Absorption by *Chlorella Vulgaris* Beijer. of Phosphorus Compounds Released by the Submerged Macrophyte *Potamogeton Pectinatus* L.

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Abstract

The green alga, *Chlorella vulgaris* Beijerinck was used to study uptake of phosphorus compounds released by the stems and leaves (foliage) and the roots of a submerged freshwater macrophyte, *Potamogeton pectinatus* L. *Chlorella* was observed to absorb more of the phosphorus compounds released by the foliage and the roots of *Potamogeton* under light than under dark conditions and, furthermore, to show a greater affinity for phosphorus compounds released by the roots of this hydrophyte compared to those of the foliage. In freshwater lakes, phosphorus compounds released by submerged macrophytes may be an important source of phosphorus for phytoplankton.

Introduction

A number of researchers have shown that dissolved organic phosphorus (DOP) is released by marine algae (cf. Fogg, 1971) while Lean (1973) in his investigation on the phosphorus cycle in the epilimnion of a freshwater lake, found that an organic phosphorus compound of low molecular mass is released by phytoplankton. Lean and Nalewajko (1975) also established that the principal phosphorus flux was an exchange of phosphate between the limnetic water and four species of freshwater algae investigated.

Concerning the release of phosphorus compounds by submerged marine macrophytes, McRoy and Barsdate (1970) found that *Zostera marina* releases an unidentified phosphorus compound into the surrounding water, while Reimold (1972) reports that for each gram of fresh plant tissue *Spartina alterniflora* releases 380 μg dissolved inorganic phosphorus (DIP) per tidal cycle. Reimold (1972) also stressed the importance of further studies on the fate of these released phosphorus compounds in aquatic ecosystems.

With regard to the release of phosphorus compounds by submerged freshwater macrophytes, Swanepoel and Vermaak (1977) have shown that some of the ^{32}P absorbed by the foliage and roots of *Potamogeton pectinatus*, following transportation, is released into the surrounding medium by the roots and foliage, respectively.

In the present investigation *Chlorella vulgaris* which was available in its axenic condition was used as test organism to determine the ability of this alga to absorb phosphorus released by both the foliage and the roots of *Potamogeton pectinatus*.

TABLE 1
WATER CHEMISTRY OF THE AQUARIUM WATER IN WHICH THE *POTAMOGETON PECTINATUS* PLANTS WERE GROWN

pH	8,35
Conductivity	400,00 μS cm^{-1}
Dissolved oxygen	8,55 mg ℓ^{-1}
Chloride	40,00 mg ℓ^{-1}
Total hardness as $CaCO_3$	140,00 mg ℓ^{-1}
Ammonia – N	0,50 mg ℓ^{-1}
Nitrite – N	0,13 mg ℓ^{-1}
Nitrate – N	0,25 mg ℓ^{-1}
Soluble reactive phosphorus	0,05 mg ℓ^{-1}

Methods

Potamogeton pectinatus plants were grown in an aquarium from tubers collected from a laboratory stream ecosystem in which a variety of aquatic macrophytes were cultivated (Vermaak *et al.*, 1976). The aquarium, filled with water collected from the laboratory stream ecosystem, was housed in a growth cabinet provided with cool white fluorescent lamps. A photoperiod of 14 h was used at an incubation temperature of 22°C. Some physico-chemical analysis made of the aquarium water according to APHA (1971) is provided in Table 1. Nine days after planting of the tubers, young plants were already 9 to 12 cm in size and suitable for experiments.

After removal, a plant from the aquarium was thoroughly cleaned of epiphytes and placed in a sterilized culture vessel in which the foliar parts were separated from the roots (Swanepoel and Vermaak, 1977). A water sample from the aquarium in which the plants were grown was then passed through a 0,22 mm membrane filter to exclude bacterial contamination and an aliquot of 130 mℓ added to both the upper and lower compartments of two culture vessels. 5 μCi (18,5 x 10^4 Bq) of carrier-free radio-active phosphorus, as phosphate, were then added to the upper compartment of one vessel and the same activity to the lower compartment of one vessel and the same activity to the lower compartment of the other. After incubation for 6 h at 22°C under 20 000 lx in a Conviron growth cabinet, a 1 mℓ water sample was withdrawn from the upper compartment of the vessel into which the radio-isotope had been added to the

lower compartment and the same volume from the lower compartment of that vessel into which $^{32}PO_4$ had been added to the upper compartment. The radio-activity of the released phosphorus compounds ($Z^{32}P$) was subsequently determined on the water samples in a liquid scintillation spectrometer. 10 mℓ Aliquots of the medium containg $Z^{32}P$ released by the roots of *Potamogeton* (root-$Z^{32}P$) were next transferred to 4 triplicate sets of autoclaved vials. The same volumes of the medium containing $Z^{32}P$ released by the foliage of the pond weed (foliar-$Z^{32}P$) were transferred to another 4 sets of autoclaved vials. To each of these 8 sets of vials were added 2 mℓ of an axenic *Chlorella vulgaris* culture kept in a phosphate-free BG-11 medium (Stanier *et al*, 1971). Four sets of the vials of which 2 contained root-$Z^{32}P$ and another 2 foliar-$Z^{32}P$ were then incubated for 6 h in the light whilst the other 4 sets were incubated for the same period in the dark. After incubation, the contents of each vial was separately passed through a 0,45 μm membrane filter and the radioactivity of 1 mℓ of the filtrate of each determined. The membrane filters containing the algae were then rinsed with an aliquot of non-radio-active incubation medium previously passed through a 0,22 μm membrane filter. The filters with the algae were directly placed into vials containing 20 mℓ of a water soluble scintillation cocktail (Scintisol Complete, Isolab) and the radioactivity determined.

The necessary corrections for quenching and radio-active decay were made. Results were expressed in disintegrations per minute per mℓ.

Results and Discussion

Phosphorus compounds ($Z^{32}P$) released by both the foliage and roots of the submerged macrophyte *Potamogeton pectinatus* are absorbed by *Chlorella vulgaris* under both light and dark conditions (Table 2). *Chlorella* absorbs, however, more $Z^{32}P$ when incubated in the light than in the dark (Table 2, column B), a phenomenon which may be related to photosynthetic activity. From Table 2 (column A) it is further evident that the ^{32}P-activity of the *Chlorella* incubation medium derived from the roots of *Potamogeton* (root $Z^{32}P$) is substancially higher than that derived from the foliage (foliar $Z^{32}P$) of this hydrophyte. In other experiments in which the uptake and release of ^{32}P by *P. pectinatus* were studied (cf. Swanepoel and Vermaak, 1977), it was observed that foliar absorption consistently exceeded root absorption and that approximately 20 times more $Z^{32}P$ was released by the roots than by the leaves.

During both light and dark incubation, *Chlorella* absorbed more or less 40 times more root-$Z^{32}P$ than foliar-$Z^{32}P$. This phenomenon may partially be ascribed to the higher $Z^{32}P$ concentration of the incubation medium derived from the root compartment of the culture vessel. However, when the ^{32}P-activity in the algae is expressed as a percentage of the initial $Z^{32}P$-activity of the incubation media an approximate ratio of 2:1 is still obtained. This clearly indicates that the algae absorbed relatively more of the $Z^{32}P$ released by the roots of *P. pectinatus*.

From the results obtained it is clear that *Chlorella* does absorb phosphorus compounds released by *Potamogeton*. In freshwater lakes phosphorus compounds released by the foliage of submerged macrophytes may well be an important source of phosphorus to phytoplankton and epiphytes associated with submerged macrophytes. The fact that the phosphorus released by the roots of *P. pectinatus*, and thus possibly other aquatic macrophytes, appears to be readily accessible to algae may perhaps account for the frequent development of dense mats of benthic algae observed on the substrate in water bodies such as Germiston Lake in the Witwatersrand area. Another factor which may further contribute to this phenomenon is the development of roots by *Potamogeton pectinatus* above the substrate where organic particles are usually in suspension for a depth of several centimetres and where the phosphorus may be released by the roots directly into the water above the sediment.

More information on the possible interplay between macrophytes, epiphytes, benthic and limnetic algae with respect to released phosphorus compounds may throw more light on this aspect of the phosphorus cycle in a freshwater ecosystem and should therefore be investigated.

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TABLE 2

^{32}P-ACTIVITY OF THE INCUBATION MEDIUM ($Z^{32}P$) USED FOR *CHLORELLA VULGARIS* (A); AVERAGE VALUES OF THE ^{32}P-ACTIVITY OF THE ALGAE FOLLOWING INCUBATION FOR 6 h UNDER BOTH LIGHT AND DARK CONDITIONS (B); AND ^{32}P-ACTIVITY OF THE ALGAE EXPRESSED AS A PERCENTAGE OF THE INITIAL ACTIVITY OF THE INCUBATION MEDIUM (C)

	A		B	C
$Z^{32}P$ released by	(dpm* 10 mℓ$^{-1}$)	Incubation	(dpm* 2 mℓ$^{-1}$)	(B/A %)
Foliage	446	Light	17,1	3,83
		Dark	5,4	1,21
Roots	7 972	Light	646	8,10
		Dark	231	2,90

*dpm = disintegrations per minute

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