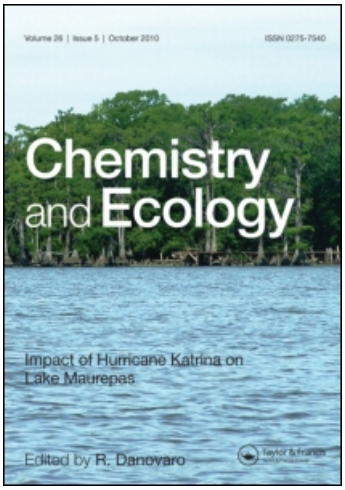

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Transport of Inorganic Phosphate in the Marine Seagrass *Posidonia Oceanica* By ^{32}P-Phosphate As Tracer

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TRANSPORT OF INORGANIC PHOSPHATE IN THE MARINE SEAGRASS *POSIDONIA OCEANICA* BY ^{32}P-PHOSPHATE AS TRACER

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Uptake and loss of inorganic phosphate by *Posidonia oceanica* leaf tissue has been studied in *in vitro* experiments. Experimental data have shown that a steady state of inorganic phosphate uptake (about 40 nmol mg^{-1} dry wt.) is attained after 48 hours. In particular high accumulation (over 1000-fold the natural level in sea water) and slow loss (biological half-life, 65 days) of inorganic phosphate has been evaluated. Moreover the effect of three different metabolic inhibitors (sodium monovanadate, sodium azide, 2,4-dinitrophenol) have been tested. Results of this effect and the high degree of inorganic phosphate accumulation in leaf tissue have demonstrated that inorganic phosphate carrier is energy dependent. Furthermore, the inorganic phosphate uptake is probably influenced by bivalent cations (Ca^{+2}, Mg^{+2}) but the mechanism is still uncertain.

Preliminary kinetic study has shown interesting results. In particular, k_m estimated value (2.8 μmol l^{-1}) has demonstrated the existence of a relatively high uptake rate (V_{max}) at low DIP concentration while the kinetic study of inorganic phosphate loss from leaf tissues has shown a low value of the biological half life (about 60–70 days). This evidence could be significant for the existence of a complex distribution of inorganic phosphate in the leaf tissues.

KEY WORDS: ^{32}P-phosphate, phosphate transport, seagrass, *Posidonia oceanica.*

INTRODUCTION

In water ecosystems, different phosphorus species exist at low concentrations (0.1 – 1 μmol). Phosphorus with other important nutrients like nitrogen derivatives leads to serious eutrophication phenomena when a high extent of nourishing materials are present in the water column. The main causes of eutrophication derive from autogenous sources (plants senescence) and particularly from anthropomorphic sources (use of fertilisers in agriculture, detergents).

Several investigations have been carried out regarding nutrient dynamics (Hocking *et al.*, 1981; Kistritz, 1978; Kirkman *et al.*, 1979; Brix and Lingby, 1985) and nutrient concentration in some marine phanerogams. In particular, a recent study carried out on *Thalassia testudinum* (Fourqurean *et al.*, 1992) has shown a close correlation between intra-tissular uptake of phosphorilated nutrients and phosphate concentrations in water. Other authors (Delgado, 1986; Delgado and Vidal, 1989) have

demonstrated the response of *Posidonia oceanica* in causing heterogeneity and modification of distribution of dissolved inorganic phosphorus (DIP) in the water column.

Preliminary investigations on uptake of inorganic phosphate by *Posidonia oceanica* (Fresi and Saggiomo, 1981) have demonstrated the existence of a relation between phosphorus uptake and its external concentrations.

Nevertheless, the knowledge about inorganic phosphate transport in this macrophyte is rather short as well as its potentiality as a "sentinel accumulator" of inorganic phosphate levels in water column.

MATERIALS AND METHODS

Sampling

Samples of *Posidonia oceanica* were collected in June at 5 m depth in the northern coastline of the Latium Region (Italy). The plant samples were placed in plastic bags filled with fresh sea water for transport, were gently rubbed and rinsed with tap water to remove adhering sediment, debris and epiphytes. These plants were then placed in an appropriately equipped glass tank, containing 300 litres of continuously aerated and filtered sea water. A constant temperature of $18°C \pm 1°C$ was maintained and a photo-period of 12 h was provided using a light intensity of 2.5 mW cm^{-2}. Sea water was added with commercial sodium hypochloride (final concentration 6 μg ml^{-1}) to prevent the growth of epiphytes. At this concentration, as shown below, hypochloride did not affect inorganic phosphate uptake.

Inorganic, organic and total phosphate analysis

Leaf samples of 24 cm length, rinsed with distilled water, were cut in 3-cm sections and pulverised by liquid nitrogen. Inorganic phosphate was extracted by 1M $HClO_4$ for 20 min at 4°C, to avoid enzyme catalysed ATP hydrolysis. The inorganic phosphate content in 1 ml of the supernatant, and the total phosphate content after acidic oxidative combustion of the leaf sections, were estimated by spectrophotometric analysis (Van Veldhoven and Mannaerts, 1987) using a Carl Zeiss PMQ-II Spectrophotometer. The organic phosphate was calculated by subtracting the inorganic phosphate content from the total value.

Analysis of inorganic phosphate uptake

a) Incubation and measurement Leaf tissues (sections 3-cm long) rinsed with double distilled water were left in a ^{32}P-phosphate solution at high specific activity (70,000 cpm ml^{-1}) for a long time period (about 48 hours). The net uptake of ^{32}P tracer were measured according to the method described by Walker and Pittman (1976).

According to this method leaf tissues were blotted and an experimental protocol was developed to remove *free space* interference (Briggs, 1957). This protocol was

applied to allow rapid washing and transfer of each leaf section through vessels containing fresh sea water. Tracer loss of ^{32}P in the eluting solutions could be determined in relatively short time and could be calculated by liquid scintillation analysis (β Counter Camberra-Packard 460 C). Correction for radioactive decay of the isotope was made for all the samples. For all the experiments a temperature of $18°C \pm 1°C$ was maintained. Preliminary experiments showed that free space interference is eliminated in 20–30 min time. The ^{32}P net uptake was determined on the basis of a simple mathematical method.

b) Analysis of inorganic phosphate uptake versus time Samples of 3-cm leaf sections (11 mg dry wt.*) were left in a solution containing $NaH_2{}^{32}PO_4$ in 15 ml of filtered sea water for different periods: 6, 12, 24, 48, 72, 96 hours. The ^{32}P-phosphate uptake (cpm mg^{-1}) was analysed according to the method described above (*a*). Analyses of radioactivity were performed separately on leaf sections, incubation and eluting solutions by liquid scintillation technique.

c) Inorganic phosphate uptake versus DIP concentration The analysis was performed as described in *b*) but adding NaH_2PO_4 in each solution for leaf incubation (10, 50, 100, 500, 1000, 5000 nmol ml^{-1}) for 48 hours (time necessary to attain steady-state).

d) Effect of sodium hypochloride on DIP uptake The experimental procedure described in the previous paragraph has been used. The inorganic phosphate uptake was analysed in sea water with sodium hypochloride (6 μg ml^{-1}) and compared with the same experiment carried out in sea water without hypochloride added.

e) Effect of some bivalent cation (Ca^{+2}, Mg^{+2}) The analysis was performed as described in *c*) using saline solutions of different composition (soln. A: 3.4% NaCl, 10mM $CaCl_2$, pH = 8; soln. B: 3.4% NaCl, 0.0052 mM $MgCl_2$, pH = 8; soln. C: 3.4% NaCl, 10 mM $CaCl_2$, 0.0052 mM $MgCl_2$, pH = 8) as an incubation medium.

f) Effect of some metabolic inhibitor (2,4-dinitrophenol, sodium azide, sodium monovanadate) The analysis was performed as described in *c*) but adding in separate experiments: 0.1 mM 2,4-dinitrophenol (phosphate transport inhibitor not specific), 1 mM sodium azide (phosphate transport inhibitor at tonoplaste), 0.1 mM sodium monovanadate (phosphate transport inhibitor at plasmalemma) in 15 ml of filtered sea water for 48 hours.

Kinetic study on inorganic phosphate uptake (Estimation of K_m)

The analysis was performed as described in c) adding NaH_2PO_4 (5, 10, 15, 20 nmol ml^{-1}) in 15 ml of filtered sea water for 2 hours. After this time phosphate uptake was analysed.

The uptake rate (nmol P h^{-1} mg^{-1}) was analysed by a rearranged form of the Michaelis-Menten equation.

*dry wt. is specified and not included in text that follows.

Kinetic study on inorganic phosphate loss (Estimation of biological half-life, T_b)

Samples of 3-cm leaf sections were left with moderate stirring in a solution containing 5 mM NaH_2PO_4 in 15 ml of filtered sea water for 48 hours.

After incubation, 3 leaf sections were analysed for inorganic phosphate content by spectrophotometry. The remaining leaf sections (20 samples, about 1 g wet wt.) were used for inorganic phosphate loss analysis. Consequently, they were transferred in 15 ml of sea water solution and stirred for 4 hours. Then 1 ml of this solution was analysed by spectrophotometry to estimate inorganic phosphate loss. At the same time leaf sections were transferred in another 15 ml of sea water again and stirred for 8 hours and 1 ml of this solution was analysed by spectrophotometry. This procedure was repeated again after 1, 2, 5, 14, 15 days from the start of the experiment. After 15 days, the experiment was stopped to avoid the possible putrefaction of leaf tissues. On the basis of the inorganic phosphate loss data a simple mathematical extrapolation of T_b was evaluated.

RESULTS

Table I lists the natural values of inorganic phosphate distribution from the leaf base to the tip. It can be observed that the highest inorganic phosphate content was estimated in the first 6 cm of each leaf. This is not surprising since this tissue is constituted of actively regenerating cells (meristematic tissue).

Figure 1 shows ^{32}P tissue uptake *vs.* time. The plot profile shows that increasing incubation time the ^{32}P-tracer uptake by leaf sections rises and levels-off at steady-state value after 48 h.

Table II shows dose-response analysis of the inorganic phosphate uptake process (nmol mg^{-1}) by leaf section compared with dose response analysis estimated with 0.1 mM 2,4-dinitrophenol, 1 mM sodium azide and 0.1 mM sodium monovanadate. It can be observed that with increasing inorganic phosphate concentration in sea water without inhibitors, the uptake by leaves rises and levels-off at a steady-state

Table I Inorganic, organic and total phosphate concentration (nmol P mg^{-1}) in 3-cm sections of the leaf of *Posidonia oceanica* at different distances (cm) from the leaf base (mean value ± standard deviation; $n = 3$).

Distance (cm)	*Inorganic P (nmol mg^{-1})*	*Total P (nmol mg^{-1})*	*Organic P (nmol mg^{-1})*
0–3	21.0 ± 2.0	55.4 ± 6.0	34.4 ± 3.0
3–6	19.4 ± 2.0	54.4 ± 5.5	35.0 ± 3.0
6–9	13.4 ± 1.3	50.9 ± 5.0	37.5 ± 4.0
9–12	13.1 ± 1.3	43.9 ± 4.0	30.8 ± 3.0
12–15	11.4 ± 1.1	46.4 ± 4.5	35.0 ± 3.5
15–18	11.2 ± 1.0	40.3 ± 4.0	29.1 ± 3.0
18–21	13.8 ± 1.5	41.0 ± 4.0	27.2 ± 3.0
21–24	10.3 ± 1.0	43.0 ± 4.0	32.7 ± 3.0
24–27	10.9 ± 1.0	46.9 ± 4.5	36.0 ± 3.5

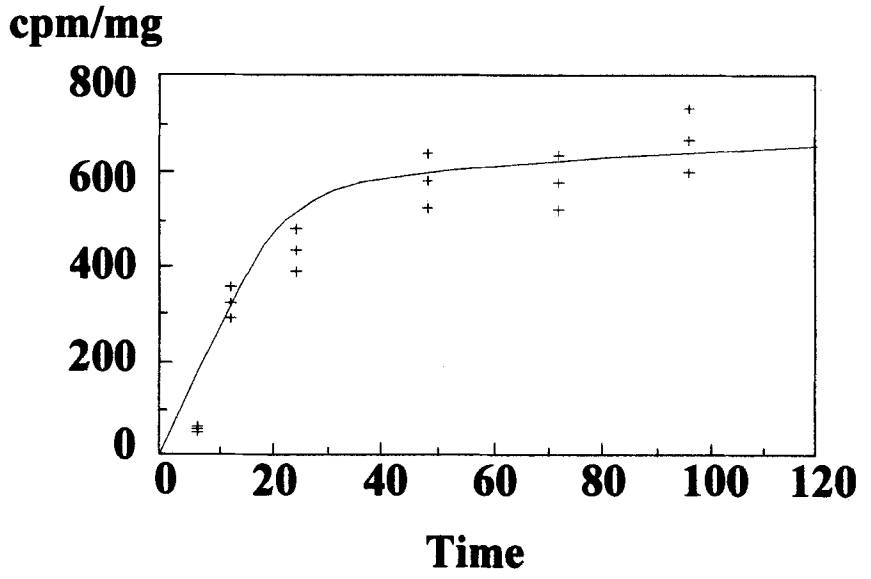

Figure 1 ^{32}P-phosphate uptake (cpm mg^{-1} dry wt.) *vs.* time (hours).

Table II Inorganic phosphate uptake (nmol P mg^{-1}) in *Posidonia oceanica* leaf sections, *vs.* inorganic phosphate (nmol ml^{-1}). Effects of three different inhibitors: 0.1 mM sodium monovanadate, 1 mM sodium azide, 0.1 mM 2,4-dinitrophenol (mean value ± standard deviation; $n = 3$).

Incubation (48 hrs) (nmol ml^{-1})	*Uptake without inhibitor (nmol mg^{-1})*	*Uptake with sodium monovanadate (nmol mg^{-1})*	*Uptake with sodium azide (nmol mg^{-1})*	*Uptake with 2,4-dinitrophenol (nmol mg^{-1})*
0	0	0	0	0
10	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.01	0.3 ± 0.05
50	1.6 ± 0.2	0.9 ± 0.1	0.3 ± 0.005	1.3 ± 0.1
100	3.4 ± 0.3	1.3 ± 0.1	0.9 ± 0.1	3.1 ± 0.5
500	18.2 ± 2.0	1.9 ± 0.2	1.0 ± 0.1	6.7 ± 0.7
1000	32.9 ± 3.0	2.0 ± 0.2	1.4 ± 0.1	13.5 ± 1.5
5000	40.0 ± 4.0	2.6 ± 0.3	2.1 ± 0.2	16.6 ± 1.7

value (about 40 nmol mg^{-1}). On the other hand, the effect of the different inhibitors results in a considerable reduction of steady-state value. In particular sodium azide and sodium monovanadate seem to inhibit inorganic phosphate transport much more than 2,4-dinitrophenol. While the latter stops inorganic phosphate transport, sodium azide and sodium monovanadate inhibit its accumulation in the tissues.

Table III shows dose-response analysis of the inorganic phosphate uptake (nmol P mg^{-1}) by leaf sections measured in sea water compared with dose response curve estimated in sea water with sodium hypochloride (6 μg ml^{-1}). Data values demonstrate that no valuable effect on inorganic phosphate uptake can be detected.

Table III Inorganic phosphate uptake (nmol P mg^{-1}) in *Posidonia oceanica* leaf sections *vs.* inorganic phosphate (nmol ml^{-1}). Effects of NaClO (6 μg ml^{-1}) (mean value ± standard deviation; $n = 3$).

Incubation (48 hrs) (nmol ml^{-1})	*Uptake without NaClO (nmol mg^{-1})*	*Uptake with NaClO (nmol mg^{-1})*
0	0	0
10	0.1 ± 0.01	0.1 ± 0.01
50	1.6 ± 0.2	2.9 ± 0.3
100	3.4 ± 0.3	5.1 ± 0.5
500	18.2 ± 2.0	15.8 ± 1.5
1000	32.9 ± 3.0	28.9 ± 3.0
5000	40.0 ± 4.0	37.0 ± 3.5

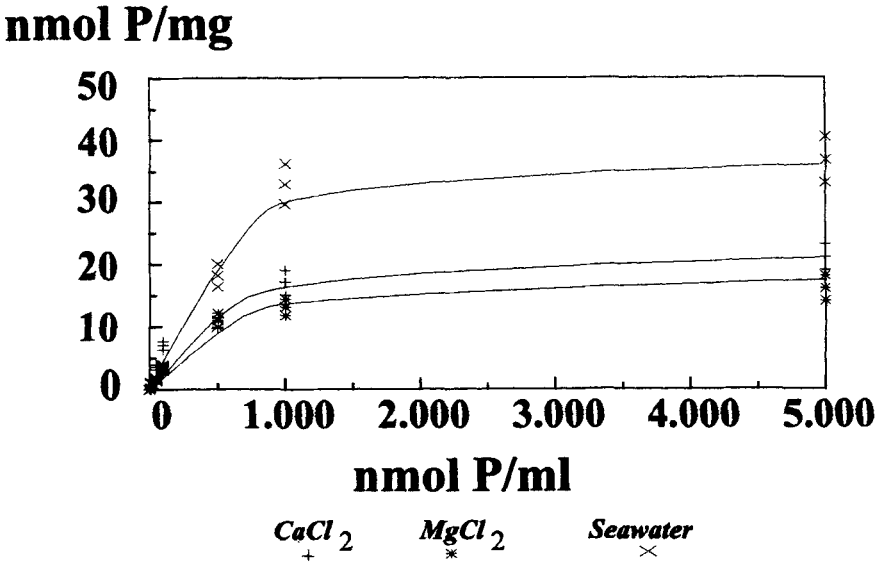

Figure 2 Inorganic phosphate uptake (nmol P mg^{-1} dry wt.) in *Posidonia oceanica* leaf sections *vs.* inorganic phosphate (nmol ml^{-1}). Effects of two different bivalent cations: Ca^{+2}, Mg^{+2} (mean value ± standard deviation; $n = 3$).

Figure 2 shows dose-response curve of the inorganic phosphate uptake process by leaf sections measured in sea water compared with dose response curve estimated in saline solution containing respectively Ca^{+2} and Mg^{+2}. It can be observed that steady-state value of uptake is attained at about 40 nmol mg^{-1} in sea water, at 20 nmol mg^{-1} in Ca^{+2}-saline, and at 18 nmol mg^{-1} in Mg^{+2}-saline.

Figure 3 shows the Lineweaver-Burk graph for the calculation of the Michaelis-Menten constant ($K_m = 2.8$ μmol l^{-1}). The data obtained show that the V_{max} is reached at low concentration of inorganic phosphate in the water.

In Table IV are reported inorganic phosphate accumulation data values in sea water (nmol 15 ml^{-1}) and inorganic phosphate residual data values (nmol mg^{-1} wet wt.) in *Posidonia oceanica* leaf tissue over time (days). The biological half-life (T_b) was estimated about 65 days. If we consider the relative short duration of the experiment (to avoid leaf tissue degeneration), the T_b estimated is quite uncertain but it may be considered indicative to describe the inorganic phosphate loss. Moreover, this T_b value could represent an important starting point to demonstrate a complex inorganic phosphate distribution across leaf tissue compartments.

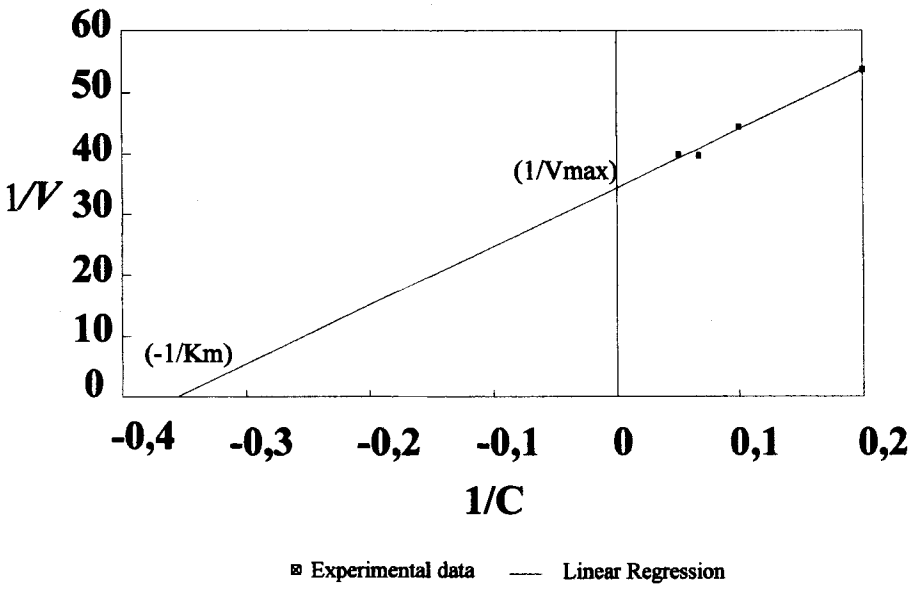

Figure 3 Graphic extrapolation of the K_m value.

Table IV Inorganic phosphate accumulation (nmol P 15 ml^{-1}) in sea water and inorganic phosphate residual (nmol P g^{-1} wet wt.) in *Posidonia oceanica* leaf sections *vs.* time (days). The value 8000 nmol g^{-1} is referred to maximal inorganic phosphate uptake after 48 h of incubation at 5000 nmol P ml^{-1} (mean value ± standard deviation; $n = 3$).

Time (days)	*P accumulation nmol 15 ml^{-1}) (sea water)*	*P residual (nmol g^{-1} w.wt.) (leaf tissue)*	*%*
0	0	8000	100
4(*)	748 ± 68	7252	90.7
8(*)	892 ± 87	7108	88.9
1	1056 ± 96	6944	86.8
2	1099 ± 120	6901	86.3
5	1261 ± 130	6739	84.2
14	1957 ± 195	6043	75.5
15	2013 ± 200	5987	74.8

*Hours.

DISCUSSION

The results obtained with these *in vitro* experiments show that *Posidonia oceanica* takes up a large amount of inorganic phosphate by leaf tissue and probably accumulates it in the vacuole. All data values seem to demonstrate that the uptake is dependent on DIP concentration. In particular, by increasing DIP concentration up to 5000 nmol ml^{-1}, there is a 4-fold increase in the deal rate. This preliminary evidence is confirmed by comparing inorganic phosphate concentration in sea water (about 1 nmol ml^{-1}) with the basal concentration in the leaf tissue (about 1-2 μmol g^{-1} wet wt.). The concentration ratio is about 1000. Experiments on the effects of three metabolic inhibitors of nutrient uptake (2,4-dinitrophenol, sodium azide, sodium monovanadate) show a strong inhibitory effect as indicated by lower steady-state level of inorganic phosphate uptake. This result has a great interest in demonstrating the existence of a energy dependent transport.

Sodium azide and sodium monovanadate strongly inhibit inorganic phosphate uptake, much more than 2,4-dinitrophenol. This phenomenon is strictly connected to a different inhibition mechanism. Indeed 2,4-dinitrophenol inhibits inorganic phosphate transport while sodium azide and sodium monovanadate block accumulation processes in the cytosol and in the vacuole. Moreover, the inorganic phosphate uptake is probably influenced by bivalent cations such Mg^{+2} and Ca^{+2} but the mechanism is still uncertain.

Preliminary kinetic investigations on the inorganic phosphate uptake show interesting results. In particular, K_m estimated value (2.8 μmol l^{-1}) demonstrates the existence of a relatively high uptake rate (V_{max}) at low DIP concentration while the kinetic study of inorganic phosphate loss from leaf tissues shows a low value of the biological half life (about 60–70 days). This evidence could be significant for the existence of a complex distribution of inorganic phosphate in the leaf tissues.

On the basis of these results it is possible to discuss two preliminary conclusions: *Posidonia oceanica* highly concentrates DIP in the leaf tissues; high rate of inorganic phosphate uptake matches with slow rate of loss.

These data are in agreement with the analysis carried out by Fourqurean *et al.* (1992) on *Thalassia testudinum.* Indeed in both *Thalassia testudinum* and *Posidonia oceanica* the inorganic phosphate content depends on the DIP levels in external water column. This evidence could be considered of great interest as it shows that this angiosperm "memorises" inorganic phosphate concentration at a site. These preliminary results combined with an appropriate collection strategy propose this angiosperm as a potential sentinel accumulator of inorganic phosphate in marine coastal ecosystems.

References

Briggs, G. E. (1957) Some aspects of free space in plant tissue. *New Phytologist* **56**: 305–324.

Brix, H. and Lyngby, J. E. (1985) Uptake and translocation of phosphorus in eelgrass (*Zostera marina*). *Marine Biology* **90**: 111–116.

Delgado, O. (1986) Contenido de fosforo en los tejidos de fanerogamas marinas del Mediterraneo occidental y su relacion con la dinamica de cada especie. *Oecologia aquatica* **8**: 139–151.

Delgado, O. and Vidal, M. (1989) Phosphorus cycling in Mediterranean seagrass ecosystems: phosphorus content of vegetal tissues and sediment. *International Workshop on Posidonia Beds*, Boudouresque C.F., edit. Meinesz A., Fresi E. and Gravez. V. GIS Posidonie publ., **2**: 93–100.

Fourqurean, J. W., Zieman, J. C., Powell G. V. N. (1992) Phosphorus limitation of primary production in Florida Bay: Evidence from C:N:P ratios of the dominant seagrass *Thalassia testudinum. Limnol. Oceanogr.* **37**: 162–171.

Fresi, E. and Sagggiomo, V. (1981) Phosphorus uptake and transfer in *Posidonia oceanica (L)* Delile. *Rapp. P.V. Commis. Internation. Explor. Sci. Mer.. Medit.* **27**: 187–188.

Hocking, P. J., Cambridge, M. L. and McComb A. J. (1981) The nitrogen and phosphorus nutrition of developing plants of two seagrasses, *Posidonia australis and Posidonia sinuosa. Aquatic Botany* **11**: 245–261.

Kirkman, H., Griffiths, F. B. and Parker R. R. (1979) The release of reactive phosphate by a *Posidonia australis* seagrass community. *Aquatic Botany* **6**: 329–337.

Kistritz, R.U. (1978) Recycling of nutrients in an enclosed aquatic community of decomposing macrophytes *(Myriophyllum spicatum). Oikos* **3**: 561–569.

Van Veldhoven, P. P and Mannaerts G. P. (1987) Inorganic and organic phosphate measurements in the nanomolar range. *Anal. Biochemistry* **161**: 45–48.

Walker, N. A. and Pitmann, M. G. (1976) Measurements of fluxes across membranes, 94–126. In: *Transport in Plants II*, Part A, Cells; Edited by U. Little and M. Pitmann, Springer-Verlag Berlin, Heidelberg, N.Y.