



TÍTULO DEL TRABAJO

“Nitrogen absorption by *Sparganium erectum* L. and *Typha domingensis* (Pers.) Steudel grown as floaters”

TÍTULO RESUMIDO

“Nitrogen absorption by macrophytes grown as floaters”

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FIGURAS Y TABLAS

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NITROGEN ABSORPTION BY *Sparganium erectum* L. AND *Typha domingensis* (Pers.) Steudel GROWN AS FLOATERS

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ABSTRACT

The aim of this work was to study the uptake of nitrogen by young plants of *Sparganium erectum* L. y *Typha domingensis* (Pers.) Steudel in the period that follows their implantation in an induced floating system. The effect of the N-form was studied by isotopic tracers. Results showed that both species rapidly absorb N from the nutrient medium whether it is in the ammonia or nitric form. In the species studied, the rate of N absorption per unit of dry weight was similar for the two mineral N forms. *S. erectum* was revealed more efficient than *T. domingensis* in the removal of N.

KEY-WORDS: Macrophytes, floaters, nitrogen, isotopes.

ESTUDIO ISOTÓPICO DE LA ABSORCIÓN DE NITRÓGENO POR *Sparganium erectum* L. Y *Typha domingensis* (Pers.) Steudel DESARROLLADAS EN FLOTACIÓN

RESUMEN

En este trabajo se estudia mediante trazadores isotópicos la absorción de nitrógeno en forma amoniacal y nítrica por plantas jóvenes de *Sparganium erectum* L. y *Typha domingensis* (Pers.) Steudel durante el período inmediatamente posterior a su implantación en un sistema de flotación inducida. Los resultados mostraron que las dos especies absorbían rápidamente nitrógeno procedente de las dos formas aplicadas. En una misma especie, la tasa de absorción por unidad de peso seco fue similar para cualquiera de las dos formas aplicadas de nitrógeno. *S. erectum* resultó ser más eficiente que *T. domingensis* en la remoción del nitrógeno.

PALABRAS CLAVE: Macrofitas, flotantes, nitrógeno, isótopos.

1. INTRODUCTION

Constructed wetlands are wastewater treatment systems based on natural processes of depuration stimulated by plants. Physical, chemical and biological mechanisms interact in these systems resulting in the removal of water contaminants. Constructed wetlands are especially well suited for organic-type wastewater produced by small villages or by agricultural activities. One of the most common contaminant in this type of wastewater is the nitrogen, which can be in the organic nitrogen form (N_{org}) or in the mineralized nitrogen form ($N-NH_4$ and $N-NO_3$, mostly). The proportion of N_{org} over total nitrogen can be high in municipal raw wastewaters and farming effluents, but it is transformed to ammonium nitrogen sooner or later as a result of organic matter degradation. Mineral nitrogen is one the prime contaminants causing eutrophication of water resources and, the same as other water contaminants, it has to be removed before the discharge of the wastewater to the environment.

On the whole, three types of constructed wetlands for wastewater treatment are recognized: i) surface flow wetlands, also known as free water surface wetlands (FWS), ii) subsurface flow wetlands (SFW) or vegetated submerged beds, and iii) aquatic systems. Plant species used for wastewater treatment vary from one type to another. FWS and SFW are usually vegetated with emergent plants, like cattails (*Typha* spp), that are rooted in the bottom of the wetland. The third type of wastewater treatment system is vegetated with species exhibiting natural floating habit, like the water hyacinth (*Eichhornia crassipes*).

More recently, a mixed system named 'Floating Macrophytes Filter' has been proposed to treat wastewater. In that system, emergent plants are grown as floaters to join the advantages of the systems based on emergent plants and those based on aquatic plants. Knowledge of the

performance of emergent plants grown as floaters is a subject of growing interest for the development of this new system of wastewater treatment.

This study is framed within the research and development line of the wastewater treatment system 'Floating Macrophytes Filter' by the Polytechnic University of Madrid. Young plants of *Sparganium erectum* L. and *Typha domingensis* (Pers.) Steudel grown as floaters are studied for nitrogen absorption. Specific objectives of this work were i) to determine if just after the implantation these plants uptake nitrogen and ii) if nitrogen absorption is dependent on the mineralized nitrogen form.

2. MATERIAL AND METHODS

2.1. Experimental design and plant material

The experiment was carried out using the stable nitrogen isotope ^{15}N as a tracer. In order to reproduce the same growing conditions in every trial, the nitrogen source formula was the same, NH_4NO_3 and it was applied to the nutrient medium at a same dose.

The experimental design was the following: 2 macrophytes species (*Sparganium erectum* L., *Typha domingensis* (Pers.) Steudel) x 3 treatments ($^{15}\text{NO}_3\text{NH}_4$, $^{15}\text{NH}_4\text{NO}_3$, NH_4NO_3) x 4 replications.

Plants for this study came from the macrophytes nursery of the Agroenergy Group (GA) sited at the Polytechnic University of Madrid (Spain). *T. domingensis* plants were produced from seed in cell trays filled with a substrate 5 made up of 50% sand+50% peat. *S. erectum* plants were produced by vegetative propagation, given that the seeds produced from this species in the GA experimental conditions are not viable. At the phenological stage of 3-4 well-developed leaves -about 10-15 cm plant length- the plants were taken off the cells in order to remove every substrate particulate. Then the plants were allowed to acclimatize in nutritive solution for 14 days. For that purpose the nutritive solution was prepared from a soluble fertilizer to the concentration of 50 ppm N, P_2O_5 and K_2O ; micronutrients Fe, Mn, B, Cu, Zn, Mo were also included in the formulae of that fertilizer. At the end of the acclimatization period, a random sampling (n=4) was performed in order to characterize the plant biomass at the starting point of the experiment.

2.2. Experiment conditions

The experiment was carried out in a controlled environment cabinet. Growing conditions were the following: 25°C-20°C light-dark temperature, light-dark cycle of 16 to 8 h, fluorescence light source (Gro-Lux 58 W lights; average PAR $53 \mu\text{mol m}^{-2} \text{s}^{-1}$). The trials were performed in square grass containers sizing 19 x 19 x 9 cm, in which 1 L nutritive solution without N had been poured in. Just before introducing the plants into the containers, 50 mg of the planned nitrogen source were added per litre of nutritive solution. The N-treatments were $^{15}\text{NO}_3\text{NH}_4$ with 9% ^{15}N (at%) as N- $^{15}\text{NO}_3$ and $^{15}\text{NH}_4\text{NO}_3$ with 10 at% as N- $^{15}\text{NH}_4$; besides, a control treatment with NH_4NO_3 natural enrichment was performed.

Just after adding the N-source, four young plants of the same species -previously acclimatized in the growth cabinet- were transferred per container. The container water surface was kept free and in order to compensate losses by evapotranspiration, N-free nutritive solution was periodically added as required.

The N-free nutritive solution used for the trials was prepared at the laboratory, with MgSO_4 ($2 \cdot 10^{-3}$ M), KH_2PO_4 ($1 \cdot 10^{-3}$ M), CaCl_2 ($5 \cdot 10^{-3}$ M) and KCl ($5 \cdot 10^{-3}$ M). A microelements solution was prepared from KCl (1.864 mg/L), H_3BO_3 (773 mg/L), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (423 mg/L), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (288 mg/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (63 mg/L), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{44} \cdot 4 \text{H}_2\text{O}$ (9.2 mg/L) and

FeCl_3 (4.09 mg/L), which was added at a rate of 2 ml/L nutritive solution. Additionally, 1 ml algicide ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 4 g/L) was added as well.

Plants were allowed to grow under the abovementioned conditions for two weeks. On day 14 the whole plant material was harvested to proceed to the analytic determinations.

2.3. Analytic methods

Each plant was separately evaluated and analysed. Shoot and roots were cut into pieces and dried at 105°C in an air-fan oven; then, they were finely ground using an IKA analytic grinder mod. A10.

Isotopic analysis were conducted following the ANCA/MS methodology. This involves interfacing an automatic nitrogen/carbon elemental analyser (ANCA) to a mass spectrometer (MS) for isotope ratio (Mulvaney 1993), operated by PC. Conveniently calibrated, the system provides data of nitrogen content (N% on dry weight basis, ANCA) and isotope ratio ($^{15}\text{N}/\text{N}_t$ as at %) from a single sample. In this experiment we used an elemental analyser model NA 2000 of FISON Inst., coupled to a mass spectrometer DELTA-plus of Finnigan MAT with an interface ConFlo II of Finnigan MAT. All the samples were analysed with 3 replications. Sampling size was about 2 mg dry weight, accurately determined to 0.01 mg precision.

3. RESULTS AND DISCUSSION

Table 1 shows the results of plant characterization at the starting point of the experiment. The average dry weight of *T. domingensis* plants was about 75% higher than *S. erectum* in spite of the fact that the plant size was similar (? 15 cm shoot length); this is attributed to the lower biomass density of the latter. On the contrary, the N% of *S. erectum* was higher than that of *T. domingensis*.

The results obtained after the complexation of the tracer trials are given in Table 2. In the experiment conditions, the plants grew more than 50% (dry weight basis) over the study period. The nitrogen content of *S. erectum* biomass was kept higher than that of *T. domingensis*. Non-significant differences were found between the N% of a same species; this result was expected from the fact that the nitrogen source and nitrogen dose was the same for every treatment (50 mg $\text{NH}_4\text{NO}_3/\text{L}$ nutritive solution). The results of at% showed that both species took up ^{15}N from the nutritive solution; this indicating that the young plants of *S. erectum* and *T. domingensis* are able to uptake nitrogen immediately after they are implanted in a floating system. The higher at% of *S. erectum* suggested that the rate of N absorption of that species is higher than that of *T. domingensis*, consistent with the fact that the former has higher N content.

From the results in Table 2, the average dry weight of plant biomass and the weighed values of total nitrogen content, the ^{15}N content in the plants of each treatment is calculated. The values are shown in Table 3.

The effect of the N-form is given in the values of ^{15}N content (? g $^{15}\text{N}/\text{g}$ dm) respective to the control treatment. The performance of both species was similar in that, for a same species, the ^{15}N content for the two N-treatments ($^{15}\text{N}-\text{NO}_3$ and $^{15}\text{N}-\text{NH}_4$) was similar. The values were few higher for the ammonia treatment. The fact that the tracer used for the $\text{N}-\text{NH}_4$ treatment were more enriched in ^{15}N (10 at% versus 9 at%) could explain the difference between the values of both treatments. *S. erectum* accumulated more ^{15}N per unit of dry weight than *T. domingensis*, this fact suggests that *S. erectum* is more efficient (>60%) in the removal of N.

The recovery percentage of applied tracer in plant biomass is calculated from the results of biomass dry weight and ^{15}N content, respective to the amount of ^{15}N applied in each

treatment. Table 3 shows that *Sparganium* and *Typha*, grown as floaters, yield similar % recovery (60.5%) in the ammonia N treatment; therefore, the higher N-removal efficiency of *Sparganium* compensated the effect of the dry weight -lower figures- of *Sparganium* in this experiment. This was not the case for the $^{15}\text{N-NO}_3$ treatment, in which *T. domingensis* recovered more nitrogen (76.5%) than *S. erectum* (67.8%). Lusby et al (1998), in a ^{15}N study of the fate of N-NH_4 in *Typha orientalis*, obtained 4.5 to 21% recovery for single plants (10-15 cm length) grown in sediment columns for 28 days, simulating a natural wetland.

Isotope ratio analysis of the nutritive solution sampled at the end of the experiment clearly showed the presence of the ^{15}N tracer. Average values obtained for the trials with *S. erectum* were 0.8180 at% ($\text{N-}^{15}\text{NO}_3$ treatment) y 0.8652 at% ($\text{N-}^{15}\text{NH}_4$), and for *T. domingensis* 0.6086 at% ($\text{N-}^{15}\text{NO}_3$) and 0.7613 at% ($\text{N-}^{15}\text{NH}_4$). These figures showed that nitrogen was still available in the nutritive solution at the end of the experiment. Therefore, the possible nitrogen losses by volatilization or de-nitrification -nitrogen lost from the system- plus the nitrogen recovered in the plant biomass did not exhaust the amount of nitrogen applied to the nutrient medium.

From the results of % recovery of the two N forms, the rate of N absorption is estimated. The young plants of *S. erectum* y *T. domingensis* grown as floaters in a solution containing 17.5 ppm N (50% N-NO_3 + 50% N-NH_4) absorbed 0.4488 and 0.2678 mg N/g dm/day respectively. These figures are equivalent to a N uptake of 0.2030 and 0.2115 mg N/plant/day for *S. erectum* y *T. domingensis*, respectively, given that the average biomass dry weight in the period was 0.4523 g/plant and 0.7897 g/plant, respectively.

4. CONCLUSIONS

From the results obtained in this work, the following conclusions are drawn:

- ? ?Young plants of *S. erectum* y *T. domingensis*, grown as floaters, are able to uptake nitrogen in the nitric and ammonia form after they are implanted in the system.
- ? ?For a same species, the rate of N absorption is similar when the nitrogen is supplied in the ammonia or nitric form; the treatment with N-NH_4 resulted in values very few higher than the treatment with N-NO_3 .
- ? ?The average rate of absorption of mineral N (N-NO_3 + N-NH_4) by young plants of *S. erectum* y *T. domingensis*, grown as floaters, is estimated at 0.4488 y 0.2678 mg N/g dm/day, respectively.
- ? ? *S. erectum* is more efficient in the removal of N than *T. domingensis*, no matter the mineral N form.
- ? ?The advantage of *S. erectum* of being more efficient in the removal of N may be masked by differences in biomass dry weight.

5. REFERENCES

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7. TABLAS Y FIGURAS

Table 1. Characterization of young plants of *Sparganium erectum* y *Typha domingensis* at the starting point of the experiment. DW, dry weight; N, content in total nitrogen (dry weight basis); at, atomic isotope ratio $^{15}\text{N}/\text{N}_{\text{total}}$. (n=4)

Species	Shoot			Roots		
	DW (g)	N (%)	at (%)	DW (g)	N (%)	at (%)
<i>S. erectum</i>	0.2066	2.446	0.3658	0.1549	1.919	0.3668
<i>T. domingensis</i>	0.3212	1.988	0.3665	0.3143	1.650	0.3655

Table 2. Characterization of young plants of *Sparganium erectum* y *Typha domingensis* at the end of the experiment. DW, dry weight; N, content in total nitrogen (dry weight basis); at, atomic isotope ratio $^{15}\text{N}/\text{N}_{\text{total}}$. (n=4)

Treatment	Species	Shoot			Roots		
		DW (g)	N (%)	at (%)	DW (g)	N (%)	at (%)
N- $^{15}\text{NO}_3$	<i>S. erectum</i>	0.3275	2.363	1.3716	0.2257	1.882	1.7357
	<i>T. domingensis</i>	0.5773	1.697	1.2577	0.4678	1.578	1.3018
N- $^{15}\text{NH}_4$	<i>S. erectum</i>	0.3492	2.259	1.4776	0.1839	2.079	1.7468
	<i>T. domingensis</i>	0.4556	1.650	1.3048	0.3873	1.529	1.4995
Control	<i>S. erectum</i>	0.3481	2.003	0.3667	0.2090	1.977	0.3653
	<i>T. domingensis</i>	0.5481	1.444	0.3660	0.5293	1.531	0.3654

Table 3. Balance of the experiment. DW, plant biomass dry weight; N, plant biomass content in nitrogen (weighed mean); ? at, isotope ratio increment respective to control; %recovery, recovery of added ^{15}N in plant biomass; dm, dry matter.

Treatment	Species	DW (g)	N (%)	? at (%)	^{15}N derived from tracer ($\mu\text{g } ^{15}\text{N}/\text{g dm}$)	% recovery
N- $^{15}\text{NO}_3$	<i>S. erectum</i>	2.2126	2.176	1.156	251.6	67.76
	<i>T. domingensis</i>	4.1803	1.644	0.915	150.4	76.54
N- $^{15}\text{NH}_4$	<i>S. erectum</i>	2.1325	2.201	1.204	265.1	62.17
	<i>T. domingensis</i>	3.3715	1.564	1.014	158.7	58.82