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Effect of Fe^{2+}, Mn^{2+}, Zn^{2+} and Pb^{2+} on H^+/K^+ fluxes in excised *Pistia stratiotes* roots

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Absract

Pistia stratiotes is used for the epuration of domestic sewage in the Biyem Assi phytopurification station. During the process, Fe^{2+}, Mn^{2+}, Zn^{2+} and Pb^{2+} are absorbed in substantial amounts by the plant. These metals modify the H^+/K^+ exchange system at the root level. H^+ efflux is inhibited by Fe^{2+} and by Zn^{2+} and enhanced by Mn^{2+} and Pb^{2+}. K^+ influx is inhibited by Fe^{2+}, by Zn^{2+} and by Pb^{2+} and enhanced by Mn^{2+}. It is shown that the purification capacity of *Pistia stratiotes* can vary with the composition of the heavy metals in the surrounding medium.

Introduction

Pistia stratiotes is a floating aquatic weed which has been useful to human beings for a long time (Fittman *et al.* 1975). In Nigeria and China for example, it is believed that this weed has medicinal values. In certain areas, it is used as fodder for animals. *P. stratiotes* prevents water losses by evaporation and in Cameroon, it is commonly used for epuration of domestic sewage. Most of the research available on *P. stratiotes* concerns mainly the occurrence, biomass production, physiology of growth and the mineral composition of the plant (Little and Henson 1967, Idhar and Sharma 1980, Allenby 1981).

Work has been conducted in our laboratory on the influence of some heavy metals in the Biyem Assi sewage on the activity of the H^+/K^+ exchange system in excised *P. stratiotes* roots.

H^+/K^+ exchange is a critical membrane function that contributes to the control of intracellular pH, transmembrane potential difference and the proton motive force which is essential for solute transport (*e.g.* Sze and Churchill 1981, Spanswick 1985). The presence of heavy metal ions in excess amounts in a habitat interferes with the uptake and use of nutrients (Foy *et al.* 1978). Besides, they modify cell wall plasticity (Smith and Raven 1979) and enzyme activities associated with the

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plasmalemma (Leonard 1984, Omokolo *et al.* 1986). Generally, plants react to such conditions by changing the pH of the habitat through H^+ extrusion which modifies the solubility and the activity of the ions.

We present here our findings on the effects of Zn^{2+}, Fe^{2+}, Mn^{2+} and Pb^{2+} ions on the H^+/K^+ exchange. These mineral elements are absorbed in substantial amounts by *P. stratiotes* as the sewage flows from the first through the fifth pond (Agendia 1987). Such a research could come out with a set of conditions for maximum purification by *P. stratiotes*.

Materials and methods

Young *Pistia stratiotes* plants were randomly harvested from the Biyem Assi phytopurification ponds and carried with some water from the growing medium to the laboratory everyday for experimentation. Previous results have shown that the amounts of polluting heavy metals are almost constant in the phytopurification ponds. There *Pistia stratiotes*, *Enydra fluctuans*, *Hydrocotyle ranunculoides* and *Heteranthera callifolia* are well adapted in this habitat (Agendia 1987).

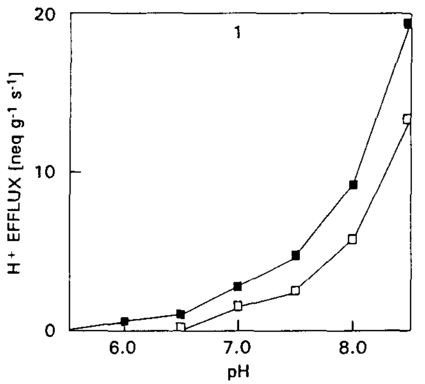
3 g of healthy roots were selected and excised from the plants, washed thoroughly with distilled water and lightly blotted with tissue paper. They were then placed in a 150 cm^3 beaker. The assay medium (80 cm^3) consisted of 25 mM KCl with or without 5 mM $CaSO_4$, pH 8, supplemented with graded concentrations of Fe-EDTA× $FeSO_4$ (0 - 0.75 mM), $MnSO_4$ (0 - 5 mM), $ZnSO_4$ (0 - 1.25 mM) or $PbSO_4$ (0 - 0.75 mM), respectively, as specified for each experiment. The medium was continually stirred during the experiment.

H^+ fluxes were determined by monitoring the pH of the assay medium for 30 min using a pH meter *Methrom 654* (*Methrom SA*, Herisau, Switzerland). During the assay, the pH was kept constant by titration of the solution with 10 mM Tris [hydroxymethyl]-aminomethane. K^+ fluxes were simultaneously measured using a selective electrode connected to an ion meter *Methrom 502* (*Methrom SA*, Herisau, Switzerland). After each experiment the roots were blotted by tissue paper before determination of fresh mass. Except where stated, each value (mean ± SE) was obtained from six independent experiments.

Results

H^+ secretion was recorded in both deionised water and in KCl. H^+ excretion increased with higher pH values (Fig. 1). However, more H^+ was secreted in the presence of KCl than in deionised water. KCl stimulated both H^+ efflux and K^+ influx (Fig. 2). Between 0 and 5 mM KCl, H^+ secretion increased exponentially with increasing KCl concentration in the medium. Above this concentration, the secretion decreased. K^+ influx kept increasing within the 0 - 25 mM KCl range.

Effect of Fe: Fe^{2+} progressively inhibited the extrusion of H^+ as its concentration increased in the external medium. Proton extrusion was very sensitive to this element as more than 50 % inhibition was observed for a concentration of 0.35 mM Fe (Fig. 3A). With 0.75 mM Fe, the inhibition rose up to 95 %. With $CaSO_4$ added to the medium, this inhibitory effect was significantly attenuated: a maximum of 30 % was recorded in 0.75 mM Fe.



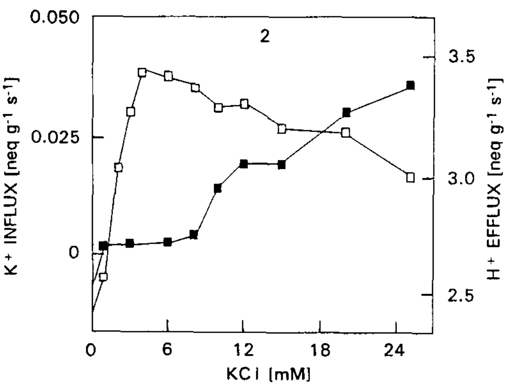


Fig. 1. Effect of pH on H^+ efflux by excised *P. stratiotes* roots in 25 mM KCl (*closed squares*) and in deionised water (*open squares*). Data are representative of a single experiment since the standard error was less than 1 % from one experiment to another.

Fig. 2. Effect of KCl on H^+ efflux (*open squares*) and K^+ influx (*closed squares*) by excised *P. stratiotes* roots at pH 8. Otherwise as in Fig. 1.

The uptake of K^+ by the root segments was also inhibited by Fe^{2+} (Fig. 3B). The inhibition was concentration dependent and increased to about 90 % for a concentration of 0.50 mM Fe. Unlike with H^+ extrusion, the presence of $CaSO_4$ even accentuated the inhibitory effect of Fe^{2+} on K^+ uptake.

Effect of Mn: Proton extrusion was stimulated by the presence of Mn^{2+} in the assay medium whether with or without $CaSO_4$ (Fig. 4A). Without $CaSO_4$, it increased from about 400 neq in 0 mM to more than 500 neq in 2 mM and 700 neq in 4.5 mM $MnSO_4$ representing a stimulation of 25 % and 75 %, respectively. When $CaSO_4$ was added to the reaction medium, H^+ extrusion rose from 400 neq in 0 mM to about 500 neq in 2 mM and 800 neq in 4.5 mM $MnSO_4$, representing a stimulation of 25 % and 100 %, respectively.

The stimulatory effect of Mn^{2+} on K^+ influx was low: about 11 % in 4.5 mM $MnSO_4$ in the presence of $CaSO_4$. Without $CaSO_4$, this effect was also low in the 0 - 3 mM range; beyond this concentration, the stimulation rose to 36 % in 4 mM $MnSO_4$ (Fig. 4B).

Effect of Zn: In the presence of KCl alone or with $CaSO_4$, H^+ efflux was inhibited by the addition of Zn^{2+} in the assay medium (Fig. 5A). The inhibition was less than 10 % in the 0.0 - 0.5 mM range and 34 to 84 % in the 0.50 - 1.25 mM range. The presence of $CaSO_4$ slightly moderated this inhibiting effect of Zn^{2+}.

K^+ uptake was also inhibited by Zn^{2+} in the presence or in the absence of $CaSO_4$ (Fig. 5B). The magnitude of the inhibition in the two cases was almost the same.

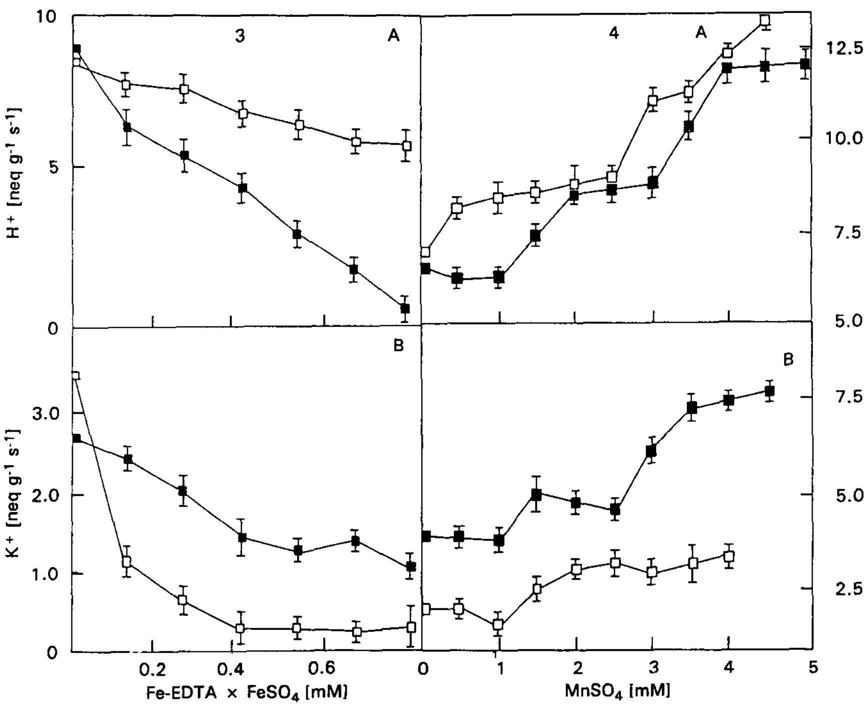


Fig. 3. Effect of Fe^{2+} on H^+ efflux (*A*) and K^+ influx (*B*) by excised *P. stratiotes* roots in 25 mM KCl with (*closed squares*) and without (*open squares*) 5 mM $CaSO_4$ at pH 8. Vertical bars show the standard error of six independent experiments.

Fig. 4. Effect of Mn^{2+} on H^+ efflux (*A*) and K^+ influx (*B*) by excised *P. stratiotes* roots in 25 mM KCl with (*closed squares*) and without (*open squares*) 5 mM $CaSO_4$ at pH 8. Otherwise as in Fig. 3.

Effect of Pb: Pb^{2+} stimulated H^+ extrusion. This stimulation increased steadily with the concentration of Pb^{2+} in the assay medium up to 0.5 mM. Beyond this concentration, H^+ efflux was almost constant. The addition of $CaSO_4$ did not have a significant effect on the extrusion process (Fig. 6A).

Contrarily, K^+ uptake was inhibited by Pb^{2+} (Fig. 6B). In the absence of $CaSO_4$, the inhibition was 6 to 11 % in the 0.15 - 0.35 mM range and 57 to 78 % in the 0.35 - 0.75 mM range. The presence of $CaSO_4$ in the medium weakened the inhibitory effect of Pb^{2+} on K^+ uptake (13 % at 0.75 mM).

Discussion

Pistia stratiotes roots secrete protons into deionised water as well as into the KCl solution. This means that proton pumping does not serve only for the active uptake of solutes. It could also serve other functions like the regulation of cytoplasmic pH and/or the regulation of the rate of uptake of other ions. In fact, the pH of the medium is of critical importance for ion absorption as it modifies the ionic state of the minerals.

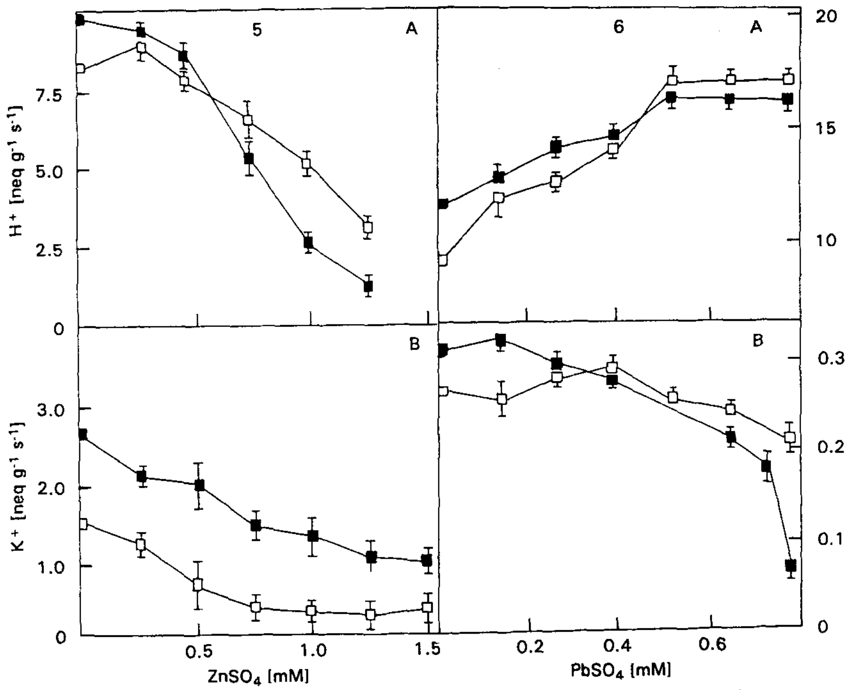


Fig. 5. Effect of Zn^{2+} on H^+ efflux (*A*) and K^+ influx (*B*) by excised *P. stratiotes* roots in 25 mM KCl with (*closed squares*) and without (*open squares*) 5 mM $CaSO_4$, pH 8. Otherwise as in Fig. 3.

Fig. 6. Effect of Pb^{2+} on H^+ efflux (*A*) and K^+ influx (*B*) by excised *P. stratiotes* roots in 25 mM KCl with (*closed squares*) and without (*open squares*) 5 mM $CaSO_4$, pH 8. Otherwise as in Fig. 3.

Previous results have shown that plants respond to metallic ion stress in different ways. Among the physiological responses, there are modification of proton pumping and changes in redox and ATPase activities associated with the plasmalemma (De Vos *et al.* 1986, Rubinstein and Stern 1986). H^+ and K^+ fluxes were influenced by the heavy metals tested. The acidification of the medium was enhanced by Pb^{2+} and by Mn^{2+} and depressed by Zn^{2+} and by Fe^{2+}. K^+ influx was lowered by Pb^{2+}, by Zn^{2+} and by Fe^{2+} ions while Mn^{2+} enhanced the process.

The variations of proton extrusion observed appear to be correlated to the composition of the medium. The ionic composition of the medium determines the uptake and the use of elements due to interactions among ions and between the latter and the membrane charges. The variation indicates the adjustment of the physiological activity of the plant to fit conditions of the environment.

Metallic ion toxicity is a pH-dependent process (Foy *et al.* 1978). Our results show that in the presence of these ions, roots react by modifying proton pumping which in turn modifies the pH of the surrounding medium. This consequently modifies the solubility and the activity of the metallic ions. All these modifications control ion absorption at the root level and constitute the primary adaptation of the plant to a given habitat. In our experiments, the magnitude of the extrusion was specific to each ion and to its concentration in the assay medium.

Our results show that except for Pb^{2+}, a modification of proton pumping was accompanied by a modification in the same manner of K^+ influx. Probably, K^+ is taken up, at least in part, for charge compensation (Sze and Churchill 1981).

The effects of metallic ions on H^+ efflux and K^+ influx were generally depressed by the addition of Ca^{2+} in the assay medium. This Ca^{2+} effect can be accounted for by its binding to negative charges of the plasmalemma where it contributes to the protection of membrane integrity.

P. stratiotes contributes to the purification of the domestic sewage by taking up some metallic ions (Agendia 1987). In our results, it is shown that the absorption capacity of this plant varies with the composition of the heavy metals in the medium. This certainly affects the purification capacity of *P. stratiotes* since H^+/K^+ exchange system is a critical process in solute transport in plants (Spanswick 1985). This implies that *P. stratiotes* should be associated with other aquatic plants having different absorption capacities to maximize the purification of sewage.

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