

## SEASONAL FLUCTUATIONS IN THE CHEMICAL CONSTITUENTS AND NUTRITIONAL VALUE OF THE ABOVE- WATER TISSUES OF WATER LETTUCE (*PISTIA STRATIOTES* L.) AT GIZA PROVINCE, EGYPT

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**Abstract.** The current research examined the potential of *P. stratiotes* to accumulate inorganic and organic nutrients from contaminated watercourses in Egypt. It also aimed to determine whether the biomass of the plant can be safely used as animal feed. Plant and water samples were taken seasonally for a year at three different sites using three randomly assigned quadrats. There were significant seasonal differences in water pH, dissolved oxygen, transparency, and water temperature. The highest biomass (314.52 g DM/m<sup>2</sup>) was recorded during spring, while the lowest (35.38 g DM/m<sup>2</sup>) during winter. Winter contributed to the highest contents of N and P (4.09 and 0.62%), while summer had the highest Ca (2.46%), and spring had the highest of Mg and Na (1.23 and 2.65%). The highest Co, Fe, Ni, and Pb values (17.28, 2517.36, 18.99, and 48.57 mg/kg) were highest in summer. The highest levels of crude protein and carbohydrates (26.47 and 42.59%), and the greatest levels of digestible crude protein, digestible energy, metabolized energy, net energy, and gross energy (21.07%, 2.79 Mcal/kg, 2.29 Mcal/kg, 1.14 Mcal/kg and 405.97 Kcal/100 g) were recorded in winter. Summer is ideal for harvesting *P. stratiotes* for phytoremediation, while winter is suitable for animal feeding.

**Keywords:** *floating macrophytes, nutrients, heavy metals, animal fodder, Al-Sero Drain*

### Introduction

Nutrient concentrations in aquatic environments have grown due to intense anthropogenic activities and socioeconomic growth, leading to ecological deterioration (Huang et al., 2020; Wu et al., 2021). Cultivated areas may discharge nutrients and heavy metals into watercourses due to excessive fertilizers and pesticides application, which exceeds the crop requirements (Kiani et al., 2021). Overabundant nutrients build up at the bottoms of wetlands, but they can be recycled back into the surrounding water and cause eutrophication (Galal et al., 2023), which is a serious issue for other aquatic biota as well as for human health (Ali et al., 2024). Additionally, heavy metal pollution in the aquatic environment is a serious concern because of their potential toxicity and accumulation in aquatic environments (Vardhan et al., 2019; Wang et al., 2024). A

number of actions can be taken to improve the quality of contaminated water, including educating the public about the correct way to dispose of untreated wastes, prosecuting those who violate water pollution laws, and remediating waste using plants (Irmawanty et al., 2023). Using free-floating aquatic plants to remediate wastewater in contaminated wetlands is an inexpensive, environmentally friendly, and natural way to manage wastewater (Yadav et al., 2023; Ali et al., 2024).

Fast and densely growing aquatic plants is an issue causing severe water loss from water bodies through evapo-transpiration, clogged gates, pumps, etc. (Al-Sherif, 2009). In addition, they deplete the water's dissolved oxygen concentration by consuming oxygen, upsetting the lifestyles of fish and other aquatic biota (Hendrasarie and Redina, 2023). They are among the main threats to global biodiversity, ecosystem functions, economic sustainability, and sustainable development (Xiong et al., 2023). They are important biological factors that have a negative impact on the environment and ecosystem health (Simberloff et al., 2013). However, in freshwater ecosystem services, aquatic plants have the ability to regulate nutrient remediation and water filtration (Manolaki et al., 2020). They are able to sequester large amounts of nutrients and heavy metals from wetlands by storing both in their roots and/or shoots, (Eid et al., 2020). Furthermore, it is thought that aquatic plants serve as an efficient collector of nutrients and heavy metals in addition to providing food for aquatic invertebrates in the aquatic ecosystem (Galal et al., 2023). It is known that some free-floating plants, like *P. stratiotes*, can bioconcentrate and absorb nutrients and heavy metals, leading to an internal concentration that is many times higher than the surrounding environment (Galal et al., 2018).

The demand for fodder production has increased due to desertification and population growth (Tanaka et al., 2017), which hamper production of food crops and the decrease agricultural land's expansion (Bruinsma, 2003). Therefore, it is crucial for developing countries to utilize alternative feed sources (Galal et al., 2023). According to Rattanasomboon et al. (2019), feeding non-traditional feedstuffs may lower feed costs for animal producers, however, this alternative feedstuff may include high fiber content, which may compromise digestion and therefore meat quality. Since aquatic plants grow quickly and are highly productive, they can accumulate significant amounts of nutrients in their biomass for both growth and reproduction (Vymazal, 2020). Harvesting biomass from above-ground plants parts is a widespread approach for managing and restoring aquatic environments by creating low-nutrient environments (Geurts et al., 2020; Galal et al., 2022). Forages, fertilizers, soil amendments, and the generation of biofuel are only a few uses for the recovered biomass that can be recycled (Plaimart et al., 2021). Thus, in addition to being used for animal forage (Galal et al., 2021), regular harvesting of aquatic plant biomass improves the effective treatment and inhibits the resorption of nutrients into the waterbody (Vymazal, 2020).

Water lettuce, or *Pistia stratiotes* L. (Araceae), is a free-floating macrophyte with a quick vegetative growth rate (Ali et al., 2021). According to Eid et al. (2021), it was included in the Global Invasive Species Database as an invasive species. Large portions of the water's surface in tropical and sub-tropical regions are entirely covered with *P. stratiotes*, which is gregariously floating on the stagnant water (Sasmal and Mondal, 2013). It thrives in ponds, lakes, irrigation canals, slowly flowing rivers, and reservoirs, frequently creating dense floating mats that extend from the waterbody's periphery toward open waters (Milićević, 2023). *P. stratiotes* is a type of free-floating aquatic macrophyte that is widely used to remove containments from wastewater before it is

released, reducing the amount of pollutants that endanger naturally occurring water bodies (Ali et al., 2024). The rationale behind the selection of *P. stratiotes* is its exceptional efficacy in eliminating both organic and inorganic contaminants including heavy metals, from agricultural, domestic, municipal, and industrial effluents, as well as drainage ditches (Wardah et al., 2022; Thakur et al., 2023).

*P. stratiotes* can deposit or absorb pollutants from the water through its different organs and is adaptable to a variety of environmental conditions (Kumar et al., 2017). Besides, in just 5, 10 and 20 days its biomass becomes double, triple, and quadruple respectively, and in a period of less than 1 month it can increase by nine times as compared to its original biomass (Ali et al., 2024). The primary goals of the current study are to examine the potential of the free-floating invasive plant, *P. stratiotes*, to accumulate inorganic and organic nutrients as well as heavy metals from contaminated watercourses. It also aimed to determine whether or not its biomass can be safely used as animal feed. This is done from the perspective of plant variation in their nutrient sequestration potential and nutritional quality. This research has the potential to advance our understanding of the interactions between *P. stratiotes* and contaminants in contaminated wetlands as well as provide insightful new information on sustainable feeding methods and phytoremediation.

## Materials and methods

### *Site and sampling design*

The research area is located in Giza Province in Egypt's South Nile Delta (Fig. 1), which is found in the hyper-arid region (UNESCO, 1977). The annual rainfall is about 87 mm, and the mean maximum and minimum air temperatures are 30.0°C and 14.8°C, respectively. The mean evaporation is 6.9 mm/day (Piche), while the mean annual relative humidity is 45.5%, and the mean annual wind speed is 3.9 m/s (NASA-POWER, 2020).

Plant sampling was conducted in monospecific and pure stands of *P. stratiotes* at three sites (Site 1: Lat. 30° 03' 18.88" N, Long. 31° 08' 17.56" E; Site 2: Lat. 30° 03' 15.73" N, Long. 31° 08' 28.20" E; Site 3: Lat. 30° 03' 30.00" N, Long. 31° 08' 14.00" E. Site 1 is located ~ 400 m north of Site 2 and ~ 400 m south of Site 3 (Fig. 1) along Al-Sero Drain (a characteristic drainage channel in the South Nile Delta) in Giza Province. Three randomly dispersed quadrats, each measuring 0.5 × 0.5 m, were used to sampling all *P. stratiotes* individuals within each quadrat (n = 36). The biomass of *P. stratiotes* plants was sampled seasonally from spring 2018 to winter 2019 at each selected site. Shoots were separated in the lab, weighed, and then carefully cleaned with tap water over a 4 mm mesh screen to reduce material loss. They were then oven dried at 70°C to a consistent weight and weighed to calculate the biomass value as g DM/m<sup>2</sup>.

### *Plant samples analyses*

#### *Photosynthetic pigments*

Three fresh plant leaves were taken from each quadrat and mixed to form three composite samples for the examination of plant pigments. After extracting the chlorophyll and carotenoids from a 2 g fresh weight of leaves in 50% acetone under total darkness and storing the leaves overnight at 4°C, the samples were removed and subjected to spectrophotometric (CECIL CE 1021) measurements at three different

wave lengths: 453, 644, and 663 nm (Allen, 1989). The following formulas were used to determine each pigment fraction's concentration:

$$\text{Chlorophyll a} = (10.3 \times E_{663}) - (0.918 \times E_{644}) \quad (\text{Eq.1})$$

$$\text{Chlorophyll b} = (19.7 \times E_{644}) - (3.87 \times E_{663}) \quad (\text{Eq.2})$$

$$\text{Carotenoids} = (4.2 \times E_{453}) - (0.0264 \times \text{chl. a} + 0.426 \times \text{chl. b}) \quad (\text{Eq.3})$$

The values of pigments were then expressed as mg/g fresh weight.

### Macro- and micro-nutrients

Plant samples were prepared by extracting P, Ca, Mg, Na, and K using a mixed-acid digestion procedure ( $\text{HNO}_3\text{:HClO}_4\text{:HF}$ , 1:1:2, v:v:v) from 0.5 to 1 g of plant shoots. Atomic Absorption Spectrophotometer (Shimadzu AA-6200) was used to estimate Ca, Mg, Na, and K. A spectrophotometer (CECIL CE 1021) was used to determine the total P using the molybdenum blue method, and a CHN Elemental Analyzer (Yanako CHN Corder MT-5 and Auto Sampler MTA-3) using the indophenol blue method was utilized to determine the total N content (Allen, 1989; APHA, 1998). Furthermore, Atomic Absorption Spectrophotometer (Shimadzu AA-6200) was used to determine Zn, Fe, Cu, Cd, Mn, Ni, Pb, and Co in accordance with Allen's (1989) guidelines for plant analysis. The operational parameters and instrumental settings were modified in compliance with the manufacturer's recommendations.



**Figure 1.** Map of the study area indicating the locations of the three sampling sites (★)

### Organic nutrients

In order to determine the content of ash (%), a gram of the dry sample was heated at 550°C for 2 h in a muffle furnace, or until the weight remained constant. According to Allen (1989), crude lipids were extracted from *P. stratiotes* plant shoots using ether, and crude fibers were extracted using the Soxhlet method. N-concentration was multiplied by a factor of 6.25 to estimate the total protein content, in accordance with Adesogon et

al. (2000). Le Houérou's (1980) equation was used to evaluate the amount of carbohydrates (NFE):

$$\text{NFE (in \% dry matter)} = 100 - (\text{TP} + \text{CF} + \text{crude fat} + \text{ash}) \quad (\text{Eq.4})$$

Given that TP: total protein and CF = crude fiber. Digestible crude protein (DCP) was estimated according to the equation (Demarquilly and Weiss, 1970):

$$\text{DCP (in \% dry matter)} = 0.929 \times \text{TP (in \% dry matter)} - 3.52 \quad (\text{Eq.5})$$

Total digestible nutrients (TDN) were estimated according to the equation applied by Naga and El-Shazly (1971):

$$\text{TDN (in \% dry matter)} = 0.623 \times (100 + 1.25 \times \text{EE}) - (\text{CP} \times 0.72) \quad (\text{Eq.6})$$

where EE and CP are the ether extract (crude lipids) and crude protein percentages, respectively. Digestible energy (DE) was evaluated following this equation (NRC, 1984):

$$\begin{aligned} \text{DE (Mcal/kg)} = & 0.0504 \times \text{TP (\%)} + 0.077 \times \text{EE (\%)} + 0.02 \times \text{CF (\%)} \\ & + 0.000377 \times (\text{NFE})^2 (\%) + 0.011 \times (\text{NFE}) (\%) - 0.152 \end{aligned} \quad (\text{Eq.7})$$

Metabolized energy (ME) was calculated as (Garrett, 1980):

$$\text{ME} = 0.82 \times \text{DE} \quad (\text{Eq.8})$$

$$\text{Net energy (NE)} = 0.50 \times \text{ME (Le Houérou, 1980)} \quad (\text{Eq.9})$$

Moreover, gross energy (GE) was calculated following the equation (NRC, 1984):

$$\begin{aligned} \text{GE (Kcal 100/g)} = & 5.72 \times \text{TP (\%)} + 9.5 \times \text{EE (\%)} + 4.79 \times \text{CF (\%)} \\ & + 4.03 \times \text{NFE (\%)} \end{aligned} \quad (\text{Eq.10})$$

### **Water sampling and analysis**

At every sampling site, three water samples were collected during different seasons, near the sampling quadrats. The water samples were obtained by using plastic bottles to create composite samples that encompassed the water surface down to a depth of 50 cm. These samples were then transported to the laboratory, where they were combined to create a single composite sample for each site during each season. A levelling rod was used to assess the water's level in the field, and a 25-cm-diameter Secchi disc was used to measure the water's transparency. water temperature and dissolved oxygen (DO) were measured immediately, salinity (EC) was measured with an electric conductivity meter (60 Sensor Operating Instruction Corning); and pH values were measured with a pH meter (Model 9107 BN, ORION type). The Atomic Absorption Spectrophotometer (Shimadzu AA-6200) was used to determine the nutrient elements (P, Ca, Mg, Na, and K) and heavy metals (Zn, Fe, Cu, Cd, Mn, Ni, Pb, and Co) in accordance with the Standard Procedures for Examination of Water and Wastewater (Allen, 1989; APHA, 1998).

### Statistical analysis

The data underwent assessments to determine if it followed a normal distribution and exhibited homogeneity of variance. Shapiro-Wilk's W test and Levene's test were used for these evaluations, respectively. In cases where necessary, log transformation was applied. Analysis of variance (ANOVA) was conducted to assess variations in plant and water parameters across different seasons. All statistical analyses were performed using Prism version 9.0.

## Results

### Water characteristics

Water in the research region was analyzed physico-chemically, and the results showed seasonally significant differences in water pH, dissolved oxygen (DO), transparency, and temperature (WT), while there were no significant differences in water level (WL) and EC (Fig. 2). Winter was found to significantly have the lowest WT (23.18°C), the greatest DO (11.73 mg/L), and the highest water pH (8.14). In addition, spring had the highest water EC (29.31 mS/cm) and water level (104.00 cm), while summer had the lowest pH (7.13), EC (13.99 mS/cm), DO (3.17 mg/L), and water level (82.50 cm), but significantly the highest WT (31.59°C) and water transparency (24.83 cm). Moreover, seasonal significant fluctuations were found in all studied elements according to the examination of water inorganic nutrients. Springtime contributed significantly to the lowest values of water N and K (2.75 and 3.55 mg/L) but the greatest values of water Ca, Mg, and Na (49.67, 123.07, and 252.78 mg/L, respectively). Furthermore, water P and K values were significantly highest (12.86 and 19.59 mg/L) during the winter, while water N value was highest (28.75 mg/L) during autumn and P was lowest (11.07 mg/L) in the summer. It was discovered that the concentration of inorganic nutrients (mg/L) in the water was distributed as follows: Na > Mg > Ca > N > K > P.

Significant seasonal fluctuations were seen in the investigated heavy metal in the water analysis (Fig. 2). The results showed that while spring had the lowest Co (0.002 mg/L), it had the greatest concentrations of water Cd, Cu, Ni, Pb, and Zn (0.009, 0.014, 0.049, 0.338, and 0.038 mg/L, respectively). Furthermore, autumn had the highest water Fe and Mn contents (0.749 and 0.265 mg/L), while winter had the highest Co concentration (0.021 mg/L). Noteworthy, the sequence in which the concentration of heavy metals in water decreased was Fe > Pb > Mn > Ni > Zn > Co > Cu > Cd.

### Plant biomass

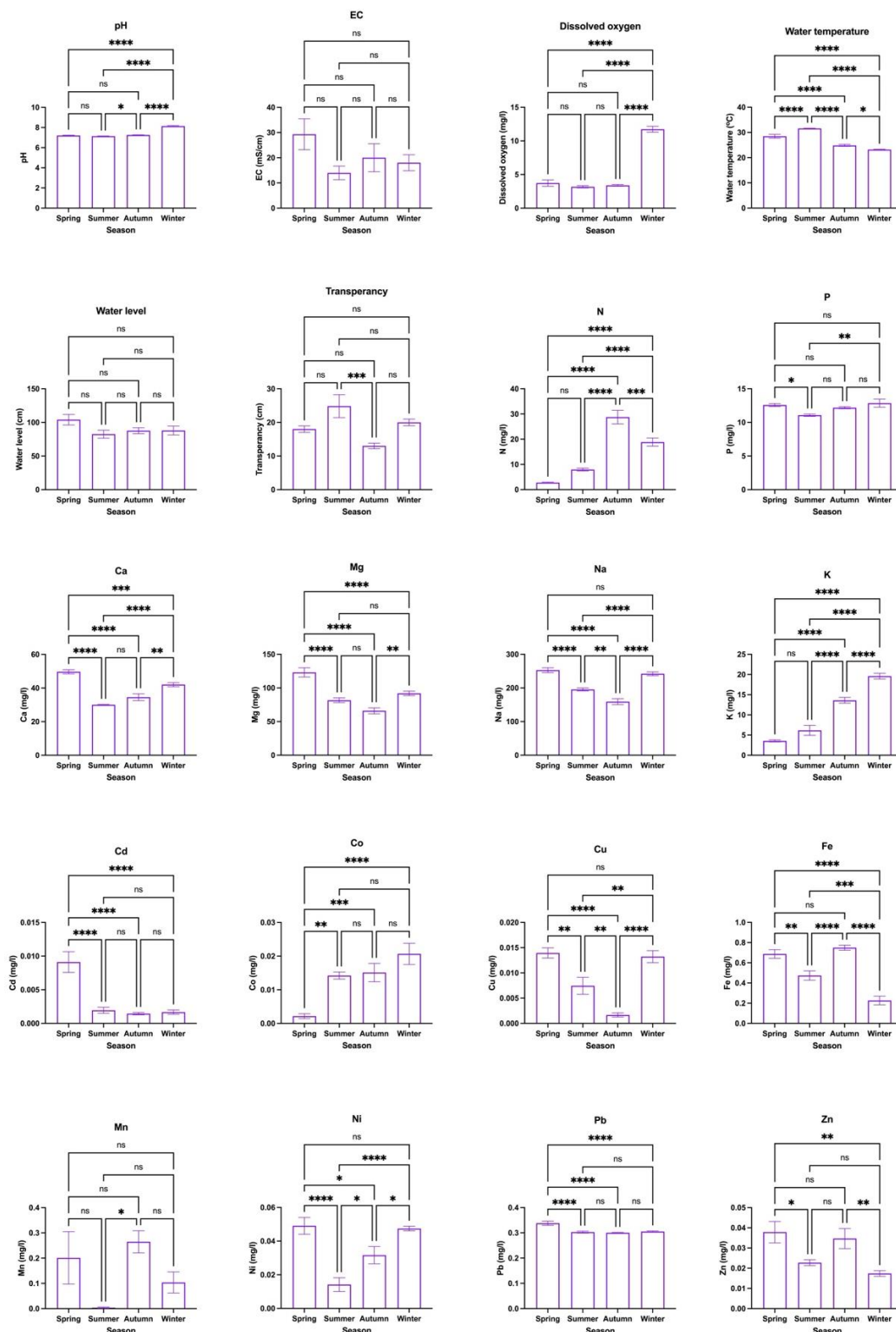
The biomass data of the above-water organs of *P. stratiotes* indicated significant seasonal fluctuations (Fig. 3). The highest plant biomass (314.52 g DM/m<sup>2</sup>) was recorded during spring, while significantly the lowest (35.38 g DM/m<sup>2</sup>) were recorded during winter.

### Plant analysis

#### Photosynthetic pigments

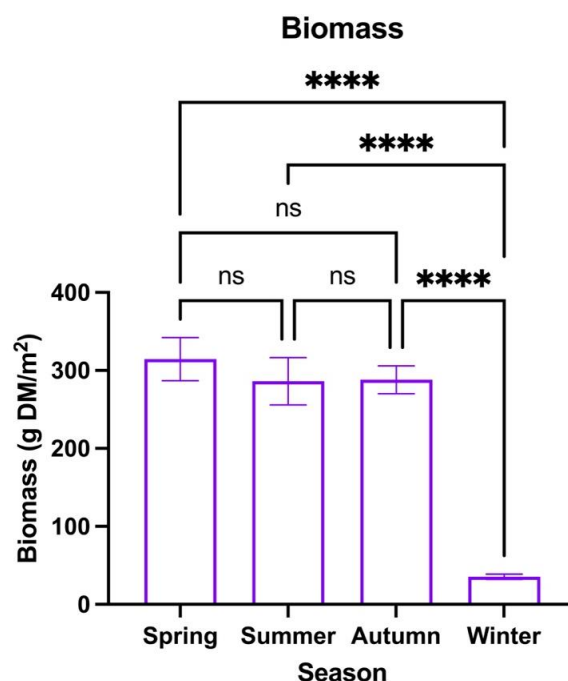
Chlorophyll a, chlorophyll b, and carotenoids showed substantial seasonal fluctuations in the pigment analysis data of *P. stratiotes* leaves (Fig. 4). Autumn yielded the greatest leaf chlorophyll a, chlorophyll b, and carotenoids concentration (0.399, 0.231, and 0.876 mg/g FW). On the other hand, the lowest carotenoids level

(0.394 mg/g FW) was recorded in summer and the lowest chlorophyll a and b contents (0.251 and 0.126 mg/g FW) were detected in the spring.

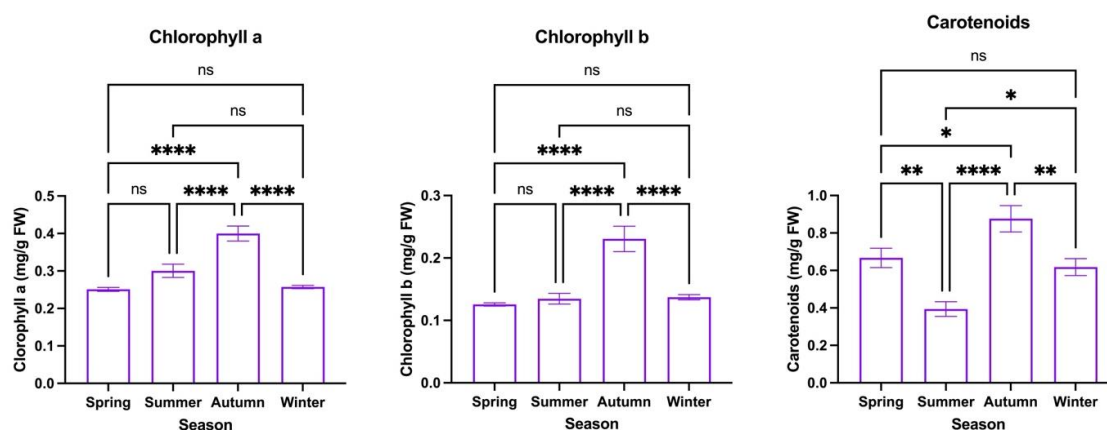


**Figure 2.** Seasonal variation (mean ± standard error, n = 18) in water characteristics of Al-Sero Drain supporting *Pistia stratiotes* population in the South Nile Delta, Egypt. One-way ANOVA comparisons were displayed on the graphs. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ )





**Figure 3.** Seasonal variation (mean  $\pm$  standard error,  $n = 18$ ) in biomass of *Pistia stratiotes* population grown in Al-Sero Drain (South Nile Delta, Egypt). One-way ANOVA comparisons were displayed on the graph. \*\*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ )

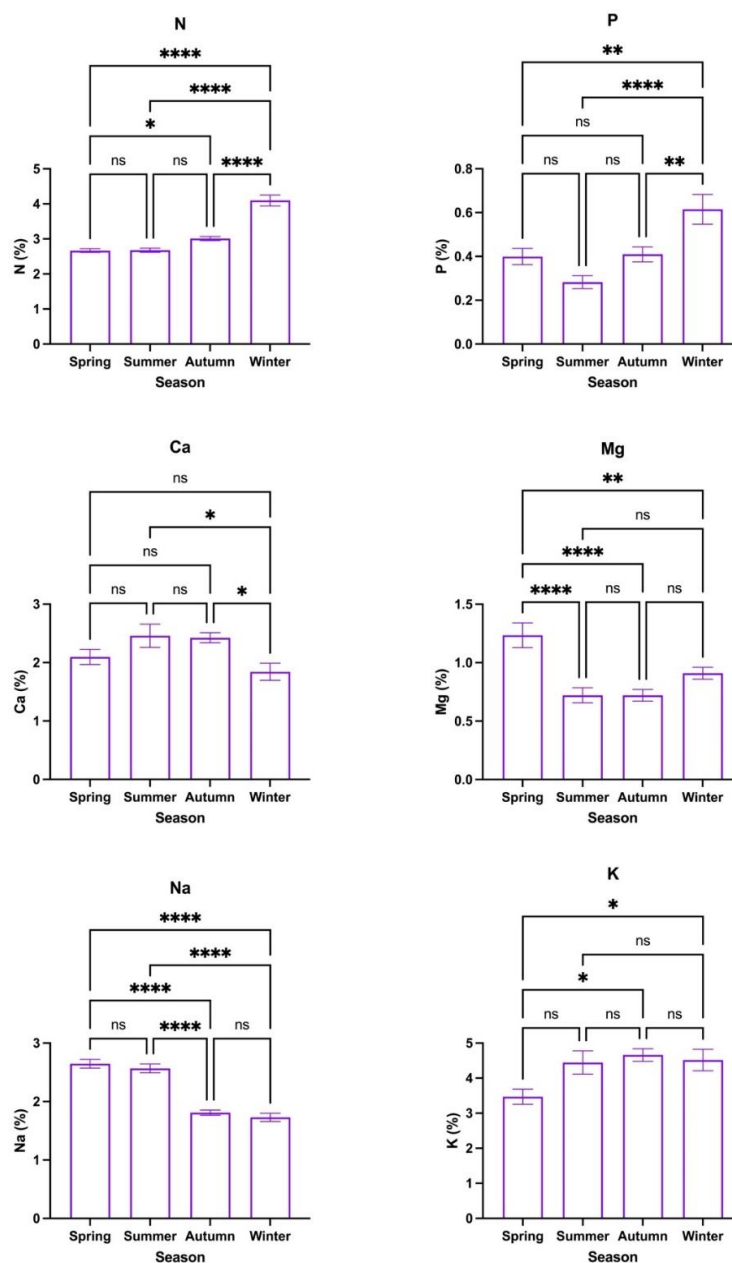


**Figure 4.** Seasonal variation (mean  $\pm$  standard error,  $n = 18$ ) in photosynthetic pigments of *Pistia stratiotes* population grown in Al-Sero Drain (South Nile Delta, Egypt). One-way ANOVA comparisons were displayed on the graphs. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ )

### Macro- and micro-nutrients

Seasonally significant fluctuations were found in the examined macro- and micro-nutrients in the above-water leaves of *P. stratiotes* (Fig. 5). Winter season significantly contributed to the highest plant N and P (4.09 and 0.62%, respectively) and the lowest Ca and Na (1.84 and 1.73%), while summer had the highest Ca (2.46%) and the lowest P and Mg (0.28 and 0.72%). Additionally, the highest concentration of Mg and Na (1.23 and 2.65%) were recorded during spring and K (4.66%) in autumn.



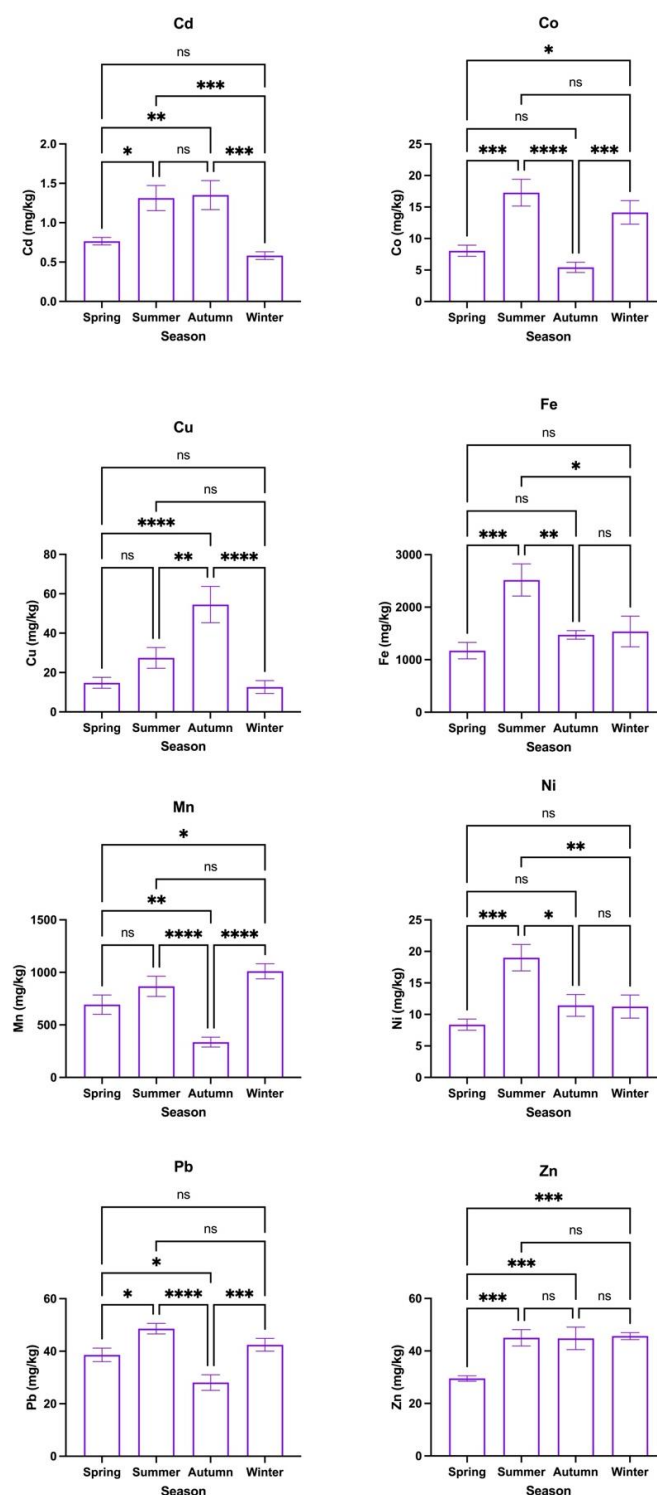


**Figure 5.** Seasonal variation (mean  $\pm$  standard error,  $n = 36$ ) in inorganic nutrients of *Pistia stratiotes* population grown in Al-Sero Drain (South Nile Delta, Egypt). One-way ANOVA comparisons were displayed on the graphs. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ )

### Heavy metals

All estimated heavy metals in the *P. stratiotes*' above-water shoots showed seasonally significant fluctuations (Fig. 6). The highest plant Co, Fe, Ni, and Pb values (17.28, 2517.36, 18.99, and 48.57 mg/kg, respectively) were shown to be substantially the highest in the summer. In addition, autumn produced the lowest amounts of shoot Co, Mn, and Pb (5.43, 336.23, and 28.06 mg/kg), and the highest amounts of plant Cd and Cu (1.35 and 54.51 mg/kg). Furthermore, the lowest Cd and Cu (0.58 and 12.62 mg/kg) were recorded by the plant with the highest Mn and Zn (1010.13 and

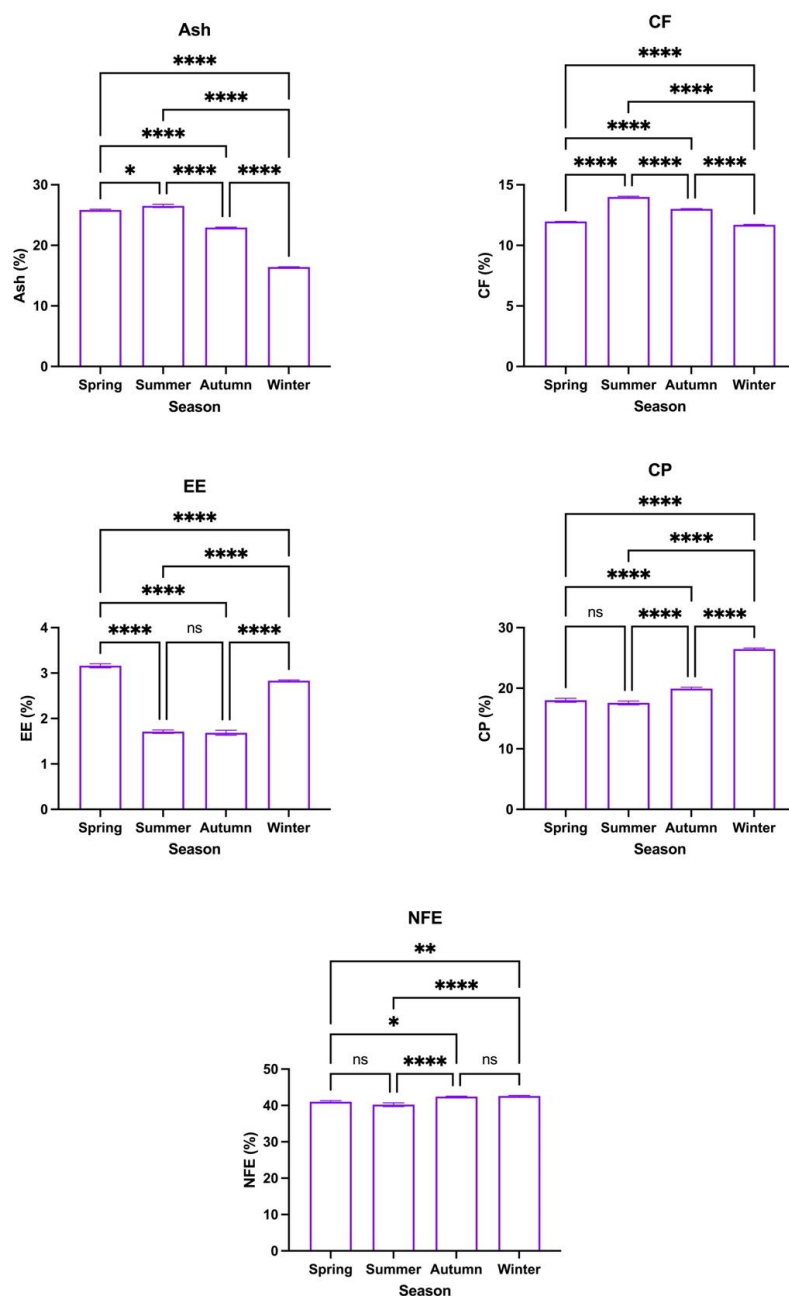
45.65 mg/kg) throughout the winter. It was determined that the concentrations of heavy metals that accumulated in *P. stratiotes*' shoot were in the following order: Fe > Mn > Zn > Pb > Cu > Ni > Co > Cd.



**Figure 6.** Seasonal variation (mean ± standard error, n = 36) in heavy metals of *Pistia stratiotes* population grown in Al-Sero Drain (South Nile Delta, Egypt). One-way ANOVA comparisons were displayed on the graphs. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ )

## Organic nutrients

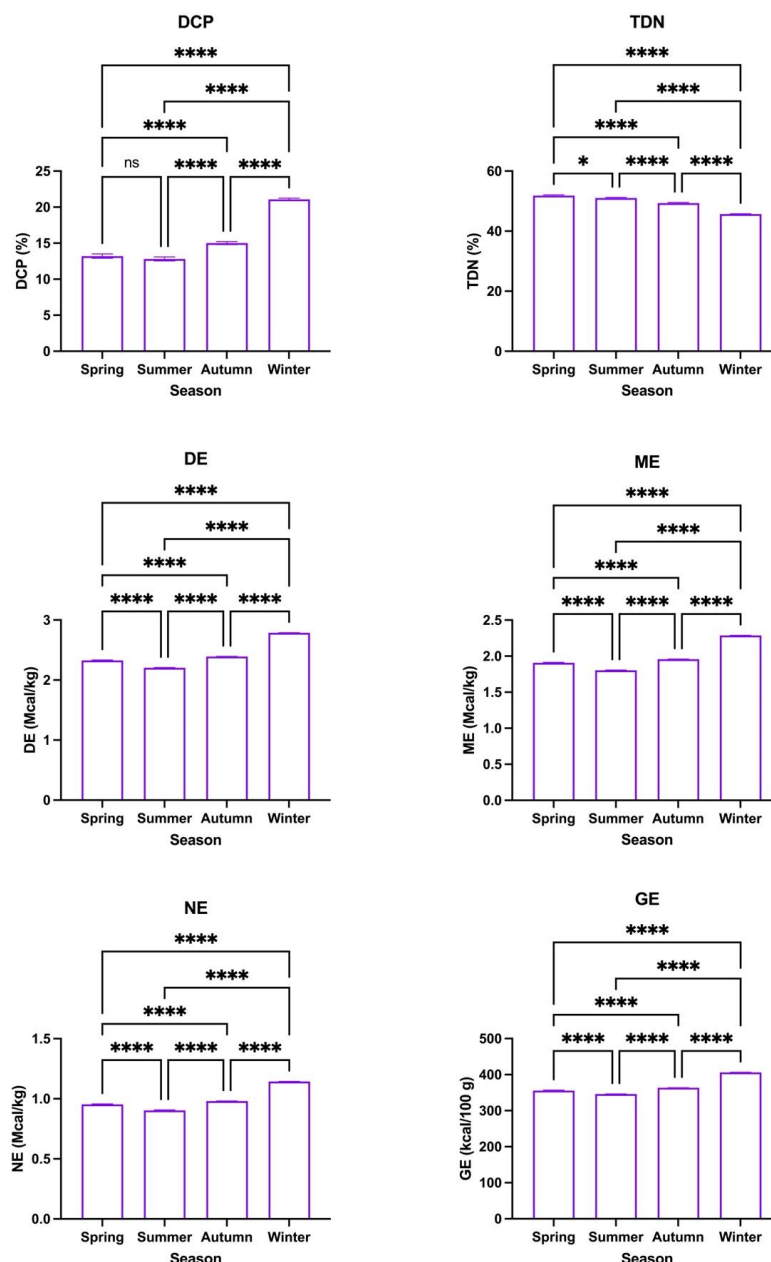
Seasonally significant fluctuations in the examined organic nutritional elements of *P. stratiotes*' above-water tissues were found (Fig. 7). Summer was found to have the lowest levels of CP and NFE (17.58 and 40.21%, respectively), but the greatest values of ash content and CF (26.51 and 13.99%). In addition, wintertime recorded the highest levels of shoot CP and NFE (26.47 and 42.59%) and the lowest levels of ash and CF (16.41 and 11.70%, respectively).



**Figure 7.** Seasonal variation (mean  $\pm$  standard error,  $n = 18$ ) in ash and organic nutrients of *Pistia stratiotes* population grown in Al-Sero Drain (South Nile Delta, Egypt). One-way ANOVA comparisons were displayed on the graphs. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ ); CF: crude fiber; EE: ether extract; CP: crude protein; NFE: nitrogen free extract (carbohydrate content)

## Nutritional value

Seasonally significant fluctuations in the nutritive qualities of *P. stratiotes* shoots were observed (Fig. 8). Winter was clearly responsible for the lowest plant TDN (45.66%) and the greatest levels of DCP, DE, ME, NE, and GE (21.07%, 2.79 Mcal/kg, 2.29 Mcal/kg, 1.14 Mcal/kg and 405.97 Kcal/100 g, respectively). Summertime, however, produced the lowest DCP, DE, ME, NE, and GE (12.81%, 2.20 Mcal/kg, 1.80 Mcal/Kg, 0.90 Mcal/kg, and 345.87 Kcal/100 g). Furthermore, springtime recorded the greatest TDN value (51.84%).



**Figure 8.** Seasonal variation (mean  $\pm$  standard error,  $n = 18$ ) in nutritive value of *Pistia stratiotes* population grown in Al-Sero Drain (South Nile Delta, Egypt). One-way ANOVA comparisons were displayed on the graphs. \* $p < 0.05$ ; \*\*\*\* $p < 0.0001$ ; ns: not significant (i.e.,  $p > 0.05$ ); DCP: digestible crude protein; TDN: total digestible nutrients; DE: digestible energy; ME: metabolized energy; NE: net energy; GE: gross energy

## Discussion

Water ecosystems have certain patterns of physical and chemical characteristics, which are determined largely by the climatic, geomorphologic, and geochemical conditions prevailing in the drainage basin and the underlying aquifer (Eid et al., 2020). Various attributes of water are correlated positively with aquatic plant distribution; these include alkalinity, salinity and phosphate concentration (Khedr and Serag, 1998). Winter was found to significantly have the greatest pH and DO (8.14 and 11.73 mg/L), while summer had the lowest (7.13 and 3.17 mg/L). These fluctuations may be attributed to the photosynthetic activities of phytoplankton and aquatic plants, and respiration of animals and plants as well as variations in temperature (Galal and Farahat, 2015). Springtime contributed significantly to the greatest contents of water Ca, Mg, and Na, while winter had the highest P and K and autumn had the highest N content, indicating that eutrophication increased during cold rather than hot seasons in accordance with Galal et al. (2019) and Ali et al. (2021) on the same species. Dakhil et al. (2016) attributed the high nutrient contents in Al-Sero Drain to domestic and agricultural drainage from the adjacent area. The nutrient elements' concentration in the water fell in the order: Na > Mg > Ca > N > K > P, which is similar to that recorded in Lake Burullus (Eid et al., 2020) and in the main watercourses in Greater Cairo, Egypt (Ghazi et al., 2019). Furthermore, the heavy metal concentrations in water of Al-Sero Drain did not exceed the standard levels of the US Environmental Protection Agency (EPA, 2006); and the Egyptian standards according to Egyptian Environmental Affairs Agency (EEAA, 2008); as well as the toxic levels reported by Allen (1989).

The estimation of biomass is a pre-requisite for the study of dry matter flow and plant functioning (Galal and Farahat, 2015). *P. stratiotes* reached the maximum above-water biomass during spring, which has a significant survival value because the plant can avoid the competition for light and nutrients with the other aquatic plants, which have their peak growth in summer (*Typha domingensis* in July, Eid et al., 2012; *Phragmites australis* in August, Eid et al., 2010). According to Galal et al. (2019), variations in *P. stratiotes* biomass can be attributed to nutrient availability and environmental factors, such as habitat conditions, which vary with latitude, such as temperature, day length, solar radiation, and growing season length. The maximum biomass recorded in the present study was 314.52 gDM/m<sup>2</sup>, which was lower than 358.4 gDM/m<sup>2</sup> recorded by Ali et al. (2021) in Egyptian drains, and 700.0 and 430.0 gDM/m<sup>2</sup> recorded by Hall and Okali (1974) in Ghana and Dewalds and Lounibos (1990) in Florida, respectively. The plant biomass showed notable reduction in winter season characterized by high concentrations of water heavy metals, particularly Cu, Co and Ni. and this is agreed with Hadi et al. (2014), who attributed the biomass reduction to heavy metals present in water. It was reported that heavy metals such as Cu, Co and Cr (Ghazi et al., 2019), Ni and Cd (Batoool et al., 2014) inhibit plant biomass and productivity by the reducing photosynthetic pigments and photosynthetic activity. In the same context, the highest values of chlorophyll a, chlorophyll b, and carotenoids were recorded during autumn. Leaves chlorophyll plays a very important role in absorbing light to carry out photosynthesis, the more the amount of chlorophyll in the leaves, the photosynthesis process will run well so that plants can produce photosynthates in large quantities (Makaruku et al., 2023). Chlorophyll fluorescence parameters were found to be strongly correlated with whole-plant growth and increases its biomass (Wang et al., 2024). The decrease in chlorophyll content in plant leaves under adverse stress directly affects plant photo-synthesis, inhibiting plant growth. Furthermore, our investigation revealed a

collapse phase of *P. stratiotes* biomass during winter and explosive growth during the remaining seasons (with no significant differences) in accordance with the studies of Hall and Okali (1974), Galal et al. (2018), and Ali et al. (2021) on the same species. According to Galal et al. (2023) and Eid et al. (2019), low air temperature in winter is an important limiting factor determining the growth of floating macrophytes.

Aquatic macrophytes play a crucial role by creating a favorable environment for a variety of complex chemical, biological, and physical processes that contribute to the removal and degradation of nutrients (Abdallah et al., 2020). It is known that nutrients N, P and K are primary macronutrients that are needed more by plants than other nutrients (Makaruku et al., 2023). Winter season significantly contributed to the highest plant N and P, while summer had the highest Ca, spring had the highest Mg and Na, autumn had the highest K. These results coincided with Ali et al. (2021) and Galal et al. (2019) on the same species. The highest plant N and P concentration associated with lower biomass may be attributed to the utilization of these nutrients in building up plant biomass in the growing season during summer, while in the winter season they can be stored in the tissues. As reported by Irfan (2014), the increase of plant N enhances plant biomass till maturity, while further increase only enhance the nitrogen storage in plant tissues. Therefore, N cannot be assigned as the single factor for plant growth and biomass yield. It is worth to note that the order of inorganic nutrients concentration (%) in *P. stratiotes* shoot was  $K > N > Ca > Na > Mg > P$  in line with Ali et al. (2021) and Dakhil et al. (2016) on *P. stratiotes*. According to Ali et al. (2021), the nutrient storage capacity of *P. stratiotes* was generally high respecting the nutrient loadings in the surface water of Al-Sero Drain, which is a potential factor affecting the nutrient storages of the target plant. It is worth noting that summer is the ideal season for harvesting *P. stratiotes* for remediating the maximum nutrient content from aquatic ecosystems.

The high accumulation potential of *P. stratiotes* to trace metals was reported by many authors (Thilakar et al., 2012; Galal and Farahat, 2015; Galal et al., 2018; Irmawanty et al., 2023). The highest plant Co, Fe, Ni, and Pb values were shown to be substantially the highest in the summer, while the highest Cd and Cu were attained in autumn. Similar results were reported by Galal et al. (2018) on the same species. The high concentrations of these heavy metals were associated with high biomass accumulation, which indicated that summer and autumn are ideal seasons for eliminating and monitoring pollution in aquatic ecosystems through harvesting *P. stratiotes* biomass. Moreover, *P. stratiotes* accumulated Fe and Mn concentrations that exceeded, 1000 mg/kg in its harvestable shoots, consequently it is considered as a hyperaccumulator for these metals (Nanda and Abraham, 2013; Irmawanty et al., 2023). According to Thilakar et al. (2012), *P. stratiotes* is a natural hyperaccumulator of many trace and toxic metals, and can be effectively employed in phytoremediation of polluted wetlands. Moreover, this plant had the ability to accumulate high concentrations of Pb, Cu, Ni and Co in its shoot, particularly in summer, which exceed the safe concentrations in normal plants (Allen, 1989; Nagajyoti et al., 2010; Chiroma et al., 2014). These results were in line with Irmawanty et al. (2023), who reported that *P. stratiotes* has the potential to accumulate heavy metals, such as Cu, Ni, Pb, and Zn in their above-water tissues. Therefore, *P. stratiotes* can be considered a potential candidate for heavy metals phytoremediation without negative impacts since it can counteract any effect induced by the pollutants through antioxidant and non-antioxidant responses (Ugya and Meguellati, 2023). In line with Galal et al. (2018), the concentrations of heavy metals that accumulated in *P. stratiotes*' shoot were in the following order:  $Fe > Mn > Zn > Pb > Cu > Ni > Co > Cd$ .

Total protein and crude fibers are regarded as indicators of the nutritional quality of the diet for grazing animals (Heneidy, 2002). Summer was found to have the highest levels of ash content and CF, while wintertime recorded the highest levels of shoot CP and NFE, besides the greatest levels of DCP, DE, ME, NE, and GE. Based on the recommendation of Galal et al. (2022) and Geurts et al. (2020), plants should be harvested when the protein content is highest for high forage quality. Consequently, *P. stratiotes* should be harvested during winter to be used as fodder, where its above-water parts had the highest protein content (26.47%) and relatively high CF (11.69%) associated with the lowest and safe concentrations of toxic metals. These results were in line with Galal et al. (2022) on the emergent sedge *Cyperus alopecuroides*. Besides, Makaruku et al. (2023) reported that *P. stratiotes* contains high fiber, protein, nutritional value, and biomass contents to be used as a suitable animal fodder. Moreover, *P. stratiotes* contained a high level of protein that exceeded the minimum protein content (6%-12%) required for animal feed (Shaltout et al., 2016) and the contents (2.7%-13.4%) required for some rough fodder (Shoukry, 1992) as well as the value of *Trifolium alexandrinum* (16.2% Chauhan et al., 1980), *Phragmites australis* (6.7% El-Kady, 2002), *Persicaria salicifolia* (10.2%), *Eichhornia crassipes* (10.3%), *Ceratophyllum demersum* (14.1%) and *Azolla filiculoides* (17.2%) (Shaltout et al., 2010, 2012, 2014). Moreover, the ether extract (crude lipids) content lies within the scale (0.5%-3.1%) of some rough fodder (Shoukry, 1992). According to El-Beheiry (2009), the TDN is an appropriate measure for the animals' feed energy. In the present study, the highest TDN content (51.84%) of the above water shoots of *P. stratiotes* was comparable to the diet requirements (50.0%) of breeding cattle (NRC, 1984). Besides, the mean value of DE (up to 2.79 Mcal/kg) save the amount (2.70 Mcal/kg) required by sheep (NRC, 1985), while the ME (2.29 Mcal/kg) approximated the breeding cattle and sheep requirements (NRC, 1984, 1985). It is also important to be taken into consideration the potential of this species to accumulate heavy metal pollutants in its tissues. Therefore, care should be taken when harvesting *P. stratiotes* during summer and autumn to be used as animal fodder, where heavy metals concentration was toxic. However, the plant shoots can be used as fodder in winter as the concentrations of heavy metals did not exceed the permissible levels (Allen, 1989; Nagajyoti et al., 2010; Chiroma et al., 2014). It is worth noting that the nutritional values of *P. stratiotes* above-water shoots lie within the range of nutritive value of sheep (NRC, 1975), goat (NRC, 1981), dairy cattle (NRC, 1978) and beef cattle (NRC, 1984).

## Conclusion

*P. stratiotes* reached the maximum above-water biomass during spring, while the lowest was recorded during winter associated with the highest plant contents of N and P. The highest plant Co, Fe, Ni, and Pb values were shown to be substantially the highest in the summer, while the highest Cd and Cu were attained in autumn. Wintertime recorded the highest levels of shoot CP and NFE, besides the greatest levels of DCP, DE, ME, NE, and GE. the nutritional values of *P. stratiotes* above-water shoots lie within the range of nutritive value of sheep, goat, dairy cattle, and beef cattle. Finally, summer is the ideal season for harvesting *P. stratiotes* for remediating the maximum nutrient and heavy metal contents from aquatic ecosystems, while winter is suitable for utilizing the harvested plants for animal feeding.



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