations. This trend suggests that the active sorption sites were initially underutilized, but as nutrient availability increased, more sites became accessible, resulting in greater uptake efficiency (Yuzhen *et al.*, 2023). Similar trends have been reported by many authors , they noted that *L. gibba* is nitrophilic and tolerant of high nitrogen levels, and that microbial conversion and sedimentation also contribute significantly to N and P removal (Du Jardin, 2015; Delaide *et al.*, 2019; Varga *et al.*, 2023).

Effect of Wastewater Dilution and Seasonality

Wastewater dilution influenced removal efficiency, with lower removal at low concentrations and improved uptake at higher nutrient levels. Seasonal variation further modulated this process, as abiotic stresses (temperature extremes, photoperiods) reduced growth rates and uptake efficiency. Duckweed fronds

often displayed visual stress indicators such as anthocyanin accumulation (red coloration) and necrosis during harsh conditions, consistent with earlier observations (Paolacci *et al.*, 2016) and (Coughlan *et al.*, 2022). Initially, fronds were dark green with short roots, but as pollutants were consumed, plants became pale with elongated roots, reflecting nutrient limitation (Carvajal *et al.*, 2023).

Nitrogen Removal

Nitrogen (especially NO_3^-) removal was higher in this study than reported by (Agus *et al.*, 2024)*)* Mechanisms included assimilation into biomass, nitrification-denitrification, volatilization of NH_3, and microbial biofilm activity in the root zone (Zhu *et al.*, 2025). Although wastewater in this study contained relatively low NO_3^- (due to a high proportion of gray water), removal remained substantial. This supports previous findings that duckweed can remove up to two-thirds of nitrogen directly, with the remainder attributed to microbial denitrification and sedimentation (Zhao *et al.*, 2015; Jiang *et al.*, 2025).

Phosphorus removal

Phosphate (PO_4^{3-}) removal was strongly influenced by wastewater strength, redox potential, pH, and temperature, in agreement with data obtained in a study done by Kristina and Markus (2025). Removal mechanisms included uptake by duck-weed biomass, adsorption to organic matter, precipitation with cations (Priya *et al.*, 2012; Mohedano *et al.*, 2012; Shen *et al.*, 2019). Our findings corroborate previous reports that duckweed accounts for ~75% of N and P removal in such systems, with microbial processes playing a supporting role.

Table (3): Effect of agricultural and sewage water treatments on carbohydrate, protein, and amino acid yield of *Lima gibba* at different concentration across seasonal growth cycles.

Treatment (Conc.)	Carbohydrate Yield (g/m^2/day)						Protein Yield (g/m^2/day)						Amino acid Yield (g/m^2/day)					
	Agri (g/g.d.wt)			Sewage (g/g.d.wt)			Agri (g/g.d.wt)			Sewage (g/g.d.wt)			Agri (g/g.d.wt)			Sewage (g/g.d.wt)		
	Seasons																	
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Cont (T0)	0.54	0.21	0.44	0.32	0.78	1.07	1.58	0.49	1.50	1.14	1.31	3.42	0.13	0.03	0.05	0.04	0.05	0.39
T1 (1:3)	0.83	1.27	0.55	1.10	1.62	4.27	1.60	2.77	1.64	2.07	5.00	14.44	0.16	0.20	0.08	0.10	0.25	1.02
T2 (1:1)	4.47	1.72	0.76	3.60	3.09	4.06	10.15	3.81	2.44	6.61	7.47	16.33	0.78	0.23	0.13	0.55	0.45	0.62
T3 (3:1)	13.75	2.27	2.47	5.32	2.50	3.98	20.86	3.85	4.29	8.79	8.50	18.24	1.60	0.30	0.39	0.69	0.50	0.61
T4 (3:0)	1.90	0.76	1.43	0.71	0.62	2.30	1.22	1.52	4.63	1.28	1.91	12.26	0.16	0.17	0.58	0.17	0.16	0.33
Mean	5.24	1.51	1.30	2.68	1.96	3.65	8.46	2.99	3.25	4.69	5.72	15.32	0.68	0.23	0.29	0.38	0.34	0.65

Agri., Agriculture wastewater. T1, T2, T3 and T4, different dilution of wastewater as factor of water concentration.

Sulphate and cation removal

SO_4^{2-} removal followed the order T0 > T1 > T2 > T3 > T4, indicating greater uptake at lower dilutions. Duckweed assimilation of sulphate contributes to the biosynthesis of sulfur amino acids, essential for protein formation (Abou Seeda *et al.,* 2020). Similar findings were reported by (Li *et al.,* 2025). Cation uptake ($Ca^{2+}Mg^{2+}$, K^+, Na^+) was also significant, as these ions are physiologically important for growth and contribute to ionic balance in aquatic systems (Yang *et al.,* 2024).

Biomass Production and Growth Performance

Biomass productivity varied with nutrient concentration. *L. gibba* produced higher yields in nutrient-rich wastewater compared to control water. However, extremely high nutrient levels inhibited growth, likely due to biofilm formation on roots and toxic effects of excess organic matter (Chen *et al.,* 2025). Seasonal growth patterns revealed initial stress (yellowing, necrosis), followed by recovery as degraded fronds released nutrients for regrowth. These results are in agreement with (Escobar & Escobar, 2017), who highlighted duckweed's adaptability in nutrient-rich conditions.

Biochemical yields and applications

Protein and carbohydrate yields were significantly higher in wastewater setups than in controls. High protein content highlights the potential of *L. gibba* as an aquaculture feed resource, though care must be taken regarding heavy metal accumulation. Elevated carbohydrate levels suggest utility in bioenergy production (Desai *et al.,* 2024; Ge *et al.,* 2012). Whereas, under SG, T3 and T2 are more effective water strengths for S1, S2 and S3, respectively. All recorded data for treatments and their interactions were recorded (Table 3). Duckweed starch accumulation under nutrient stress provides additional advantages for biofuel applications. Biomass productivity and biochemical profiles were influenced by nutrient load, temperature, and photoperiod, consistent with (Merah *et al.,* 2025).

Implications for wastewater treatment and resource recovery

Overall, *L. gibba* effectively removed N, P, SO_4^{2-}, and cations, while producing high-value biomass rich in protein and carbohydrates. This dual role makes duckweed a promising candidate for integrated wastewater treatment and resource recovery systems (Takacs *et al.*, 2025). However, optimization of factors such as plant density, hydraulic retention time, light, and seasonal management is essential for large-scale applications. Future studies should focus on defining optimum nutrient ranges, assessing long-term productivity, and minimizing potential risks such as greenhouse gas emissions (Mohedano *et al.,* 2019; Kupper *et al.,* 2020).

CONCLUSION

This study highlights the need for further research to demonstrate *Lemna gibba*'s potential as an effective solution for wastewater remediation and to harvest a large biomass production with high nutritive values. The crisis of freshwater is one of the growing concerns at the beginning of the twenty-first century that needs the integrate duckweed *Lemna gibba* as a powerful tool into wastewater-duckweed sites, contributing to a circular economy and supporting sustainable aquaculture. Duckweed may be the most effective plant for purifying water. Because of its high rate of rapid growth, high dry matter accumulation, and excellent environmental adaptability. Duckweed is a cheap and eco-friendly way to stop pollution in the environment and protect terrestrial and aquatic ecosystems through phytoremediation. Offer promising, eco-friendly solutions for wastewater purification and nutrient recovery through phytoremediation. Adaptability and high biomass yield enable efficient removal of various ionic elements and anthropogenic pollution, making them suitable for industrial and municipal water treatment. Water resources are under threat because of the growing population. The increasing generation of wastewater effluents (municipal, industrial, and agricultural) in developing countries, especially in Egypt, has the potential to serve as an alternative to freshwater resources for reuse in other purposes (crop and vegetables), as one of the big challenges for our country, especially after the Grand Ethiopian Renaissance Dam (GERD). Lastly, duckweeds have made a great contribution in wastewater purification as a contaminant-absorbing agent, producing high amounts of fresh and dry biomass with high nutritive values. The data showed the highest ion removal efficiency in AG than SG, and Vice versa for the biomass production and metabolites. The second season (S2) and high water strength (T3) were the most active parameters. Overall, *L. gibba* demonstrated strong phytoremediation capacity and produced nutrient-rich biomass suitable for aquaculture or bioresource applications. Therefore, further studies could be directed to conduct on utilizing duckweeds in different wastewater effluents.

REFERENCES

ABOU SEEDA, M. A., ABOU EL-NOUR, E. A. A. Y., A.A, & GAD MERVATSAHA, R. Z., M. (2020). Importance of sulfur and its roles in Plants physiology: A Review. Current Science International., 09 (2) Pages*:* (2), 198–231. https://doi.org/DOI: 10.36632/csi/2020.9.2.18

Agus, W., Evi, S., Awalina, S., Wardah, W., Kukuh, N., Yuni , P., Hastuti , and Tjandra , C. (2024). The impact of shade net use on total nitrogen removal by duckweed (*Lemna perpusilla*) at different levels of catfish farming effluent Aquacultural Engineering, 107 1–12. https://doi.org/https://doi.org/10.1016/j.aquaeng.2024.102456

AL-AMEEN, A. A. J., & AL-TAEE, T. T. N. (2022). Phytoremediation of Drug Industry Wastewater using Nineveh *Lemna* spp. (Duckweed). *Indian Journal of Ecology 49*(20), 194–199.

BOULOS, L. v. p. (2005). Flora of Egypt (Vol. 4). Al-Hadara publishing. Cairo.

CARVAJAL, J., ENRIQUE, V., PAOLA, G., MON-TANA, J., HERNANDO, P. S., ELBERTHAL, JOS´E , A., MERCHAN, P., & MARGARITA, A. L. (2023). Macronutrient omission influences mor-phological parameters, growth, and yield in *Arracacia xanthorrhiza 9*(2), 13062–13074. https://doi.org/10.1016/j.heliyon.2023.e13062

CHEN, G., YANG, F., JUN, H., YONGGUI, Z., QI LI, LAI, F., XU, Y., TIAN, X., HE, K., JIN, Y., TANAC, L., & ZHAO, H. (2018). Duckweed systems for eutrophic water purification through converting wastewater nutrients to high-starch biomass: comparative evaluation of three different genera (*Spirodela polyrhiz*a, *Lemna mino*r and *Landoltia punctat*a) in monoculture or polyculture The Royal Society of Chemistry advanced, 8, 17927–17937. https://doi.org/ DOI: 10.1039/c8ra01856a

CHEN, L., ZHAO, M., LI, X., LI, Y., & WU, J. (2025). Effect of High-Strength Wastewater on Formation Process and Characteristics of Hydrophyte Periphytic Biofilms. *Sustainability*, *17*, 2654–2671. https://doi.org/https://doi.org/10.3390/su17062654

COUGHLAN , N. E., WALSH, E., BOLGE , P. B., BURNELL, G., O'LEARY, N., MARIA, O. M., SIMONA, P., DAVID, W., & MARCEL, A. K. J. (2022). Duckweed bioreactors: Challenges and opportunities for large-scale indoor cultivation of Lemnaceae. *Journal of Cleaner Production, 336*, 130285–130295. https://doi.org/10.1016/j.jclepro.2021.130285.

DELAIDE, B., TEERLINCK, S., DECOMBEL, A., & BLEYAERT, P. (2019). Effect of wastewater from a pikeperch (Sander lucioperca L.) recirculated aquaculture system on hydroponic tomato production and quality. *Agricultural Water Management,*, *226*, , 1–9. . https://doi.org/10.1016/j.agwat.2019.105814.

DESAI, S., BELEKAR, P., PATHAN, S., & PAWAR, A. (2024). Enhanced Phytoremediation and Resource Recovery: The Efficacy of Duckweed in Mitigating Wastewater Pollution *MICRO ENVIRONER* *4*(1), 12–16. https://doi.org/https://doi.org/10.54458/mev.v4i01.00041

DU JARDIN, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae;*, *196*, 3–14. https://doi.org/ 10.1016/j.scienta.2015.09.021

DUBOIS, M., GILLES, K., HAMILTON, J., ROBERTS, P., & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem*, *28*, 350–356.

EL-EZABY, K., SOLIMAN, A., HASABALLAH, A. 2023. 'Rice Straw as a Sustainable Treatment Medium for Grey Wastewater: A Case Study in Damietta, Egypt', Catrina: The International Journal of Environmental Sciences, 27(1), pp. 93-104. doi: 10.21608/cat.2023.208767.1173

EL SHAFEI, A., ZAHRAN, H., NASR, M., & EL NOZAHI, D. 2024. Efficacy of the Electro-coagulation Treatment of Agricultural Drainage Water. Catrina: The International Journal of Environmental Sciences, 33(1), 1-9. doi: 10.21608/cat.2024.287086.1280

ENG, H. H., AND SIAU, H. M. 2025. Duckweed: Growth factor and applications in nonfood, food, and health. *Food Safe Health*, *3*(2), 144–154. https://doi.org/https://doi.org/10.1002/fsh3.12078

ESCOBAR, C., & ESCOBAR, A. 2017. Duckweed: A tiny aquatic plant with enormous potential for bioregenerative life support systems. 47th International Conference on Environmental Systems,

FOROUGHI, M., NAJAFI, P., TOGHIANI, S., TOGHIANI, A., & HONARJOO, N. (2013). Nitrogen Removals by *Ceratophyllum demersum* from Wastewater. Journal of Residuals Science & Technology.;10:2. *Pp 63-68.* , *10* (2), 63–68. https://doi.org/10(2):063-066

FOUROUNJIAN, P., FAKHOORIAN, T., & CAO, X. H. (2020). Importance of Duckweeds in Basic Research and Their Industrial Applications; . Springer: Cham, Switzerland.

GAUR, R. Z., & SUTHAR, S. (2017). Nutrient scaling of duckweed (*Spirodela polyrhiza*) biomass in urban wastewater and its utility in anaerobic co-digestion. *Process Safety and Environmental Protection*, *107*, 138–146. https://doi.org/10.1016/j.psep.2017.02.005

GAVRILESCU, M., DEMNEROVA, K., AAMAND, J., AGATHOS, S., & FAVA, F. (2015). Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *N Biotechnol*, *32*(1), 147–156. https://doi.org/10.1016/j.nbt.2014.01.001

GE, X., ZHANG, N., PHILLIPS, G. C., & XU, J. (2012). Growing Lemna minor in agriculture wastewater and converting the duckweed biomass to ethanol. *Bioresource Technology*, *124* 485–488. https://doi.org/DOI: 10.1016/j.biortech.2012.08.050

GHALWA, N. M., KODEH, F. S., FARHAT, N. B., & EL NAJJAR, D. M. (2023). Treatment of Grey Water Using Electrochemical Methods: A Comparison between Combined Electrocoagulation and Electrooxidation. *Journal of Environmental Engineering Studies*, *8*(1), 61–74.

JACKSON , M. (1960). *Soil chemical analysis.* Verlag: Prentice Hall, Inc., Englewood Cliffs, NJ., 498 S. DM 39.40.

JIANG, S., HUANG, H., YONGLI , C., JIANHUA, X., ZIYUAN, L., & SHUANGFEI, W. (2025). Optimizing Nitrogen Removal Through Coupled Simultaneous Nitrification-Denitrification and Sulfur Autotrophic Denitrification: Microbial Community Dynamics and Functional Pathways in Mariculture Tailwater. Treatment Water, 17(5), 683– 700. https://doi.org/ https://doi.org/10.3390/w17050683

KRISHNA, B.-K. C., & POLPRASERT, C. (2008). An integrated kinetic model for organic and nutrient removal by duckweed-based wastewater treatment (DUBWAT) system. Ecological Engineering, 34(3), 243-250. https://doi.org/10.1016/j.ecoleng.2008.08.013

KRISTINA, S., & MARKUS, E. (2025). Simultaneous nutrient removal and selective recovery of nitrogen and phosphorus from municipal wastewater: A novel combined ion exchange, surface precipitation process from proof of concept to pilot scale. Journal of Environmental Chemical Engineering. , 13(4), 117408 –117419. https://doi.org/https://doi.org/10.1016/j.jece.2025.117408.

KUBOTA, K., OTANI, T., HARIU, T., JIN, T., TAGAWA, T., MORIKAWA, M., & LI, Y. Y. 2024. Evaluation of sewage treatment performance and duckweed biomass production efficiency in a primary sedimentation basin. JournalJournal of Water Process Engineering, 65, 1–7. https://doi.org/https://doi.org/10.1016/j.jwpe.2024.105818

KUMAR, S. S., MAGESWARI, S. U., DHANASEKAR, S., PRASAATH, R. G., KUMAR, S. D., & YUVARAAJ, K. R. 2021. Domestic water treatment an Indian perspective. *Journal of Physics: Conference Series,*, *2054*(1), 1–7. https://doi.org/https://doi.org/10.1088/1742-6596/2054/1/012076

KUPPER, T., H¨ANI, C., NEFTEL, A., KINCAID, C., BÜHLER, M., AMON, B., & VANDERZAAG, A. (2020). Ammonia and greenhouse gas emissions from slurry storage – a review. *Agriculture Ecosystem Environment*, *300*, 106963–106981. https://doi.org/https://doi.org/10.1016/j.agee.2020.106963.

LI, B., GU, B., YANG, Z., & ZHANG, T. (2018). The role of submerged macrophytes in phytoremediation of arsenic from contaminated water: A case study on Vallisneria natans (Lour.) Hara. *Ecotoxicology and Environmental Safety*, *165*, 224– 231. https://doi.org/10.1016/j.ecoenv.2018.09.023

Li, J., Gao, Y., Xie, Y., & al., e. (2025). Optimal cultivation concentration of duckweed for pollutant removal from biogas slurry. *Scientific reports 15*, 5193–5211. https://doi.org/ https://doi.org/10.1038/s41598-025-89524-w

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*, *93*, 262–265.

MERAH, A., BENAHMED, K., AMIRA, F., & HALIS, Y. (2025). Organic Manure Tests on the Yield and Nutritional Content of the Duckweed *(Lemna minor* L.) Cultivated in the Geothermal Waters in Southeastern Algeria. *Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.*, *29*(3), 697 – 716.

MOHEDANO, R. A., COSTA, R. H. R., TAVARES, F., & FILHO, P. B. (2012). High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. Bioresource Technology, 112, 98–104. https://doi.org/https://doi.org/10.1016/j.biortech.2012.02.083.

MOHEDANO, R. A., TONON, G., COSTA, R. H. R., PELISSARI, C., & FILHO, P. B. (2019). Does duckweed ponds used for wastewater treatment emit or sequester greenhouse gases? Science Total Environment. , 691, 1043–1050. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.07.169.

MOORE, S., & STIEN, W. (1948). Photometric ninhydrine method for use in the chromatography for amino acids. *J. Biol. Chem*, *17*, 367–388.

NOAMAN, A., EL-NAGGAR, A., EL-TANTAWY, S., MASHALY, M. (2025). 'Biodiversity of Microfauna Dwelling Skin and Gills of Clarias gariepinus Burchell, 1822 from Polluted and Fragmented Habitats', Catrina: The International Journal of Environmental Sciences, 35(1), pp. 1-16. doi: 10.21608/cat.2025.302096.1301

OMAR, B., ZALOUK, R., SOLIMAN, S., & ELGAMMAL, M. (20323). Controlling Disinfection ByProducts using Chlorine Profile and CT Tables at EL-Nobarya Drinking Water Treatment Plant, Egypt, . Catrina: The International Journal of Environmental Sciences,, 27(1), 33–34. https://doi.org/ https://doi: 10.21608/cat.2023.194317.1158.

PAOLACCI, S., HARRISON, S., & JANSEN, M. (2016). A comparative study of the nutrient responses of the invasive duckweed Lemna minuta, and the native, co-generic species *Lemna mino*r. Aquatic Botany *143*, 47–53. https://doi.org/10.1016/J.AQUABOT.2016.07.004

PAPADOPOULOS, F. H., & TSIHRINTZIS, V. A. (2011). Assessment of a full-scale duckweed pond system for septage treatment. Environ Technol, 32(7-8), 795–804. https://doi.org/10.1080/09593330.2010.514009

PRIYA, A., AVISHEK, K., & PATHAK, G. (2012). Assessing the potentials of Lemna minor in the treatment of domestic wastewater at pilot scale. Environmental Monitoring and Assessment, 184(7), 4301–4307. https://doi.org/DOI: 10.1007/s10661-011-2265-6

SCHWARZENBACH, G., and BIEDERMANN, W. (1948). Komplexone X. Erdalkalikomplexe von 0.6 - Dioxyazofarbstoffen. *Helv.* Chem. Acta, 31, 678 – 687.

SHEN, M., YIN, Z., XIA, D., ZHAO, Q., & KANG, Y. (2019). Combination of heterotrophic nitrifying bacterium and duckweed (*Lemna gibba* L.) enhances ammonium nitrogen removal efficiency in aquaculture water via mutual growth promotion. . *Journal of General and Applied Microbiology*, *65*, 151–160. https://doi.org/https://doi.org/10.2323/jgam.2018.08.002.

STEFANAKIS, A., AKRATOS , C., & TSIHRINTZIS, V. (2014). *Vertical Flow Constructed Wetlands: Eco-Engineering Systems for Wastewater and Sludge Treatment*. Elsever Inc. https://doi.org/doi:10.1016/b978-0-12-404612-2.00008-8

SUSHMITHA, M., CHANAKYA, H., & KHUNTIA, H. K. (2019). Efficient grey water treatment and reuse options for India. A Review: Waste Water Recycling Management,, *3*, 143–149. https://doi.org/https://doi.org/110.1007/1978-19811013261910-06.

TÄCKHOLM, V. (1974). *Students Flora of Egypt*. Cairo University.

TAKACS, K., VEGH, R., MEDNYANSZKY, Z., HADDAD, J., ALLAF, K., DU, M., CHEN, K., KAN, J., CAI, T., MOLNAR, P., BARSONY, P., MACZO, A., ZALAN, Z., & DALMADI, I. (2025). New Insights into Duckweed as an Alternative Source of Food and Feed: Key Components and Potential Technological Solutions to Increase Their Digestibility and Bioaccessibility Applied . Sciences, 15, 884–916. https://doi.org/https://doi.org/10.3390/app15020884

TAVARES, F. D. A., LAPOLLI, F. R., ROUBACH, R., JUNGLES, M. K., FRACALOSSI, D. M., & MORAES, A. M. D. (2010). Use of domestic effluent through duckweeds and red tilapia farmingin integrated system. Pan-American Journal of Aquatic Sciences, *5*(1), 1–10.

UN HABITAT. (2023). *The global report on sanitation and wastewater managmentin cities and human settlements United Nations Human Settlement Programme*.

VARGA , M., ŽUNA PFEIFFER, T., BEGOVIĆL, I., SELMA, M., HORVATIĆ, J., MILOLOŽA, T., & ČAMAGAJEVAC IVNA, Š. (2023). Physiological Response of Nutrient-Stressed *Lemna gibba* to Pulse Colloidal Silver Treatment. *Plants (Basel) ;*, *12*(6), 1367–1382. https://doi.org/10.3390/plants12061367

VERMA, R., & SUTHAR, S. (2014). Synchronized urban wastewater treatment and biomass production using duckweed *Lemna gibba* L. *Ecological Engineering 64*, 337–343.

WILLIAMS, V., & TWINE, S. (1960). *Flame photometric method for sodium, potassium and calcium* (K. Paech, Tracey, M. Vced, Ed. Vol. V). Springer - Verlay, Berlin. (Original work published Modern Methods of plant Analysis)WOODS, J.T., & MELLON, M. G. (1958). *Chlorostannous reduced molybdophosphoric blue colour method in sulphuric acid system* (M. Jackson, Ed.). Prentice - Hall International, London.

WWAP. (2017). Wastewater: the untapped resource: the United Nations world water development report,PARIS.

YANG, M., ZHOU, D., HANG, H., CHEN, S., LIU, H., SU, J., LV, H., JIA, H., & ZHAO, G. (2024). Effects of Balancing Exchangeable Cations Ca, Mg, and K on the Growth of Tomato Seedlings (Solanum lycopersicum L.) Based on Increased Soil Cation Exchange Capacity *Agronomy*, *14*(3), 629–642. https://doi.org/https://doi.org/10.3390/agronomy14030629

Yuzhen, Z., Anton, S., Olena, K., ianming, X., & Nikolai, B. (2023). Duckweeds for Phytoremediation of Polluted Water. Plants (Basel) ;, 12(3), 589–608. https://doi.org/10.3390/plants-12030589

ZHAO, Y., FANG, Y., JIN, Y., HUANG, J., MA, X., HE, K., HE, Z., WANG, F., & ZHAO, H. (2015). Microbial community and removal of nitrogen via the addition of a carrier in a pilot-scale duckweed-based wastewater treatment system. Bioresource Technology, 179, 549–558. https://doi.org/https://doi.org/10.1016/j.biortech.2014.12.037

ZHU, M., MINHUI, M., CHEN, S., YUAN, R., & WANG, S. (2025). Study on the Synchronous Removal of Nitrogen and Phosphorus by Autotrophic/Heterotrophic Denitrification in the Presence of Pyrite *Molecules*, *30*,, 2412–2434. https://doi.org/https://doi.org/10.3390/molecules30112412.

ZIEGLER, P., ADELMANN, K., ZIMMER, S., SCHMIDT, C., & APPENROTH, K. J. (2015). Relative in vitro growth rates of duckweeds (Lemnaceae). The most rapidly growing higher plants. Plant. *Biology,*, *17*(1), 33–41. https://doi.org/https://doi.org/10.1111/plb.12184

تكامل ديناميكيات المواسم ومعالجة مياه الصرف مع انتاج الكتلة الحيوية لنبات عدس الماء لمعالجة مائية مستدامة

ناصر أحمد بركات، شيرى أشرف حبيب ووليد وحيد نظير
قسم النبات والميكروبيولوجى كلية العلوم جامعة المنيا – المنيا- مصر

الملخص العربي

مع استمرار ارتفاع عدد سكان العالم، أصبح من الأهمية مواجهة مشكلة انعدام الأمن الغذائي. وفي هذا الصدد جذبت تقنيات الزراعة بدون تربة مثل الأنظمة المائية والأنظمة الهوائية الكثير من الاهتمام كإجابات قابلة للتطبيق لحل مشاكل تلو مصـــادر الميــاه. عدس الماء نبات مائي كبير صديق للبيئة يتميز بقدرة عالية لمعالجة مياه الصرف وتعظيم الاستفادة من كتلته الحيوية. أشارت دراسة تكامل ديناميكيات التباين الموسمي ونوع المياه المستخدمة تحت ظروف الصوبة الزجاجية باستخدام نوعين من مياه الصرف الزراعي والصحي بتركيز خمس مستويات لثلاث مواسم متباينة النمو مدتها ست أشهر لكفاءة إزالة المغذيات ودلائل مظاهر النمو النباتي. وقد أظهرت النتائج أن كفاءة إزالة العناصر الذائبة أعلى في مياه الصرف الزراعي عنها في الصرف الصحي فيما عدا نسبة إزالة الكلوريدات والكبريتات الذائبة. كما أن أنتاج الكتلة الحيوية الطازجة كانت أعلى في النباتات النامية في مياه الصرف الصحي عن الزراعي والعكس بالنسبة للوزن الجاف كما تأثر معدلي النمو النسبي ونمو الكتلة الجافه بالتغاير الموسمي والذي أوضح أن موسم النمو الوسطى (2) هو أعلى المواسم إنتاجية وأن التخفيف رقم (3) هو الأكثر فاعلية. كذلك أيضا أظهرت نتائج قياس الدلائل الفسيو كيميائية لمستخلص النسيج الجاف للنبات أن تراكم البروتينات والسكريات أعلى تحت تأثير مياه الصرف الصحي من الزراعي. والخلاصة فإن هذه النتائج أظهرت قدرة كفاءه فاعلية هذا النبات في معالجة مياه الصرف المستخدمة وتراكم كتلة حيوية غنيه كمورد حيوي وهذا يرشح بقوه استخدام هذا النبات كمصدر قليل التكلفة لبيئة مائية مستدامة.