IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 14, Issue 8 Ser. III (August 2020), PP 47-55
www.iosrjournals.org

Comparative analysis of the response of three macrophytes under different ammonium concentrations: *insights into the role of growth forms.*

Annah Timinah Nasimiyu[1,2], Wyckliffe Ayoma Ochieng[1,2]&Cornelius M. Kyalo[2]

[1]*Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, Hubei, P.R China.*
[2]*University of Chinese Academy of Sciences, 100049, Beijing, PR China*.

Abstract:
Background*:Ammonium (NH_4^+) is an inorganic form of nitrogen that is necessary for the growth and development of aquatic plants. However, NH_4^+ is toxic when its concentrations exceed plant requirement and the extent of its toxicity is poorly understood.*
Materials and Methods*: This study sought to compare the responses of three macrophytes (Ceratophyllum demersum, Vallisneria natans, and Potamogeton maackianus) to varying NH_4^+ concentrations with respect to their varying growth forms, under four different Ammonium (NH_4^+) concentrations (control – 0mg/L, low – 0.1mg/L, medium – 15mg/L and high – 50mg/L). Ammonium stress was assessed using the parameters; Chlorophyll a and b, Protein content, Chlorophyll fluorescence (Fv/Fm), Free amino acids (FAA), and Soluble sugars.*
Results*: It was observed that FAA and Protein content increased significantly ($P<0.05$) with increased Ammonium concentrations regardless of the species. However, both Chlorophyll content and Soluble sugars exhibited a general decrease with increased NH_4^+ concentrations, except for C. demersum in which the Chlorophyll content increased. Generally, there were varied responses of macrophytes to elevated ammonium concentrations aligned with growth forms, with the canopy-forming P. maackianus and rootless C. demersum exhibiting significant tolerance while the rosette-forming V. natans was sensitive. This is attributed to differences in their ability to intercept light whereby the rosette-forming V. natans is short and prone to low light conditions which lead to insufficient carbohydrates production, a requirement to detoxify ammonium toxicity.*
Conclusion: *In conclusion, P. maackianus and C. demersum may feasibly be preferred in the practical restoration of shallow aquatic ecosystems experiencing high NH_4^+ toxicity.*
Key Words*: Ammonium toxicity, Growth forms, Macrophytes, Phytoremediation, Restoration.*

Date of Submission: 12-08-2020 Date of Acceptance: 28-08-2020

I. Introduction

Macrophytes are large aquatic plants that are categorized into four primary life forms (emergent, submerged, floating and free-floating) (Chambers et al. 2008)and are essential in aquatic ecosystems in providing habitats for fish, aquatic invertebrates and birds (Dvoiaki et al. 1982, Martin and Valentine 2012). Studying aquatic macrophytes is vital in understanding the ecological status of aquatic ecosystems, including the characterization of water quality (Ghavzan et al. 2006). Therefore, macrophytes have increasingly attracted research attention, specifically based on their ability to sequestrate many pollutants and provide aeration services in contaminated water bodies (Boyd 1970). Unlike conventional clean-up techniques, macrophytes perform natural phytoremediation processes within aquatic systems in a cost-effective and environmentally sustainable manner (Shah et al. 2014). This is due to their fast growth which translates to huge biomass, the capability to accumulate large amounts of diverse pollutants in nature, easier and rapid propagation, and abundant root system that increases surface area for absorption of contaminants (Jahnke et al. 1991, Tripathi and Shukla 1991).

Despite the important role played by macrophytes, they are declining globally due to exposure to high eutrophication in aquatic systems. Eutrophication is largely attributed to the presence of excessive macro-nutrients, primarily nitrogen and phosphorus (O'Hare et al. 2018), in water bodies.Nitrogen is a vital nutrient for plant growth and yield (Miller and Cramer 2005), including the provision of building blocks for the synthesis of biomolecules such as proteins and nucleic acids (Masclaux-Daubresse et al. 2010).In addition to being a

nutrient, nitrogen is a systematic signal that regulates genome-wide gene expression in leaf expansion, root morphology, seed dormancy, and floral induction (Hachiya and Sakakibara 2017). Nitrogen uptake by aquatic flora is mainly through either inorganic (nitrate and ammonium) or organic (e.g., urea, amino acids, peptides) forms (Miller and Cramer 2005, Liu and von Wiren 2017). As major components of nitrogen, Nitrate (NO_3^-) and Ammonium (NH_4^+) are crucial for the growth and development of aquatic plants in freshwater ecosystems. However, $NH_4^+$is the primary source of inorganic nitrogen for plants in most natural and agricultural ecosystems (Song et al. 2017). The higher preference of NH_4^+ is attributed to its lower energy requirement during assimilation as compared to the NO_3^-(Sun et al. 2014). Since NH_4^+ does not require reduction before uptake into plant cells, its uptake by macrophytes is often higher as compared to NO_3^-(Brix et al. 2002, Tylova-Munzarova et al. 2005, Fang et al. 2007).

However, despite ammonium being an important source of Nitrogen, a crucial component in plants which apart from other roles encourages the uptake and utilization of other nutrients including K and P (Leghari et al. 2016), it has diverse effects on plant functioning. Ammonium is beneficial to plants at lower to moderate concentration since it promotes their growth and development (Li et al. 2007, Qin et al. 2013, Ellis et al. 2015, Liu and von Wiren 2017). Consequently, NH_4^+ enrichment in an aquatic environment influences carbon and nitrogen metabolism, especially in eutrophic conditions where carbohydrate production could be limited by low irradiance (Cao et al. 2009, Apudo et al. 2016).However, at high concentrations, it becomes toxic by triggering oxidative stress, causes internal carbon-nitrogen imbalance and inhibiting photosynthesis in aquatic plants (Smolders et al. 2000, Britto and Kronzucker 2002, Cao et al. 2004, Li et al. 2007, Neuberg et al. 2010). A case study on aquatic plant toxicology (Shengqi et al. 2012) illustrated that high ammonium causes plant stress, resulting in decreased chlorophyll content and metabolites (soluble sugars and methane dicarboxylic aldehyde (MDA)).

The phytoremediation potential of macrophyte species is majorly determined by the rate of ammonium uptake and assimilation into the plant (Greenway 2003). Further, the rate of NH_4^+ uptake by plants is influenced by the plant type, age, as younger plants have been found to contain more nitrogen content (Kinidi and Salleh 2017). Notwithstanding, external factors such as salinity and temperature influence the performance of NH_4^+ uptake by plants. For instance, it was reported that macrophytes show neither growth nor pollutant removal (including NH_4^+) at temperatures below 10^0C (Shah et al. 2015). However, the temperatures between 15^0C and 38^0C were found to exhibit high macrophytes' growth and therefore reported the most suitable for the treatment of municipal wastewater. Although plants are generally affected by ammonium stress, previous studies report that the degree of stress varies with some species exhibiting tolerance while others display great sensitivity to ammonium (Marino and Moran 2019). For instance, the species *P. lucens* and *V. natans* (Wang et al. 2008, Zhu et al. 2014) exhibit sensitivity to high ammonium concentrations while *Myriophylum spicatum* and *C. demersum* are reported to exhibit some tolerance (Xian et al. 2020).

Increased NH_4^+ concentration in water bodies has led to the degradation of water quality, lowered the ecological integrity of most aquatic systems (Cadmus et al. 2016) and contributed to the decline of submerged macrophytes within eutrophic lakes globally (Britto and Kronzucker 2002, Cao et al. 2007). Since most urban wastewater contains high concentrations of nutrients, primarily Nitrogen and Phosphorus, ammonium-tolerant macrophytes could be used as a natural treatment through incorporation into wetland restoration designs (Shah et al. 2014). Therefore, to conclusively understand the potential of macrophytes in phytoremediation, investigation of more physiological responses to aquatic pollutants is crucial to inform wetland restoration programs. The present study compared the responses of three macrophytes to varying NH_4^+ concentrations with respect to their varying growth forms, seeking to test the hypothesis that the canopy-forming and rootless forms exhibit tolerance to high ammonium concentrations than rosette-forming species.

II. Material and Methods

Plant materials: This study treated three macrophytes; *P. maackianus*, *V. natans*, and *C. demersum* (Fig. 1) to four different ammonium concentrations to test their response and tolerance capability. The three species were selected for experimental design because of their diverse distribution in mesotrophic and eutrophic lakes around the world, their varied life forms (*C. demersum* – rootless, *V. natans* – rosette type and *P. maackianus* – canopy-forming) and their perceived capacity for ecological restoration of degraded lakes (Liu et al. 2019). The plant materials were collected from Lake Erhai Yunnan-Guizhou, China. Lake Erhai is a eutrophic subtropical plateau lake (Location: 25°36′–25°58′N, 100°06′–100°18′E) with a surface area of 249.76 km2, an elevation of 1974 m, maximum depth of 20.7 m and a mean depth of 10.5 m (Wang et al. 2015; Lin et al. 2016).

Ceratophyllum demersum *Vallisneria natans* *Potamogeton maackianus*

Fig. 1 Images of the three macrophyte species used in this study.

Experimental design: The plants collected from Erhai Lake were rinsed and acclimatized in distilled water for 24 hours. Later, 10 g of healthy plants were cultured in 1.25 L with different ammonium treatments (0 mg/L, 0.1 mg/L, 15 mg/L and 50 mg/L), with each treatment replicated five times for each species. In the treatments, the plants were cultured in Hoagland solution. The total sixty (60) pots (1.25 L) were kept in a growth cabinet in a control room at day/night temperatures of 20°C/15°C and illuminated with fluorescence tubes on a 14-hr light/10-hr dark photo-period. Several parameters (Free Amino Acids - FAA, Chlorophyll fluorescence - Fv/Fm, Protein Content, Soluble sugars, Chlorophyll *a* and *b*, Total Nitrogen (TN), and Ammonia) were determined in the lab from plants that were obtained randomly from each culture pots. Environmental indices: temperature, atmospheric pressure (AP), total dissolved solids (TDS), pH, salinity and dissolved oxygen (DO) in the culture pots were measured using YSI professional plus hand-held multi-parameter meter (Table 1).

Table 1 Physico-chemical conditions in the treatment containers

Treatments Tanks ID	Temp. (^{0}C)	AP mmHg	DO mgL^{-1}	C uScm^{-1}	TDS	PH mgL^{-1}	Salinity (ppt)
T0	26.7±0.6	615.2±0.08	6.0±0.4	316.2±8.9	175.4±11.0	7.8±0.96	0.15±0.004
T0.1	27.5±0.6	616.6±0.68	5.5±0.6	293.3±16.1	189.3±11.8	7.9±1.08	0.14±0.009
T15	27.7±0.5	615.1±0.06	6.9±0.5	392.04±34.9	203.3±25.2	7.8±0.81	0.18±0.02
T50	27.4±0.7	615.5±0.11	6.5±0.6	788.6±21.3	124.8±36.9	7.5±0.53	0.37±0.007

Note. T0, T0.1, T15, T50 represents ammonium concentration at 0 mg/L, 0.1 mg/L, 15 mg/L, and 50 mg/L respectively. AP, atmospheric pressure; DO, dissolved oxygen; C, conductivity; TDS, total dissolved solids

Measurement of Chlorophyll *a* and *b* and chlorophyll fluorescence (Fv/Fm): After ammonium dosing experiments, 0.1 g fresh weight of the plantlets was homogenized in 3 ml of 90% Ethanol for approximately 24 hrs in the dark to extract photosynthetic pigments. Chlorophyll *a* and *b* concentrations were determined by spectrophotometer (722 Vis Spectrophotometer, JINGHUA, Shanghai, China) at 470nm, 649nm and 665nm absorbance and calculated according to (Wellburn and Lichtenthaler 1984). Chlorophyll fluorescence is mostly used as an indicator of the maximum quantum yield of photosystem II (PSII) photochemistry. After each day of exposure to the four ammonium concentrations and before the measurement of chlorophyll fluorescence, plants were selected randomly from growth containers and subjected to 15 minutes of dark adaptation phase. The photosynthetic performance was determined by measuring the chlorophyll fluorescence variable using pulse-modulated fluorometer PAM 2100 (Walz, Germany) machine according to the method ofHuang et al. (2017).

Measurement of Protein content, Soluble sugars and Free Amino Acids: Plant material (0.5 g) were used to extract soluble protein as described by Sarasketa et al. (2016). Quantification was done using a Bradford-base dye-binding assay (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as standard. Finally, a spectrometer was used to determine the protein contents in the sample through color quantification as an absorbance reading in spectrometry. Frozen leaves (0.5 g) were homogenized in 10 ml of 90% ethanol and

kept in an incubator (24^0C) for 1 hr. The homogenates were re-extracted until clear and the supernatants used in the determination of soluble sugars using the phenol-sulfuric method (Dubois et al. 1951; Nielsen 2010). FAA were determined by the rapid colorimetry method using approximately 0.5 g of fresh weight leaves, as described by Bates et al. (1973).

Measurement of Total Nitrogen (TN) and Ammonium (NH_4^+): The TN was determined spectrophotometrically after digestion with $K_2S_20_8$ solution as described by (Huang et al. 2017). Ammonium was determined with Nessler's reagent calorimetrically by a method of ISO7150-1 (1984).

Statistical analysis: IBM SPSS software version 20 (IBM-Corporation 2011) was used for statistical analyses, with all data undergoing normality and homogeneity tests before the analyses. One-way and two-way ANOVA with Tukey Honest Significant Differences (HSD) tests were used to analyze the effects of increasing ammonium concentration on the physiological indexes (Chlorophyll *a* and *b*, free amino acids (FAA), soluble sugars, protein content and chlorophyll fluorescence (Fv/Fm)). Further, one-way ANOVA was performed to examine the variation in the Physico-chemical conditions in the culture flasks. The Statistical significance level was set at $p<0.05$. Post Hoc tests were run as a mean separation procedure.

III. Resultand discussion

Effects of ammonium concentration on Chlorophyll content and Soluble sugars

Several studies have evaluated the ammonium toxicity levels and their effects on the survival of macrophyte species. Though, the general understanding is that ammonium is only detrimental to plants at high levels, the ultimate effects depend on plant specificity and uniqueness of the prevailing conditions. In the present study, plant species and ammonium dosing concentrations had influence on the response of all species (Table 2), with evident significant effects on total chlorophyll *a* and *b*, Fv/Fm and Soluble sugars parameters. Previous observations have reported stress in aquatic macrophytes like *Myriophyllum* and *Egeria densa*(Wang et al. 2008; Saunkaew et al. 2011; Shengqi et al. 2012) with increased ammonium concentrations, tampering with total chlorophyll contents and as a result deterring the process of photosynthesis. Our results exhibit similarity in which remarkable variation of total chlorophyll concentrations amongst the three aquatic macrophytes was observed. For *V. natans* and *P. maackianus*, the chlorophyll concentration decreased significantly (p<0.05) when subjected to increased levels of ammonium concentrations (15 mg/L and 50 mg/L) in the water column, although the general chlorophyll content for *P. maackianus*was higher than the other species. Contrary, though there were significant differences ($P<0.05$) at Ck0, 0 mg/L and 0.1 mg/L of ammonium concentrations conditions for *C. demersum*, increased levels of ammonium concentrations in the water column led to a decrease of total chlorophyll content from 0 mg/L to 0.1 mg/L after which the content began to increase (Fig. 2A). Further, *V. natans* exhibited much decrease than the other two species, supporting its perceived sensitivity to ammonium in previous studies (Wang et al. 2008; Zhu et al. 2014; Yu et al. 2018). Therefore, with chlorophyll content parameter in this study being used as a primary determinant of ammonium tolerance, our hypothesis that the canopy-forming and rootless macrophytes are more tolerant than rosette-forming with respect to ammonium stress is confirmed.

A similar decreasing trend was observed for the Soluble sugars at ammonium concentrations>15 mg/L in all species (Fig. 2C), an expected result due to excessive use of carbohydrates to provide energy for detoxification reaction. This decrease in soluble sugars has been reported previously (Cao et al. 2004; Cao et al. 2011; Apudo et al. 2016) and has been attributed to the surplus enrichment of ammonium in the water column which in turn affects the C-N metabolism of submerged macrophytes. Although the decrease of soluble sugars is a negative impact, it's an expected result since the sugars are utilized in the detoxification process of the ammonium accumulation in the plant leaf cells (Yu et al. 2018). The detoxification process is important to counter the obstructive effects of ammonium on respiration and photo-phosphorylation (Vines and Wedding 1960; Tobin and Yamaya 2001).

Table 2 Two-way ANOVA results for the effects of ammonium concentrations on Chlorophyll *a* and *b*, Soluble sugars, FAA, Protein content and Fv/Fm for the three macrophytes

Dependent variables							
	Species			Ammonium concentration			
Chlorophyll *a* and *b*	16.21***			4.80**			
	Cd	Vn	Pm	0	0.1	15	50
	a	a	b	b	b	a	a
	Species*ammonium concentration 1.95^{ns}						
Free amino acids	3.02*			3.26*			
	Cd	Vn	Pm	0	0.1	15	50
	a	b	a	a	a	b	b
	Species *ammonium concentration 0.174^{ns}						
Fv/Fm	6.51***						
	Cd	Vn	Pm	0	0.1	15	50
	b	a	a	a	a	b	b

	Species*ammonium concentration 2.73*						
Soluble sugars	26.34***			4.34**			
	Cd	Vn	Pm	0	0.1	15	50
	b	a	a	c	ab	ab	a
	Species*ammonium concentration 1.96^{ns}						
Protein content	6.722**			5.02**			
	Cd	Vn	Pm	0	0.1	15	50
	ab	b	a	c	ab	ab	a
	Species*ammonium concentration 0.309^{ns}						

Note. Statistical significance indicated through asterisk (s): **P<0.05, **P<0.01, ***P<0.001,* ^{ns} *P>0.05*. a, b, c indicates Turkey HSD groups. Cd –*Ceratophyllum demersum*, Vn- *Vallisneria natans*, Pm- *Potamogeton maackianus*

Effect of ammonium concentration on FAA and Protein content

Aquatic macrophytes have been found to accumulate FAA rapidly with the increasing NH_4^+ concentration levels within the external water column (Cao et al. 2007; Xu et al. 2012). Similar observations were made in the present study in which FAA and Protein content parameters increased significantly (*P<0.*05) at ammonium concentrations of 15 mg/L and 50 mg/L regardless of the species (Fig. 2B & D). However, for *V. natans*, the levels of FAA under different Ammonium concentrations were lower compared to *C. demersum* and *P. maackianus*, a demonstration of its varied response. Interestingly, this is a positive observation in phytoremediation since the free amino acids are known to contribute to osmotic adjustment, detoxification of reactive oxygen species and protection of membrane integrity (Sharma and Dietz 2006; Pisani et al. 2007). Further, since the accumulation of free amino acids deters protein denaturation, prevents water loss by sustaining cell turgor and maintains membrane integrity of aquatic macrophytes plant tissue (Kim et al. 2004; Neuberg et al. 2010), the three species studied here have the potential for use in the ecological restoration of polluted wetlands. Several studies have confirmed that proteins induce resistance to heavy metals by the formation of free radicals in metabolic reactions and their oxidative stress, hence protecting labile macromolecules against attack (Patra et al. 2001; Mishra et al. 2006). However, ammonium pollution has been reported to induce significant increase of protein accumulation in aquatic macrophytes such as *V. natans* (Wang et al. 2010; Zhu et al. 2016). Our study reports consistent results in which there was an increased proteins content in the three investigated macrophytes, suggesting the plants were experiencing stress at increased ammonium concentration.

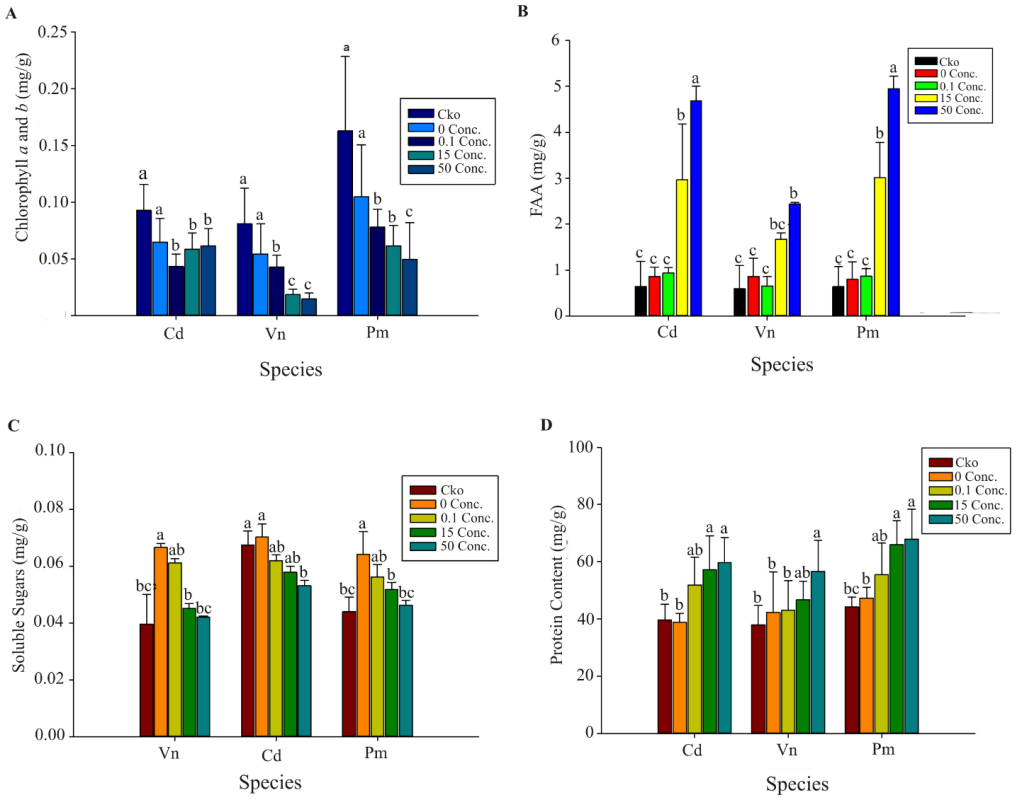

Fig. 2 Bar plots showing the effect of varying ammonium concentrations on the physiological parameters; chlorophyll *a* and *b*, FAA, soluble sugars and protein content in *C. demersum* (Cd), *V. natans* (Vn), and *P. maackianus* (Pm). The values represent means of five replicates ± SD. Bars with different letters are significantly different at *P*<0.05.

Effect of ammonium concentrations on Chlorophyll fluorescence

The species-specific responses to water column on different ammonium concentrations and their effects on photosynthetic performance were tested and the results presented in Fig. 3. High NH_4^+ concentrations on sediments and within the water column impose some undesirable effects on submerged macrophytes, including; photosynthesis inhibition, triggered oxidative stress and internal carbon-nitrogen imbalance (Smolders et al. 2000; Cao et al. 2004; Li et al. 2007; Neuberg et al. 2010). Chlorophyll fluorescence (Fv/Fm) is one of the photosynthesis-related parameter that has been previously used to investigate abiotic and biotic stress of aquatic macrophytes in aquatic ecosystem (Apudo et al. 2016). Our results show that the species *V. natans* exhibited the highest significant decrease in Fv/Fm with increased ammonium concentration within the water column, *P. maackianus* slightly significant while *C. demersum* didn't exhibit any significant decrease. Further, the Pulse Amplitude Modulated (PAM) test indicated a significant decrease of maximum quantum yield of PS11 in *V. natans*, and *P. maackianus* at increased ammonium concentrations within the water column. Since Fv/Fm is a useful determinant in the demonstration of photosynthetic capacity of macrophytes, it can be used to assess the level of damage caused by pollutants and environmental stress (Juneau and Popovic 1999; Marwood et al. 2001). Noteworthy, the rootless form (*C. demersum*) didn't exhibit any significant change in chlorophyll fluorescence, thus concluding on its tolerance nature to ammonium stress.

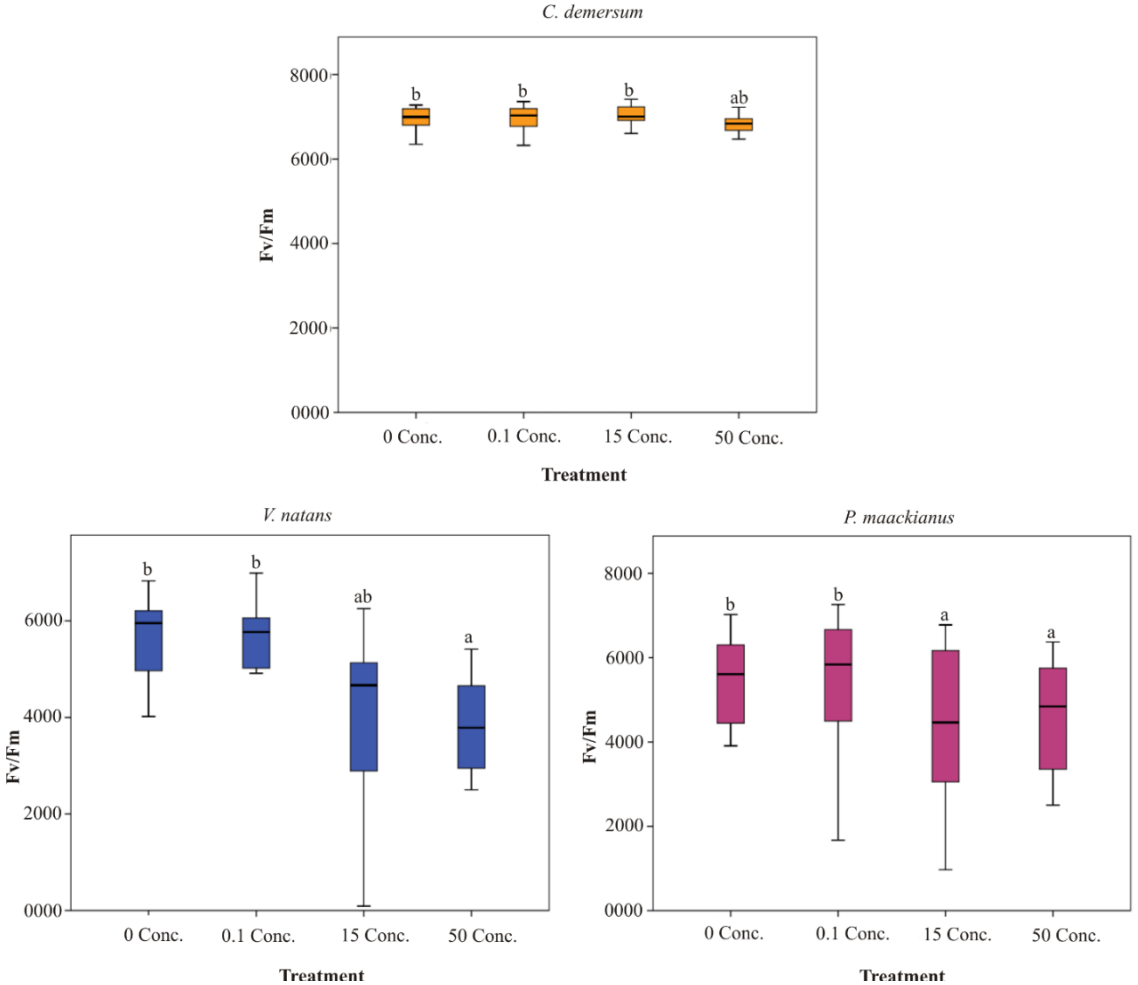

Fig. 3 Box plots showing the effect of ammonium concentrations on chlorophyll fluorescence (Fv/Fm) in *C. demersum*, *V. natans*, and *P. maackianus.*

Effect of the Species on the Ammonium and Total Nitrogen in the water

After ammonium treatments, the amount of TN and NH_4^+ was obtained from pooled samples of the three plants from each replicate. It was observed that TN and NH_4^+ increased with an increase in the ammonium dosing in the experiment, with marked changes in the NH_4^+ and TN at 0 mg/L, 0.1 mg/L, 15 mg/L and 50 mg/L Ammonium concentrations (Fig. 4).

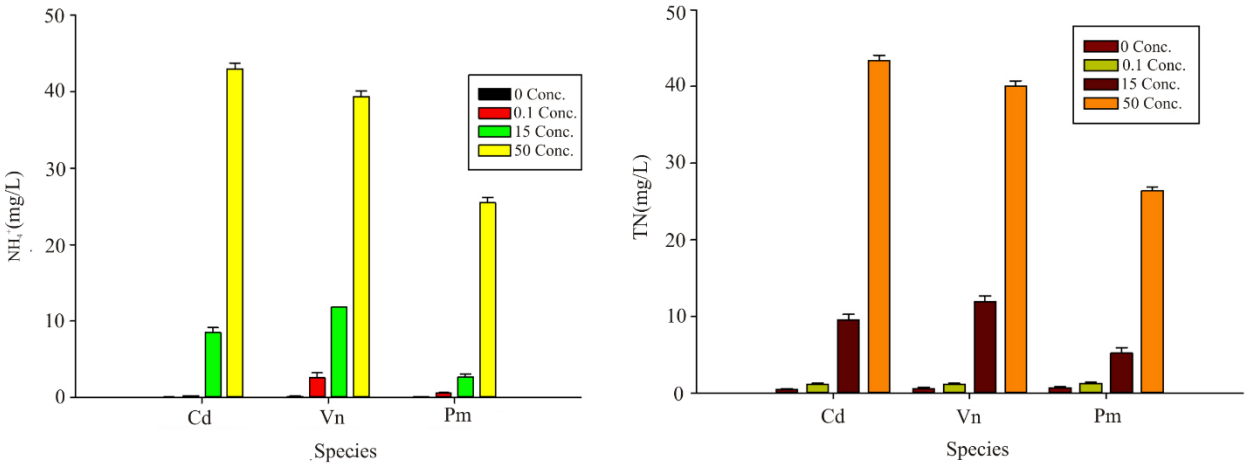

Fig. 4 The changes in the concentration of Total nitrogen (TN) and Ammonium (NH_4^+) in the water column after ammonium treatment. The data represent means± SE (*n=4*). Cd – *C. demersum,* Vn – *V. natans*and Pm – *P. maackianus.*

In summary, there were varied responses of macrophytes to elevated ammonium concentrations with significant relation to growth forms. In congruence to Yu et al. (2018) observation, the varied responses exhibited by the canopy-forming *P. maackianus*, rootless *C. demersum* and rosette-forming *V. natans* are attributed to differences in their ability to intercept light. For instance, the rosette-forming *V. natans*is short and prone to low light conditions thereby experiencing insufficient carbohydrates production which is required to detoxify ammonium toxicity (Yu et al. 2018). On the other hand, the rootless and the canopy-forming macrophytes reach water surfaces where they access relatively sufficient light for carbohydrates production, hence are able to avoid NH_4^+ toxicity within water columns (Ãzkan et al. 2010; Olsen et al. 2015). However, despite the tolerance ability of *C. demersum* and *P. maackianus* towards ammonium toxicity, their growth is normally inhibited by eutrophication since its composed of diverse pollutants (Qiu et al. 2001).

IV. Conclusion

This study provides valuable insights into potential methods of aquatic ecosystem restoration following water degradation caused by high ammonium concentrations. Here, we demonstrate that growth form is an important parameter that macrophytes use to circumvent the negative effects of high ammonium contents within the water column. The three macrophytes *C. demersum*, *V. natans*, and *P. maackianus* responded differently to NH_4^+ toxicity based on growth forms. In congruence to previous studies, our study reports *C. demersum* and *P. maackianus* to exhibit more tolerant to high ammonium concentrations while *V. natans* was sensitive to high ammonium concentration treatments, agreeing with our hypothesis. Therefore, the canopy-forming *P. maackianus* and rootless *C. demersum* species may feasibly be preferred in the practical restoration of shallow aquatic ecosystems experiencing high NH_4^+ toxicity. However, the canopy-forming species are limited in that they may alter biochemical processes by blocking light penetration, limiting gas exchange, and consequently affecting oxygen concentrations in aquatic ecosystems. In light of this, there is a need to give preference to the rootless species in studies involving phytoremediation.

References

[1]. Apudo AA, Cao Y, Wakibia J, Li W, Liu F (2016) Physiological plastic responses to acute NH_4^+–N toxicity in *Myriophyllum spicatum* L. cultured in high and low nutrient conditions. Environmental and Experimental Botany 130:79-85. doi:10.1016/j.envexpbot.2016.05.009

[2]. Ãzkan K, Jeppesen E, Johansson LS, Beklioglu M (2010) The response of periphyton and submerged macrophytes to nitrogen and phosphorus loading in shallow warm lakes: a mesocosm experiment. Freshwater Biology 55 (2):463-475. doi:10.1111/j.1365-2427.2009.02297.x

[3]. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39:205–207

[4]. Boyd CE (1970) Vascular aquatic plants for mineral nutrient removal from polluted waters. Economic Botany volume 24:95–103

[5]. Britto DT, Kronzucker HJ (2002) NH_4+ toxicity in higher plants: a critical review. Journal of Plant Physiology 159 (6):567-584. doi:10.1078/0176-1617-0774

[6]. Brix H, Dyhr-Jensen K, Lorenzen B (2002) Root-zone acidity and nitrogen source affects *Typha latifolia* L. growth and uptake kinetics of ammonium and nitrate. Journal of Experimental Botany 53 (379):2441-2450. doi:10.1093/jxb/erf106

[7]. Cadmus R, Perceval C, Grobicki A (2016) Wetland Restoration improves livelihoods in developing cities.

[8]. Cao T, Ni L, Xie P (2004) Acute Biochemical Responses of a Submersed Macrophyte, *Potamogeton crispus* L., to High Ammonium in an Aquarium Experiment. Journal of Freshwater Ecology 19 (2):279-284. doi:10.1080/02705060.2004.9664542

[9]. Cao T, Xie P, Ni L, Wu A, Zhang M, Wu S, Smolders AJP (2007) The role of NH_4^+ toxicity in the decline of the submersed macrophyte *Vallisneria natans* in lakes of the Yangtze River basin, China. Marine and Freshwater Research 58:581–587

[10]. Cao T, Xie P, Ni L, Zhang M, Xu J (2009) Carbon and nitrogen metabolism of a eutrophication tolerative macrophyte, *Potamogeton crispus*, under NH_4^+ stress and low light availability. Environmental and Experimental Botany 66 (1):74-78. doi:10.1016/j.envexpbot.2008.10.004

[11]. Cao TE, Ni L, Xie P, Xu JUN, Zhang M (2011) Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. Freshwater Biology 56 (8):1620-1629. doi:10.1111/j.1365-2427.2011.02601.x

[12]. Chambers PA, Lacoul P, Murphy KJ, Thomaz SM (2008) Global diversity of aquatic macrophytes in freshwater. In: Freshwater Animal Diversity Assessment. Developments in Hydrobiology. pp 9-26. doi:10.1007/978-1-4020-8259-7_2

[13]. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F (1951) A Colorimetric Method for the Determination of Sugars. Nature 168:167

[14]. Dvoiaki J, P E, Best H (1982) Macro-invertebrate communities associated with the macrophytes of Lake Vechten : structural and functional relationships. Hydrobiologia volume 95:115–126

[15]. Ellis BK, Craft JA, Stanford JA (2015) Long-term atmospheric deposition of nitrogen, phosphorus and sulfate in a large oligotrophic lake. PeerJ3:e841. doi:10.7717/peerj.841

[16]. Fang YY, Babourina O, Rengel Z, Yang XE, Pu PM (2007) Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. Annals of Botany 99 (2):365-370. doi:10.1093/aob/mcl264

[17]. Ghavzan NJ, Gunale VR, Mahajan DM, Shirke DR (2006) Effects of Environmental Factors on Ecology and Distribution of Aquatic Macrophytes. Asian Journal of Plant Sciences 5 (5):871-880. doi:10.3923/ajps.2006.871.880

[18]. Greenway M (2003) Suitability of macrophytes for nutrient removal from surface flow constructed wetlands receiving secondary treated sewage effluent in Queensland, Australia. 48 (2):121-128

[19]. Hachiya T, Sakakibara H (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J Exp Bot 68 (10):2501-2512. doi:10.1093/jxb/erw449

[20]. Huang W, Ma H-Y, Huang Y, Li Y, Wang G-L, Jiang Q, Wang F, Xiong A-S (2017) Comparative proteomic analysis provides novel insights into chlorophyll biosynthesis in celery under temperature stress. Physiologia Plantarum 161 (4):468-485. doi:10.1111/ppl.12609

[21]. IBM-Corporation (2011) IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp

[22]. ISO7150-1 (1984) Water Quality; Determination of Ammonium. Part 1. Manual Spectrometric Method. Geneva:ISO

[23]. Jahnke LS, Eighmy TT, Fagerberg WR (1991) Studies of *Elodea nuttallii* grown under photorespiratory conditions. I. Photosynthetic characteristics. Plant, Cell and Environment 14:147-156. doi:https://doi.org/10.1111/j.1365-3040.1991.tb01331.x

[24]. Juneau P, Popovic R (1999) Evidence for the Rapid Phytotoxicity and Environmental Stress Evaluation Using the PAM Fluorometric Method: Importance and Future Application. Ecotoxicology 8:449-455

[25]. Kim T-H, Lee B-R, Jung W-J, Kim K-Y, Avice J-C, Ourry A (2004) De novo protein synthesis in relation to ammonia and proline accumulation in water stressed white clover. Functional Plant Biology 31 (8). doi:10.1071/fp04059

[26]. Kinidi L, Salleh S (2017) Phytoremediation of Nitrogen as Green Chemistry for Wastewater Treatment System. International Journal of Chemical Engineering 2017:1-12. doi:10.1155/2017/1961205

[27]. Leghari SJ, Wahocho NA, Laghari GM, Laghari AH, MustafaBhabhan G, Talpur KH (2016) Role of Nitrogen for Plant Growth and Development: A review. Advances in Environmental Biology 10 (9):209+

[28]. Li H, Cao T, Ni L (2007) Effects of ammonium on growth, nitrogen and carbohydrate metabolism of *Potamogeton maackianus* A. Benn. Fundamental and Applied Limnology / ArchivfürHydrobiologie 170 (2):141-148. doi:10.1127/1863-9135/2007/0170-0141

[29]. Lin Q, Liu E, Zhang E, Li K, Shen J (2016) Spatial distribution, contamination and ecological risk assessment of heavy metals in surface sediments of Erhai Lake, a large eutrophic plateau lake in southwest China. Catena 145:193-203. doi:10.1016/j.catena.2016.06.003

[30]. Liu W, Tan X, Yuan G, Fu H, Peng H, Ge D, Lou Q, Zhong J (2019) Effects of ammonium pulse on the growth of three submerged macrophytes. Plos One 14 (7). doi:10.1371/journal.pone.0219161

[31]. Liu Y, von Wiren N (2017) Ammonium as a signal for physiological and morphological responses in plants. J Exp Bot 68 (10):2581-2592. doi:10.1093/jxb/erx086

[32]. Marino D, Moran JF (2019) Can Ammonium Stress Be Positive for Plant Performance? Front Plant Sci 10:1103. doi:10.3389/fpls.2019.01103

[33]. Martin CW, Valentine JF (2012) Eurasian milfoil invasion in estuaries: physical disturbance can reduce the proliferation of an aquatic nuisance species. Marine Ecology Progress Series 449:109-119. doi:10.3354/meps09515

[34]. Marwood CA, Solomon KR, Greenberg BM (2001) Chlorophyll Fluorescence as a Bioindicator of Effects on Growth in Aquatic Macrophytes from Mixtures of Polycyclic Aromatic Hydrocarbons. Environmental Toxicology and Chemistry 20 (4):890–898

[35]. Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot 105 (7):1141-1157. doi:10.1093/aob/mcq028

[36]. Miller AJ, Cramer MD (2005) Root Nitrogen Acquisition and Assimilation. Plant and Soil 274 (1-2):1-36. doi:10.1007/s11104-004-0965-1

[37]. Mishra S, Srivastava S, Tripathi RD, Govindarajan R, Kuriakose SV, Prasad MN (2006) Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiology and Biochemistry 44 (1):25-37. doi:10.1016/j.plaphy.2006.01.007

[38]. Neuberg M, Pavlíková D, Pavlík M, Balík J (2010) The effect of different nitrogen nutrition on proline and asparagine content in plant. PLANT SOIL ENVIRON 56 (7):305–311

[39]. Nielsen SS (2010) Food Analysis Laboratory Manual. In: Heldman DR (ed) Food Science Texts Series Springer, Boston, MA. doi:https://doi.org/10.1007/978-1-4419-1463-7

[40]. O'Hare MT, Baattrup-Pedersen A, Baumgarte I, Freeman A, Gunn IDM, Lazar AN, Sinclair R, Wade AJ, Bowes MJ (2018) Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. Front Plant Sci 9:451. doi:10.3389/fpls.2018.00451

[41]. Olsen S, Chan F, Li W, Zhao S, Søndergaard M, Jeppesen E (2015) Strong impact of nitrogen loading on submerged macrophytes and algae: a long-term mesocosm experiment in a shallow Chinese lake. Freshwater Biology 60 (8):1525-1536. doi:10.1111/fwb.12585

[42]. Patra RC, Swarup D, Dwivedi SK (2001) Antioxidant effects of a tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology 162:81–88

[43]. Pisani D, Benton MJ, Wilkinson M (2007) Congruence of morphological and molecular phylogenies. Acta Biotheor 55 (3):269-281. doi:10.1007/s10441-007-9015-8
[44]. Qin B, Guang G, Guangwei Z, Yunlin Z, Yuzhi S, Xiangming T, Hai X, Jianming D (2013) Lake eutrophication and its ecosystem response. Chinese Science Bulletin 59 (9): 961 970. doi:10.1007/s11434012-5560-x
[45]. Qiu D, Wu Z, Liu B, Deng J, Fu G, He F (2001) The restoration of aquatic macrophytes for improving water quality in a hypertrophic shallow lake in Hubei Province, China. Ecological Engineering 18:147–156
[46]. Sarasketa A, Gonzalez-Moro MB, Gonzalez-Murua C, Marino D (2016) Nitrogen Source and External Medium pH Interaction Differentially Affects Root and Shoot Metabolism in Arabidopsis. Front Plant Sci 7:29. doi:10.3389/fpls.2016.00029
[47]. Saunkaew P, Jampeetong A, Wangpakapattanawong P (2011) Growth, morphology, ammonium uptake and nutrient allocation of *Myriophyllum brasiliense* Cambess. under high NH4+ concentrations. Ecotoxicology 20:2011–2018. doi:10.1007/s10646-011-0744-8
[48]. Shah M, Hashmi HN, Ali A, Ghumman AR (2014) Performance assessment of aquatic macrophytes for treatment of municipal wastewater. Journal of Environmental Health Science & Engineering 12 (106)
[49]. Shah M, Hashmi HN, Ghumman AR, Zeeshan M (2015) Performance assessment of aquatic macrophytes for treatment of municipal wastewater. Journal of the South African Institution of Civil Engineering 57 (3):18-25. doi:10.17159/2309-8775/2015/v57n3a3
[50]. Sharma SS, Dietz K-J (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany 57 (4):711–726. doi:10.1093/jxb/erj073
[51]. Shengqi S, Zhou Y, Qin JG, Wang W, Yao W, Song L (2012) Physiological responses of *Egeria densa* to high ammonium concentration and nitrogen deficiency. Chemosphere 86 (5):538-545. doi:10.1016/j.chemosphere.2011.10.036
[52]. Smolders AJP, van Riel MC, Roelofs JGM (2000) Accumulation of free amino acids as an early indication for physiological stress (nitrogen overload) due to elevated ammonium levels in vital *Stratiotes aloides* L. stands. Fundamental and Applied Limnology 150 (1):169-175. doi:10.1127/archiv-hydrobiol/150/2000/169
[53]. Song Q-n, Lu H, Liu J, Yang J, Yang G-y, Yang Q-p (2017) Accessing the impacts of bamboo expansion on NPP and N cycling in evergreen broadleaved forest in subtropical China. Scientific Reports 7 (1). doi:10.1038/srep40383
[54]. Sun H, Wang W, Li J, Yang Z (2014) Growth, oxidative stress responses, and gene transcription of juvenile bighead carp (*Hypophthalmichthysnobilis*) under chronic-term exposure of ammonia. Environmental Toxicology and Chemistry 33 (8):1726-1731. doi:10.1002/etc.2613
[55]. Tobin AK, Yamaya T (2001) Cellular compartmentation of ammonium assimilation in rice and barley. Journal of Experimental Botany 52 (356):591- 604
[56]. Tripathi BD, Shukla SC (1991) Biological Treatment of Wastewater by Selected Aquatic Plants. Environmental Pollution 69: 69-78
[57]. Tylova-Munzarova E, Lorenzen B, Brix H, Votrubova O (2005) The effects of NH4+ and NO3− on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*. Aquatic Botany 81 (4):326-342. doi:10.1016/j.aquabot.2005.01.006
[58]. Vines HM, Wedding RT (1960) Some Effects of Ammonia on Plant Metabolism and a Possible Mechanism for Ammonia Toxicity. Plant Physiology 35 (6):820–825
[59]. Wang C, Zhang S, Wang P, Hou J, Li W, Zhang W (2008) Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte *Vallisneria natans* (Lour.) Hara. Aquatic Toxicology 87 (2):88-98. doi:10.1016/j.aquatox.2008.01.009
[60]. Wang Q, Li Z, Cheng S, Wu Z (2010) Influence of humic acids on the accumulation of copper and cadmium in *Vallisneria spiralis* L. from sediment. Environmental Earth Sciences 61 (6):1207-1213. doi:10.1007/s12665-009-0444-3
[61]. Wang S, Zhang L, Ni L, Zhao H, Jiao L, Yang S, Guo L, Shen J (2015) Ecological degeneration of the Erhai Lake and prevention measures. Environmental Earth Sciences 74 (5):3839-3847. doi:10.1007/s12665-015-4433-4
[62]. Wellburn AR, Lichtenthaler H (1984) Formulae and Program to Determine Total Carotenoids and Chlorophylls A and B of Leaf Extracts in Different Solvents. In: Advances in Photosynthesis Research. pp 9-12. doi:10.1007/978-94-017-6368-4_3
[63]. Xian L, Zhang Y, Cao Y, Wan T, Gong Y, Dai C, Ochieng WA, Nasimiyu AT, Li W, Liu F (2020) Glutamate dehydrogenase plays an important role in ammonium detoxification by submerged macrophytes. Sci Total Environ 722:137859. doi:10.1016/j.scitotenv.2020.137859
[64]. Xu J, Zhang J, Zhao C, Li C, Xie H, Wang S (2012) Effect of Ammonia Stress on Physiological and Biochemical Character of *Phragmites australis* in Constructed Wetland. Paper presented at the 2012 Third International Conference on Digital Manufacturing & Automation,
[65]. Yu Q, Wang HZ, Xu C, Li Y, Ma SN, Liang XM, Jeppesen E, Wang HJ (2018) Higher Tolerance of Canopy-Forming *Potamogeton crispus* Than Rosette-Forming *Vallisneria natans* to High Nitrogen Concentration as Evidenced from Experiments in 10 Ponds with Contrasting Nitrogen Levels. Frontiers in Plant Science 9:1845. doi:10.3389/fpls.2018.01845
[66]. Zhu G, Cao T, Zhang M, Ni L, Zhang X (2014) Fertile sediment and ammonium enrichment decrease the growth and biomechanical strength of submersed macrophyte *Myriophyllum spicatum* in an experiment. Hydrobiologia 727 (1):109-120. doi:10.1007/s10750-013-1792-2
[67]. Zhu Z, Song S, Li P, Jeelani N, Wang P, Yuan H, Zhang J, An S, Leng X (2016) Growth and physiological responses of submerged plant *Vallisneria natans* to water column ammonia nitrogen and sediment copper. PeerJ 4. doi:10.7717/peerj.1953

Annah Timinah Nasimiyu, et. al. "Comparative analysis of the response of three macrophytes under different ammonium concentrations: insights into the role of growth forms." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(8), (2020): pp 47-55.