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EFFECT OF SALINITY ON WASTEWATER TREATMENT ABILITY OF THREE SUBMERGED MACROPHYTES

By

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ABSTRACT

The three submerged macrophytes; ***Myriophyllum spicatum*** *L.,* ***Potamogeton pectinatus*** *L. and* ***Ceratophyllum demersum*** *L. were cultured outdoors in media containing domestic wastewater and different water salinities using a mixture of different amounts of seawater and dechlorinated tap water. This experiment was to examine their conventional use as pollutants biosorbant in culture media (in glass basins) with salinities 3.35. 6.70. 10.05 and 13.40‰. Their potential on the reduction of dissolved ammonia, phosphate and iron from domestic sewage was estimated. All test plants could tolerate salinity of 3.35‰, with highest ammonia-N and reactive-P reduction in* ***Myriophyllum media*** *(85 & 95%, respectively). Nutrients (N&P) reduction was considerably lower in the other plant media, at the same salinity. No increase in fresh weights of* ***Myriophyllum*** and ***Potamogeton*** *was detected at salinity 6.70‰.* ***Potamogeton pectinatus*** *could tolerate higher salinities (10.05 and 13.40‰) after its recovery, although with low biomass yield as well as low nutrient reduction.* ***Myriophyllum spicatum*** *was less tolerant to such high salinities and it exhibited signs of exhaustion.* ***Ceratophyllum demersum*** *was the least plant in salinity tolerance since it lost its fresh weight at salinities higher than 3.35‰. Iron concentrations were completely exhausted from all culture media at different salinity levels. Thus, it is recommended to use* ***M. spicatum*** *for treatment of such pollutants in salinity levels up to 6.70‰ more readily* ***than*** *the* *other two test plants. Further experiments should be conducted to evaluate other aquatic macrophytes as potentials for sewage treatment at different salinity levels.*

INTRODUCTION

The recycling and reuse of urban wastewater is considered today one of the most attractive options for the development of a sustainable source of water for agricultural, industrial and urban non-potable purposes. Recycling of wastewater can have the multiple benefit of protecting the environment and serving as a major source of water and nutrients for the soil.

The systems of aquatic treatment based on usage submerged, floating and emergent (rooted aquatic vascular plants) to wastewater renovation in managed pond or wetland systems. Earlier research had screened a large number of submerged, temperate climate macrophytes for their vegetative growth potential, tolerance to variations in temperature and low light levels, ability to survive wastewater's high organic and nutrient loading environments (White and Bishop, 1984). ***Elodea*** spp., ***Potamogeton*** spp., ***Ceratophyllum demersum, Salvinia molestra*** and ***hydrilla verticillata*** are the most common submerged plants found in polluted streams and were recommended by Finlayson (1984), Ozimek (1985) and Timofeeva & Stom (1988) for wastewater treatment.

Ghobrial (1994) used ***Potamogeton pectinatus, Ceratophyllum demersum, Elodea canadensis, Spirodella polyrrhiza*** (and also, ***Azolla filiculoides,*** Ghobrial, 1998a) to experiment submerged and floating macrophytes, for tertiary treatment of domestic wastewater. The author cultured these plants in media contained mixtures of dechlorinated tap water and different concentrations of domestic wastewater. These plants were found to have a potential in removing large amounts of nutrients (nitrogen and phosphorus), with additional biomass harvest and protein increase in plant tissues. Ghobrial (1998b and 2000) investigated the use of ***E. canadensis*** and ***C. demersum*** as potential for removing heavy metals from industrial and domestic wastewater respectively.

In a new trial for sewage treatments, three submerged plants namely; ***Myriophyllum spicatum***, ***Potamogeton pectinatus*** and ***Ceratophyllum demersum***, were experimented as potentials for removing pollutants (NH_4-N, PO_4-P and Fe) from domestic wastewater. These plants were cultivated in media enriched with 20% domestic sewage and different salinities using mixtures of seawater and dechlorinated tap water. The widespread species ***M. spicatum*** and ***P. pectinatus*** are chosen as they are the most abundant submerged macrophytes in the freshwater and oligohaline marshes (Grillas, 1990). They can produce high biomass (Van Wijk, 1988) and have similar growth patterns. ***M. spicatum*** is known to have a rapid and effective dispersal, mainly by plant fragments (Smith and Barko, 1990). ***C. demersum*** was chosen because it is known to be one of the disturbance- tolerant aquatic plants (Murphy ***et al.***, 1990).

The objective of this study was to quantify the impact of domestic wastewater and increased seawater (salinity) on growth performance of the three selected macrophytes. The ability of these plants to reduce dissolved ammonia, phosphates and also iron from domestic sewage was estimated.

MATERIALS AND METHODS

Water samples :

Domestic wastewater was taken from the main sewer, which collects the sewage from the western district of Alexandria City. The wastewater was left in big plastic jars to settle 24 hrs and the supernatant liquid layer was used for the experiment. Tap water was left also for three days to get dechlorinated water. Seawater was collected freshly from the Eastern Harbour of Alexandria city and then filtered.

Plant samples:

The three submerged macrophytes; the water milfoil ***Myriophyllum spicatum***, Sagopond weed ***Potamogeton pectinatus*** and the coontail ***Ceratophyllum demersum*** were collected during May (growing season) from a drainage channel in Abis village which is, more or less, away from direct exposure to agricultural pollutants. The average water temperature was 23.5°C. Plants were transported, sorted and planted in small containers filled with dechlorinated tap water and allowed to acclimate to laboratory. After the yield increase or loss (if detected) were estimated at the beginning and the end of each incubation period and the excess plants were harvested maintaining, thus, its constant weight (1g/ l medium) in each new experiment.

Dissolved ammonia was determined using phenate method as given by Koroleff (1969). Reactive phosphate was determined according to the method described by Murphy and Riley (1962). Iron metal level was measured with Perkin-Elmer model 2380 atomic absorption spectrophotometer, using flame emission according to the method mentioned in APHA (1985).

Control experiments

Parallel experiments using seawater and domestic wastewater separately were conducted to examine the effect of each kind of water on the growth of the test plants (control media). Media with same salinity dilutions, but without plants, were prepared , also, to estimate reduction efficiency of N and P (selfpurification).

Statistical analysis :

Statistical analysis including correlation coefficient and Stepwise Linear Regression equations between nutrient reduction and salinity at a confidence limit of 95% ($n = 4$, $p < 0.05$) were carried out according to Hintze (1993).

RESULTS AND DISCUSSION

Ghobrial (1994) found that the plants died within a few hours when cultured in 100% domestic wastewater due to pollution stress. This was mostly due to the suspended matters accumulated on the surfaces of plant leaves, lack of oxygen, high dissolved ammonia load and high BOD levels, which impaired photosynthetic activity. Finally the plants disintegrated and sank.

Concerning the effect of seawater, symptoms of salinity stress on ***Myriophyllum spicatum*** first appeared on the older leaves, their margins became chlorotic, twisted and finally became necrotic. These symptoms progressed to younger then more immature foliages. ***Potamogeton pectinatus*** (known to be salt tolerant) remained green in seawater media several days but the plants decayed and sank after 2 weeks. ***Ceratophyllum demersum*** leaves were detached after three days and sank on the bottom of the basins.

Table (1) shows the percentages reduction of N & P and the plants yield at different salinity levels. The three test plants tolerated low salinity level (3.35‰) although with small biomass yield, which amounted to 0.17, 0.29 and 0.10 g/g initial freshweight for ***Myriophyllum, Potamogeton*** and ***Ceratophyllum*** respectively. At this salinity level, dissolved ammonia as well as reactive phosphate were highly reduced from the media of ***M. spicatum*** but were slightly reduced from the media of the other test plants.

Haller (1974) suggested that water with salinity 3.33‰ was required for the maximum production of *Lemna*, in which sodium is needed by all plants for metabolism of carbon dioxide and chloride for photoreduction of oxygen in chloroplasts. This may explain the tolerance of all test plants to salinity 3.35‰ used in the culture media, but with low production.

Fresh weights of ***M.spicatum*** and ***P. pectinatus*** did not record any increases at salinity 6.70‰. The obvious sign of plant intolerance to higher salinity is the tissue mortality of ***C. demersum*** which lost its initial weight by 2.9%. Munns (1993) noted that plant death under high salinity conditions is caused by a rapid rise in salt concentrations in cell walls or cytoplasm when the vacuoles can no longer compartmentalize the salts. Also, increased salinity can affect plant growth by the imposition of water stress through increased osmotic potential as mentioned by Hale and Orcutt (1987).

Within seven days exposure to salinity 6.70‰, about 80% of the dissolved ammonia and 97% of reactive phosphate were removed from the media of ***M. spicatum***. Considerably low reduction of both nutrients was noticed in the other culture media.

However, the effects of salinity were temporary and disappeared with exposure to higher salinity (10.05‰). Thus, ***M. spicatum*** and ***P. pectinatus***, initially produced new leaves to replace old leaves. When such plants grew in media with salinity 10.05‰, they yielded 0.021 and 0.49 g/g initial fresh weight respectively. This was accompanied by ammonia removal of 50% from culture media of ***M. spicatum*** and 41% from media of ***P. pectinatus***. Exposure of

Table (1) : Percentage reduction of dissolved ammonia-N, phosphate-P and iron from domestic wastewater at different salinity levels.

Plant species	Duration of incubation (days	‰ Salinity		NH_4-N mg/l	PO_4-P mg/l	Fe mg/l	Plant yield or loss g/g fresh weight
Myriophyllum spicatum	7	3.35	Initial conc.	0.75	4.00	0.1	0.17
			Final conc.	0.11	0.20	---	
			% Reduction	85.3%	95.0%	100%	
	7	6.70	Initial conc.	0.41	2.00	0.05	-0.072
			Final conc.	0.08	0.05	---	
			% Reduction	80.5%	97.5%	100%	
	7	10.05	Initial conc.	0.12	2.70	0.03	0.021
			Final conc.	0.06	2.57	---	
			% Reduction	50.0%	4.8%	100%	
	7	13.40	Initial conc.	0.47	2.80	0.55	Disintigration
			Final conc.	0.09	2.10	---	
			% Reduction	80.9%	25.0%	100%	
Potamogeton pectinatus	7	3.35	Initial conc.	0.75	4.00	0.1	0.29
			Final conc.	0.37	3.62	---	
			% Reduction	50.7%	10.0%	100%	
	7	6.70	Initial conc.	0.41	2.00	0.05	-0.11
			Final conc.	0.33	1.75	---	
			% Reduction	19.5%	12.5%	100%	
	7	10.05	Initial conc.	0.12	2.70	0.03	0.49
			Final conc.	0.07	2.67	---	
			% Reduction	41.7%	1.1%	100%	
	7	13.40	Initial conc.	0.47	2.80	0.55	0.28
			Final conc.	0.42	2.45	---	
			% Reduction	10.6%	12.5%	100%	
Ceratophyllum demersum	7	3.35	Initial conc.	0.75	4.00	0.1	0.10
			Final conc.	0.56	3.90	---	
			% Reduction	25.3%	2.5%	100%	
	7	6.70	Initial conc.	0.41	2.00	0.05	-0.03
			Final conc.	0.25	1.90	---	
			% Reduction	39.0%	5.0%	100%	
	7	10.05	Initial conc	0.12	2.70	0.03	-0.04
			Final conc.	0.10	2.03	---	
			% Reduction	16.7%	24.8%	100%	
	7	13.40	Initial conc.	0.75	2.80	0.58	Disintigration
			Final conc.	N.E.	N.E	---	
			% Reduction	N.E	N.E	100%	

N.E = Not Estimated

C. demersum to salinity 10.05‰ led to a loss of about 3.62% of its initial weight, with ammonia reduction by only 16%. Reactive phosphate reduction was generally very small in all culture media at salinity 10.05‰. Salinities higher than 6.70‰ probably had negatively significant effect on PO_4-P reduction from *Myriophyllum* culture media ($r = - 0.82$, $p<0.05$).

Myriophyllum spicatum was exhausted (began to sink onto the bottom of basins) within seven days exposure to salinity 13.40‰ with ammonia reduction by 80% of the initial load. In a study of different removal mechanisms in macrophyte ponds using ***Elodea*** sp., Eighmy and Bishop (1988) found that unaccounted removal (i.e. ammonia volatilization and denitrification) was greater than plant uptake. It is to be noted that, salinity (at all levels) was inversely correlated with NH_4-N reduction from culture media of ***Myriophyllum*** ($r = - 0.35$, $p<0.05$).

Potamogeton pectinatus could withstand salinity of 13.40‰ with a biomass production of 0.28 g/g initial fresh weight with low nutrient reduction. As shown in Table (2) salinity had positive insignificant correlation with ***Potamogeton*** growth. In addition, salinity significantly affected NH_4-N reduction from its culture media ($r = -0.68$, $p<0.05$).

Ceratophyllum demersum responded negatively to high salinity (13.40‰), it showed visible signs of stress with detached fragments. On the other hand, reduction of NH_4-N from cultures of ***Ceratophyllum*** was significantly affected by salinity ($r = - 0.78$, $p<0.05$)

While the pollutants (N, P) were highly reduced from culture media of ***Myriophyllum***, it showed no any increase in its weight at salinity higher than 3.35‰. This could be referred to that the rate of plant death would eventually exceed the rate at which new plants could be produced (Munns and Termaat, 1986). However, high ammonia consumption by the test plant could be attributed to the fact that, the initial plants might be nitrogen starved as stated by Fitzgerald (1968). Also, Goulder and Boatman (1971) suggested by evidences that macrophytes might store nitrogen and use it later on.

The high consumption of reactive phosphate at the first and second salinity levels in ***Myriophyllum*** media could be explained, as Eighmy and Bishop (1989) found, by the lack of correlation between phosphate removal and growth of submerged macrophytes.

Iron was completely exhausted from all culture media at all salinity levels might be because its amounts were so small than needed by the test plants (0.2 mg/l is optimum).

The pH of the final treatment solutions ranged from 7.30 (3.35‰) to 7.12 (13.40‰).

Generally, dissolved ammonia and phosphate reductions from the control media (without plant) did not exceed 16% (for NH_4-N) and 15% (for PO_4-P). However, positively significant correlation was found between initial ammonia-N load and its reduction from culture media of ***Myriophyllum*** and ***Ceratophyllum*** ($r = 0.89$, $p<0.05$ & $r = 0.68$, $p<0.05$ respectively), while it was insignificant in ***Potamogeton*** culture media ($r = 0.17$, $p<0.05$). On the other hand, initial reactive – P load had insignificant effect on its reduction from all culture media of the test plants.

It is to be taken into consideration that self-purification processes might improve the quality of water. Bacteria as well as higher microorganisms (epiphytes) which grew on the surface of the macrophytes forming a biofilm, probably have played a major role in nutrients (N & P) consumption from sewage compared with plants role (Orth *et al.*, 1987). In addition Carpenter and Lodge (1986) mentioned that the productivity of the epiphyte complex ranges from 4 to 98% of host macrophyte productivity.

By applying the technique of Stepwise Linear Regression analysis between salinity and the reduction of estimated nutrients (N & P), the models are described as follows:

Myriophyllum spicatum :

S‰ = 15.218 - 0.092. % NH_4-N reduction

S‰. = 12.504 - 0.743 %PO_4-P reduction

S‰. = 9.045 - 22.542 g/g. Fresh weight yield

Potamogeton pectinatus **:**

S‰ = 13.166 - 0.156 % NH_4-N reduction
S‰. = 9.046 - 0.074 % PO_4-P reduction
S‰. = 7.176 + 5.049 g/g Fresh weight yield

Ceratophyllym demersum **:**

S‰ = 12.539 - 0.206 % NH_4-N reduction
S‰ = 7.943 + 0.053 % PO_4-P reduction
S‰ = 10.489 - 26.422 g/g Fresh weight yield

Table (2) : Correlation between salinity and reduction of dissolved ammonia and reactive phosphate and fresh weight yield of the test plants (n =4, p<0.05).

Plant species	**Salinity correlation with**		
	NH_4-N reduction (r)	**PO_4-P reduction (r)**	**Fresh weight yield (r)**
Myriophyllum spicatum	- 0.347	- 0.819*	- 0.530
Potamogeton pectinatus	- 0.677*	- 0.093	+ 0.293
Ceratophyllum demersum	- 0.776*	+ 0.140	- 0.940*

* Significant differences at p<0.05.

From the results computed by correlation and regression it could be indicated that, the three test plants exhibited differences in salinity tolerance which could be due to adaptation period, age of plants and the stress caused by variations in salinity as postulated by Van Wijk ***et al.*** (1988).

However, Hester ***et al.*** (1996) reported that field collection sites of relatively higher salinities may not necessarily yield clones that are more salt tolerant. Furthermore, it is unclear whether sufficient time was allowed for the plants to de-acclimate from their respective field salinites. This might explain the low salt tolerance of the test plant particularly *C.* ***demersum***.

Potamogeton pectinatus was able to withstand salinities up to 13.40‰ more frequently than ***M. spicatum***, although with low biomass production. Thus, confirming the fact that ***P. pectinatus*** is more salt tolerant than ***M. spicatum*** as found in several studies (Stanly, 1974 and Watkins & Hammerschlag, 1984). However, Haller (1974) indicated that ***M. spicatum*** was tolerant to low salinities, but toxicity (signs of death) occurred in salinity around 13.32‰. Compared to the results of the present experiment ***M. spicatum*** could survive salinities up to 10.05‰, which is lower than given by Haller (1974). Regarding the pollution stress caused by addition of domestic sewage it could be concluded that ***M. spicatum*** could withstand such salinity but with low nutrient removal (N, P).

Howard and Mendelssohn (1999) addressed the capabilities of four marsh plants to recover after salinity pulse effects. This might explain the capabilities of production of the test plants ***M. spicatum*** and in particular ***P. pectinatus*** after exposure to loss in their fresh weights at salinity 6.70‰ thereafter they recover at higher salinity (10.05‰) as shown in Table 1.

In conclusion, the three test plants exhibited different salinity tolerance. The best, which could tolerate salinity up to 13.40‰, was ***Potamogeton pectinatus*** with small yield increases followed by ***Myriophyllum spicatum***, which tolerated lower salinity (10.05‰). ***Ceratophyllum demersum*** lost a part of its fresh weight at salinity 6.70‰ and higher. Nutrients (N, P) reduction and plant growth were affected, generally, by salinity. Thus it is recommended to use ***M. spicatum*** efficiently for domestic sewage treatment at optimum salinity of 6.70‰.

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