Absorption and Transport of Pb^{2+} in Young Pea Seedlings

Seshadri Kannan and Hans Keppel

Forschungsanstalt für Landwirtschaft, Braunschweig

(Z. Naturforsch. **31 c**, 393–396 [1976]; received April 1, 1976)

Pb^{2+} Uptake

Short-term experiments were carried out to understand the mechanism of absorption and transport of Pb^{2+} in young pea seedlings. It was found that considerable fraction of Pb^{2+} absorbed could be exchanged with Ca^{2+} in the medium. Furthermore, increasing concentrations of Ca^{2+}, Mg^{2+}, or K^+ in the absorption medium significantly reduced the absorption of Pb^{2+}. Uptake of Pb^{2+} was largely non-metabolic, as shown by its insensitivity to temperature changes and the presence of a metabolic inhibitor.

Presence of Pb^{2+} in the medium was highly inihibitory to the absorption of micronutrients, *viz.*, Fe^{2+}, Mn^{2+}, and Zn^{2+}. The inhibition appeared to be of a physical nature, like blocking the entry or binding of the ions to the ion-carrier.

In recent times, there is an increasing awareness of the accumulation of pollutants in the environment. Several studies [1–5] reveal that the elements like Pb^{2+} are absorbed by plants in significant amounts and prove toxic to plants and eventually man, through the biological cycle. Some aspects of accretion of Pb^{2+} by excised barley roots and its effects on the growth of a few plant species have been investigated by Broyer *et al.* [6]. Although it is considered to be predominantly accumulated in the roots with limited translocation to other parts, a wide degree of variations between plant species is indicated [7].

We have investigated the absorptive patterns of Pb^{2+} in young pea seedlings, as also its interaction with some nutrient elements in their absorption, and the results are presented here. An understanding of the mechanisms of Pb^{2+} absorption is perhaps crucial to an appraisal of the magnitude of the environmental threat posed by elements like Pb^{2+}, directly by their toxicities, and indirectly by blocking the entry of other nutrients from the medium as evidenced here, and perhaps causing deficiency symptoms hitherto not visualized.

Materials and Methods

Pea (*Pisum sativum* L. cv. Lancet) seeds were soaked in aerated distilled water for 3 days in the dark, and grown in half-strength Hoagland's nutrient solution for 8–10 days, under 12 hour photoperiod (10,000 lx) and 20 °C. The absorption experiments were performed by exposing the roots of the seedlings to solutions containing $^{210}Pb^{2+}$ labelled $PbCl_2$ (spec. activ. 2 μCi/μmol) at pH 5.5 and 25 °C. At the end of the experimental period, the roots were desorbed for 20 min in cold (5 °C) 0.1 mM $CaCl_2$, unless otherwise indicated. In the case of uptake of Fe^{2+}, Mn^{2+} and Zn^{2+}, the desorption was carried out in cold unlabelled solutions of the respective salts, instead of $CaCl_2$. The roots and shoot were separated, radioassayed in a gamma-ray spectrometer, and the absolute amounts of the absorbed elements were calculated from the radioactivities of the standard isotope solutions. The results are the means of triplicate samples of individual seedlings, and standard errors are indicated by vertical bars in a representative experiment.

Requests for reprints should be sent to Dr. S. Kanan, Biology and Agriculture Division, Bhabha Atomic Research Centre, *Bombay 400085*, India.

Abbreviation: FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone.

Results

The roots of pea seedlings ware allowed to absorb Pb^{2+} from 0.1 mM $PbCl_2$ for 1 hour, and one set of plants was placed in deionised water, and another in a solution containing 0.1 mM $PbCl_2$ and 0.1 mM $CaCl_2$. Solution samples were drawn periodically for radioassay and the solutions were also renewed each time. From the radioactivities of the medium, the amount retained in the roots at different times were calculated and expressed as a percent of initial absorption. The amount of Pb^{2+} lost in water is that held in the 'water free space', and that exchanged with Pb^{2+} and Ca^{2+} represents that held in the 'Donnan free space' [8]. The results show that considerable amount of Pb^{2+} absorbed is in the Donnan free space, and could be exchanged in a period of about 30 min (Fig. 1).

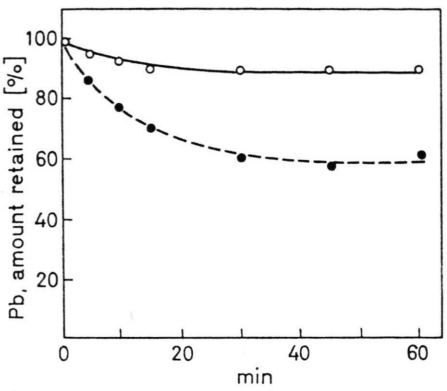

Fig. 1. The amount of Pb^{2+} retained in the roots after their suspension in either distilled water [○——○], or a solution containing 0.1 mM $PbCl_2$ and 0.1 mM $CaCl_2$ [●– –●]. The values are expressed as percent of initial absorption, and the loss into the solution was measured at different time intervals. The root were previously allowed to absorb Pb^{2+} from 0.1 mM $^{210}PbCl_2$ for 1 hour.

When the roots were exposed to 0.1 mM $PbCl_2$ for 4 hours and then transferred to different concentrations of $CaCl_2$, there was considerable loss of Pb^{2+} absorbed, and the loss was found to increase with the concentrations of $CaCl_2$. On the other hand, Pb^{2+} transported to the shoot remained more or less constant, although a very small increase was recorded with $CaCl_2$ concentrations from 1 to 50 mM (Fig. 2).

The time course of absorption of Pb^{2+} from 0.05 mM $PbCl_2$ and transport to shoot at 2 different temperatures (15 and 25 °C) is revealed in Fig. 3, and no significant difference is observed in both the absorption and transport, between the temperature treatments. In another experiment, the absorption and transport of Pb^{2+} from 0.05 mM $PbCl_2$ were measured in the absence and presence of 10^{-6} M FCCP (pH 7), and the inhibitor had no effect (Fig. 4).

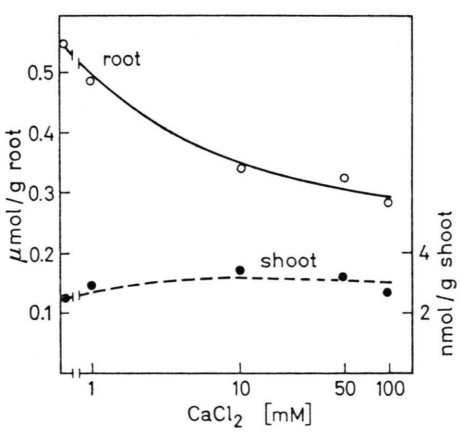

Fig. 2. The effect of post-treatment of the roots of pea seedlings with different concentrations of $CaCl_2$ for 30 min. The roots were previously exposed to 0.1 mM $^{210}PbCl_2$ for 4 hours, and the absolute amounts of Pb^{2+} retained in the roots and shoot are presented.

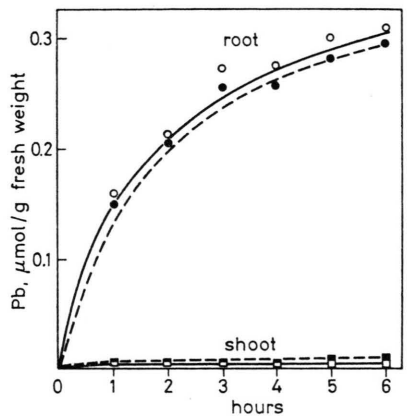

Fig. 3. The rates of absorption and transport of Pb^{2+} from 0.05 mM $^{210}PbCl_2$ at 15 °C [○–○, □–□] and 25 °C [●– –●, ■– –■], during a period of 6 hours.

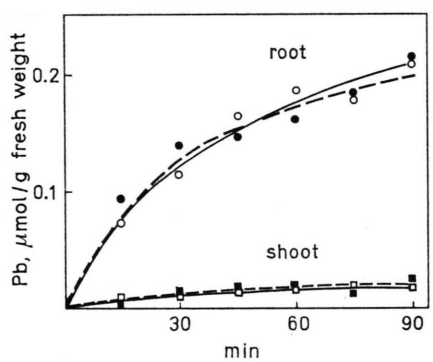

Fig. 4. The absorption and transport of Pb^{2+} from 0.05 mM $^{210}PbCl_2$ in the absence [○–○, □–□] and presence [●– –●, ■– –■] of 10^{-6} M FCCP.

Pb^{2+} uptake is found to be reduced by the presence of K^+, Ca^{2+}, or Mg^{2+} in the absorption medium (Fig. 5). While the inhibition by K^+ reached a maximum at 1 mM KCl, it increased linearly with higher concentrations of Mg^{2+} and Ca^{2+}. Fig. 6 describes the absorption and transport of Fe^{2+}, Mn^{2+}, and Zn^{2+} from 0.02 mM $^{59}FeSO_4$, $^{54}MnSO_4$, or $^{65}ZnSO_4$ (spec. activ. 2 µCi/µmol), in the absence and presence of different concentrations of $PbCl_2$. The absorption and transport were measured after 3 hours. The results show that the absorption of Mn^{2+} and

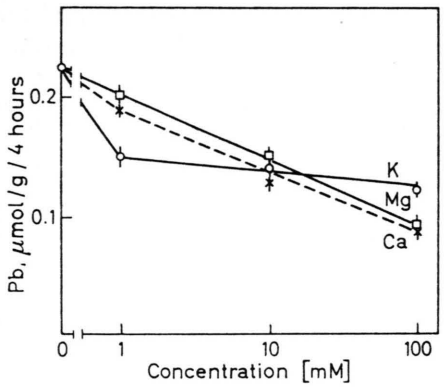

Fig. 5. The absorption of Pb^{2+} by the roots of pea seedlings from 0.05 mM $^{210}PbCl_2$ in the absence and presence of different concentrations of KCl, $MgCl_2$ or $CaCl_2$.

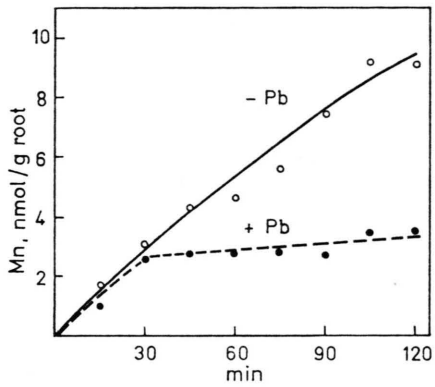

Fig. 7. Time course of absorption of Mn^{2+} from 0.02 mM $^{54}MnSO_4$ in the absence ○—○ and presence ●— —● of 0.05 mM $PbCl_2$.

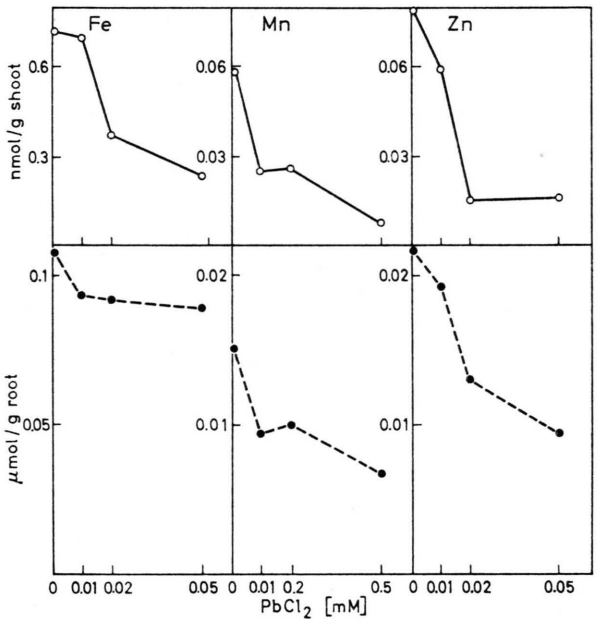

Fig. 6. The absorption and transport of Fe^{2+}, Mn^{2+} and Zn^{2+}, from 0.02 mM of $^{59}FeSO_4$, $^{54}MnSO_4$, or $^{65}ZnSO_4$ for 3 hours, in the absence and presence of different concentrations of $PbCl_2$.

Zn^{2+}, and the transport of Fe^{2+}, Mn^{2+} and Zn^{2+} to the shoot are significantly reduced by increasing concentrations of $PbCl_2$. The time course of absorption of Mn^{2+} in the absence and presence of 0.05 mM $PbCl_2$ (Fig. 7) reveals that there is a lag phase in the inhibition extending upto the first 30 min. Thereafter, the inhibition does not increase with time.

Discussion

It is found that Pb^{2+} is absorbed by pea roots in significant amounts. However, a larger percent appears to be retained in the 'apparent free space' (the sum of water and Donnan free spaces), since these are displaced by subsequent treatments (Fig. 1). What is retained in the roots then, represents the accumulation into the cytoplasmic and vacuolar compartments. The transport to the shoot is a very small fraction of what is absorbed by the roots (Fig. 2). There is evidence that even what is held within the cellular compartments could be removed by exposing the roots to increasing concentrations of $CaCl_2$ (Fig. 2). This finding, in addition to those on the insensitivity of Pb^{2+} absorption to temperature changes and an inhibitor of oxidative phosphorylation, FCCP, provide evidence that Pb^{2+} absorption is non-metabolic, as has been suggested [6]. Isermann [9] found that aqueous sprays containing CaEDTA removed large amounts of Pb^{2+} absorbed by plants, and thus reduced its transport to the shoot. Obviously the treatment caused the removal of the non-metabolically held Pb^{2+}, held in the plant foliage. Our studies show not only that the treatment of the roots with $CaCl_2$ following Pb^{2+} absorption caused an efflux of Pb^{2+} from the roots, but also that the presence of cations like Ca^{2+}, Mg^{2+}, or a monovalent cation K^+ in the absorption medium reduced the uptake (Fig. 5). These cations are known to be absorbed by carrier-mediated active processes [8], and are probably able to prevent the entry of Pb^{2+} more effectively. On the other hand, Pb^{2+} itself is inhibitory to the uptake and transport of Fe^{2+}, Mn^{2+}, and

Zn^{2+} (Fig. 6). Zn^{2+} absorption is considered to be passive [8] while Fe^{2+} and Mn^{2+} uptake are metabolic [10, 11]. The exact manner by which Pb^{2+} interferes with the absorption and transport of the micronutrients is not clear. There is a lag period of about 30 min in the inhibition of Mn^{2+} absorption (Fig. 7), and the absorption remains constant thereafter in the presence of Pb^{2+}. This suggests that Pb^{2+} causes a physical blocking for the entry of ions, or perhaps by disrupting the carrier mechanism for the micronutrients. Suchodoller and Wanner [12] studied the absorption of Pb^{2+} and Mn^{2+} by intact barley roots, and found that Mn^{2+} uptake was strongly depressed by Pb^{2+}. This inhibition was explained in terms of a greater affinity of Pb^{2+} for the adsorption sites, than that of Mn^{2+}.

The study shows that pollutants like Pb^{2+} can induce the deficiencies of micronutrients in plants even though these may be present in an available form in the medium, by interfering with their absorption by roots. There is evidence that the presence of Cd^{2+} in the medium causes chlorosis in ryegrass [13].

It is interesting to note that the time course of Pb^{2+} absorption is biphasic (Fig. 3). Similar biphasic patterns for Pb^{2+} and Mn^{2+} have been observed earlier in barley roots [12]. Hooymans [14] observed a consistent biphasic pattern for several cations and anions, and concluded that the fast phase I proceeding during the first 3 hours was reflecting largely the cytoplasmic accumulation, while the slow phase II was essentially vacuolar. In the light of these observations, it would be quite revealing to identify the compartment in the root cells where Pb^{2+} is accumulated the most. Furthermore, cell wall also appears to be a site of ultimate accumulation and concentration [15].

The senior author is grateful to the International Atomic Energy Agency and the Government of India Bhabha Atomic Research Centre, Bombay for financial support and sponsorship.

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