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# Uptake of Lead and Cadmium by Maize Seedlings and the Effect of Heavy Metals on the Activity of Phosphoenolpyruvate Carboxylase Isolated from Maize

MARTINA VOJTĚCHOVÁ and SYLVA LEBLOVÁ

Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, CS 12840 Praha 2, Czechoslovakia.

**Abstract.** Maize seeds and five-day-old maize seedlings were incubated in media containing $Pb^{2+}$ at concentrations of 50, 100, and 200 mg $l^{-1}$ and $Cd^{2+}$ at concentrations of 1, 5, 10 and 50 mg $l^{-1}$. After five days of incubation, both heavy metals were determined by means of AAS following wet mineralisation of roots and shoots. The results obtained indicate that $Pb^{2+}$ were transported to shoots from roots at a lower rate than $Cd^{2+}$.

Phosphoenolpyruvate carboxylase (PEPC) isolated from germinating maize seeds was inhibited to a comparable degree by solutions containing 0.001 mmol $l^{-1}$ $Pb^{2+}$, 0.01 mmol $l^{-1}$ $Cd^{2+}$, and 0.005 mmol $l^{-1}$ $Cu^{2+}$. The enzyme was protected against this inhibition by the addition of mercaptoethanol, the substrate (PEP), or the cofactor ($Mg^{2+}$). The inhibition increased during a 20 min incubation of the enzyme with salts of the metals. $Mn^{2+}$, $Ni^{2+}$, and $Co^{2+}$ ions could partially substitute for the metal cofactor $Mg^{2+}$. $K_m$ values for these metal ions were as follows: for $Mg^{2+}$ 0.07 mmol $l^{-1}$ in the range from 0 to 0.30 mmol $l^{-1}$ $Mg^{2+}$; 0.71 mmol $l^{-1}$ for 0.30 to 2.50 mmol $l^{-1}$ $Mg^{2+}$; for $Mn^{2+}$ 0.36 mmol $l^{-1}$; for $Ni^{2+}$ 0.34 mmol $l^{-1}$; and for $Co^{2+}$ 0.20 mmol $l^{-1}$. The activity of the enzyme reached with $Mn^{2+}$ 85 %, with $Ni^{2+}$ 65 %, and with $Co^{2+}$ 55 % of the activity recorded with $Mg^{2+}$.

Heavy metals significantly contribute to the pollution of the environment in industrial regions. The development of industrial production and the intensification of transport associated with enhanced utilization of fossil fuel affected geochemical cycles of some elements, the compounds of which started to accumulate in the biosphere (Falah-Ardakani 1984, Thomas *et al.* 1984). High concentrations of heavy metals in the soil result in increased uptake by plants (Broyer *et al.* 1972). Heavy metals then cumulate in the food chain with which they reach human beings (Lorenz 1979).

Experiments described in this paper were aimed at determining the intensity of $Pb^{2+}$ and $Cd^{2+}$ penetration into germinating maize seeds and the uptake of these ions by maize seedlings. Besides that, attention was devoted to the effect of $Pb^{2+}$, $Cd^{2+}$, and $Cu^{2+}$ on the activity of PEPC isolated from germinating maize seeds and to the possibility of substituting $Mn^{2+}$, $Ni^{2+}$, and $Co^{2+}$ ions for $Mg^{2+}$ ions which are the cofactor of phosphoenolpyruvate carboxylase.

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## MATERIAL AND METHODS

### Chemicals

These salts were used for the preparation of solutions containing heavy metals: $Pb(NO_3)_2$, $Cd(NO_3)_2 . 4 H_2O$, $Mn(NO_3)_2 . 6 H_2O$, $Ni(NO_3)_2 . 6 H_2O$, $Co(NO_3)_2 . 6 H_2O$, $Cu(NO_3)_2 . 6 H_2O$.

### Plant Material

Maize (*Zea mays* L.) cv. CE-205-S seeds served as plant material.

### Plant Cultivation

(1) Batches of 50 maize seeds were germinated at room temperature for 5 days in Petri dishes with a diameter of 15 cm on filter paper soaked with 20 ml of heavy metal salt solutions (control batch in water). Roots and shoots were sampled in triplicate after 5 days of seed germination; collected samples were mineralized.

(2) Batches of 50 maize seeds were germinated at room temperature for 5 days in Petri dishes with a diameter of 15 cm on filter paper in distilled water. After 5 days, a group of seedlings was transferred into beakers with muslin supports which made it possible to grow seedlings in metal salt solutions with only roots being submerged in the solutions. Control seedlings were growth in water. The other group of seedlings was left in Petri dishes, with heavy metal solutions moistening the whole seedling. Roots and shoots were sampled after five days; collected samples were mineralized.

### Tissue Mineralization

Weighed plant tissues (approximately 2.5 g) were heated with concentrated nitric acid (2 ml per g of plant tissue) on a sand bath. After tissue dissolution, the samples were evaporated to one-half volume. Remaining free nitric acid was decomposed by adding a few drops of concentrated $H_2O_2$. Cold samples were made up to 25 ml with distilled water and filtrated. Metal contents in the filtrates were determined by means of AAS.

### AAS Measurements

Metal contents in samples were determined using a Video II AA-AS Spectrophotometer 951 (Instrumentation Laboratory) device with the application of atomization in acetylene-air flame with a double chanel double beam arrangement. The instrument prints directly the concentration of the metal to be determined in the original sample read from a six-point calibration curve and related to the volume of the dilution solution.

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*Abbreviations used:* LDH – lactate dehydrogenase; MDH – malate dehydrogenase; NAD – nicotinamide adenine dinucleotide; OAA – oxalacetic acid; PEP – phosphoenolpyruvate; ME-mercaptoethanol.

**PEPC Isolation**

The enzyme was isolated from seeds germinated for 24 hours in the dark at room temperature using a procedure described by Leblová *et al.* (1989).

**PEPC Activity Determination**

PEPC activity was determined according to absorbance decrease at 340 nm caused by NADH oxidation in a reaction coupled with MDH:

$$PEP + HCO_3^- \xrightarrow{PEPC} OAA + P_i$$

$$OAA \xrightarrow[NAD^+ \quad NADH]{MDH} malate$$

Because oxalacetic acid is unstable in the presence of ions of bivalent metals and is decarboxylated to pyruvic acid, we added LDH to the assay mixture which reduced pyruvic acid to lactic acid with a simultaneous oxidation of NADH to $NAD^+$:

$$\text{pyruvic acid} \xrightarrow[NAD^+ \quad NADH]{LDH} \text{lactic acid}$$

The assay mixture (final volume 2 ml) contained 2.5 mmol $l^{-1}$ $Mg^{2+}$, 5 mmol $l^{-1}$ $NaHCO_3$, 1 mmol $l^{-1}$ PEP, 0.1 IU MDH, 0.2 IU LDH, 0.125 mmol $l^{-1}$ NADH, 0.1 ml of PEPC preparation and 100 mmol $l^{-1}$ Tris-HCl buffer pH 8.1. Enzyme reaction was started by adding PEP (Meyer *et al.* 1988).

**The Preparation of PEPC Apoenzyme**

The preparation of PEPC isolated with the addition of mercaptoethanol (ME) was equilibrium-dialyzed for 10 h against 3 × 1 l of 25 mmol $l^{-1}$ Tris-HCl buffer pH 8.1 containing 5 % glycerol. When PEPC was prepared with dithiothreitol which is a more potent protective agent than ME, the enzyme preparation was dialyzed for 4 hours against 2 × 1 l of 25 mmol $l^{-1}$ Tris-HCl buffer, pH 8.1, containing 5 % glycerol. Only under these conditions a complete removal of $Mg^{2+}$ ions was achieved which was confirmed by loss of activity.

**PEPC Cofactors**

The possibility of substituting other ions for $Mg^{2+}$ ions was studied by determining the activity of the demetalized enzyme preparation after adding $Mn^{2+}$, or $Ni^{2+}$, or $Co^{2+}$ into the assay mixture.

**Inactivation of PEPC with Metal Ions**

Activity decrease of the PEPC preparation was determined in 25 mmol $l^{-1}$ Tris-HCl buffer containing 5 % glycerol in dependence on the concentration of metal ions added to the assay mixture without or in presence of 5 mmol $l^{-1}$ ME. Besides that, the PEPC preparation was preincubated with metal salt solutions of various concentrations for 30 min. Samples with a volume of 0.1 ml were removed in regular intervals for PEPC activity determination. The incubation medium (1 ml) contained the enzyme preparation, metal ions, and 100 mmol $l^{-1}$ of Tris-HCl buffer, pH 8.1. In further experiments, 2.5 mmol $l^{-1}$ $Mg^{2+}$ or 1 mmol $l^{-1}$ PEP were added to the incubation medium. Blank experiment medium contained only the enzyme preparation in the buffer. All experimental incubations were carried out in a thermostable water bath at 21 °C.

TABLE 1

Lead uptake by shoots and roots of five-day-old maize seedlings.

| Lead conc. in the medium [mg $l^{-1}$] | Lead amount in shoots [μg $g^{-1}$] | Lead amount in roots [μg $g^{-1}$] | Root: shoot ratio of lead content |
|---|---|---|---|
| 0 | 2.3 | 3.20 | 1.4 |
| 50 | 3.7 | 26.55 | 7.2 |
| 100 | 4.0 | 52.80 | 13.2 |
| 200 | 7.8 | 116.35 | 14.9 |

## RESULTS AND DISCUSSION

The content of $Pb^{2+}$ and $Cd^{2+}$ ions increased in maize seedlings with increasing concentration of these ions in the cultivation medium in both five-day-old (Tables 1 and 2) and ten-day-old seedlings grown for 5 d either in Petri dishes or in beakers with muslin supports (Fig. 1). The values presented in the tables are means of three replicate determinations.

TABLE 2

Cadmium uptake by shoots and roots of five-day-old maize seedlings.

| Cd concentr. in the medium [mg $l^{-1}$] | Cd amount in shoots [μg $g^{-1}$] | Cd amount in roots [μg $g^{-1}$] | Roots: shoot ratio of Cd contents |
|---|---|---|---|
| 0 | 0.01 | 0.02 | 2.0 |
| 1 | 0.26 | 1.31 | 5.2 |
| 5 | 1.46 | 2.95 | 2.0 |
| 10 | 0.98 | 6.69 | 6.8 |
| 50 | 4.23 | 30.88 | 7.3 |

TABLE 3

The dependence of PEPC activity on the concentration of $Pb^{2+}$, $Cd^{2+}$, and $Cu^{2+}$ ions.

| Metal concentration [μmol $l^{-1}$] | PEPC activity [%] without protection | PEPC activity [%] + 5 mmol $l^{-1}$ ME |
|---|---|---|
| $Pb^{2+}$ 0 | 100 | 100 |
| $Pb^{2+}$ 1 | 58 | 77 |
| $Pb^{2+}$ 2 | 50 | 72 |
| $Pb^{2+}$ 3 | 47 | 77 |
| $Pb^{2+}$ 4 | 42 | 77 |
| $Pb^{2+}$ 5 | 33 | 77 |
| $Cd^{2+}$ 0 | 100 | 100 |
| $Cd^{2+}$ 2 | 66 | 83 |
| $Cd^{2+}$ 4 | 61 | 77 |
| $Cd^{2+}$ 6 | 61 | 77 |
| $Cd^{2+}$ 8 | 55 | 77 |
| $Cd^{2+}$ 10 | 58 | 80 |
| $Cd^{2+}$ 12 | 55 | 77 |
| $Cu^{2+}$ 0 | 100 | 100 |
| $Cu^{2+}$ 1 | 77 | 88 |
| $Cu^{2+}$ 2 | 77 | 88 |
| $Cu^{2+}$ 3 | 72 | 83 |
| $Cu^{2+}$ 4 | 66 | 83 |
| $Cu^{2+}$ 5 | 66 | 77 |
| $Cu^{2+}$ 6 | 62 | 77 |

TABLE 4

Inhibition of PEPC with $Pb^{2+}$, $Ca^{2+}$ and $Cu^{2+}$ ions, protection with $Mg^{2+}$ and PEP. Values presented in the Table are PEPC activities expressed as percentages of the activity recorded at zero time incubation.

| | Time [min] | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 30 |
| 10 μmol $l^{-1}$ $Cd^{2+}$ | 100 | 73 | 66 | 68 | 66 | 63 |
| 10 μmol $l^{-1}$ $Cd^{2+}$ + 2.5 mmol $l^{-1}$ $Mg^{2+}$ | 100 | 81 | 85 | 75 | 68 | 68 |
| 10 μmol $l^{-1}$ $Cd^{2+}$ + 1.0 mmol $l^{-1}$ PEP | 100 | 80 | 80 | 85 | 80 | 80 |
| 5 μmol $l^{-1}$ $Pb^{2+}$ | 100 | 73 | 66 | 66 | 46 | 40 |
| 5 μmol $l^{-1}$ $Pb^{2+}$ + 2.5 mmol $l^{-1}$ $Mg^{2+}$ | 100 | 82 | 72 | 69 | 64 | 65 |
| 5 μmol $l^{-1}$ $Pb^{2+}$ + 1.0 mmol $l^{-1}$ PEP | 100 | 80 | 88 | 80 | 80 | 80 |
| 5 μmol $l^{-1}$ $Cu^{2+}$ | 100 | 70 | 73 | 70 | 66 | 66 |
| 5 μmol $l^{-1}$ $Cu^{2+}$ + 2.5 mmol $l^{-1}$ $Mg^{2+}$ | 100 | 91 | 93 | 93 | 86 | 83 |
| 5 μmol $l^{-1}$ $Cu^{2+}$ + 1.0 mmol $l^{-1}$ PEP | 100 | 99 | 95 | 95 | 91 | 91 |

TABLE 5
Velocity constants of PEPC inactivation by $Pb^{2+}$, $Cd^{2+}$, and $Cu^{2+}$ ions.

| | K [$10^4$ . $s^{-1}$] |
|---|---|
| 5 μmol $l^{-1}$ $Pb^{2+}$ | 7.24 |
| 5 μmol $l^{-1}$ $Pb^{2+}$ + 2.5 mmol $l^{-1}$ $Mg^{2+}$ | 4.45 |
| 5 μmol $l^{-1}$ $Pb^{2+}$ + 1.0 mmol $l^{-1}$ PEP | 3.02 |
| 10 μmol $l^{-1}$ $Cd^{2+}$ | 5.54 |
| 10 μmol $l^{-1}$ $Cd^{2+}$ + 2.5 mmol $l^{-1}$ $Mg^{2+}$ | 3.64 |
| 10 μmol $l^{-1}$ $Cd^{2+}$ + 1.0 mmol $l^{-1}$ PEP | 3.21 |
| 5 μmol $l^{-1}$ $Cu^{2+}$ | 5.36 |
| 5 μmol $l^{-1}$ $Cu^{2+}$ + 2.5 mmol $l^{-1}$ $Mg^{2+}$ | 1.03 |
| 5 μmol $l^{-1}$ $Cu^{2+}$ + 1.0 mmol $l^{-1}$ PEP | 1.17 |

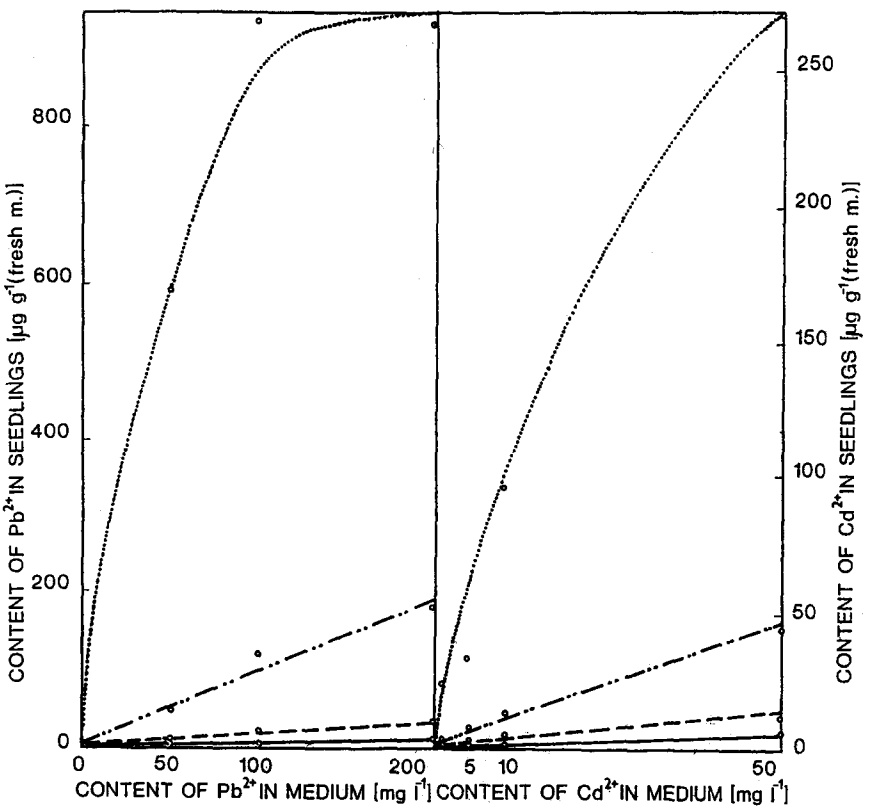


Fig. 1. Amounts of $Pb^{2+}$ or $Cd^{2+}$ in 10 d old maize seedlings grown in Petri dishes and on muslim support in dependence on $Pb^{2+}$ or $Cd^{2+}$ concentration in cultivation medium.
—— shoots, muslim; – – – shoots, dish; – . . – . . roots, dish; . . . . . roots, muslim

TABLE 6

Michaelis constants established with PEPC isolated from germinating maize seeds for $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$, and $Co^{2+}$.

| Cofactor concentr. [mmol $l^{-1}$] | Michaelis const. [mmol $l^{-1}$] | PEPC activity [%] |
|---|---|---|
| $Mg^{2+}$ 0–0.3 | 0.07 | 45 |
| $Mg^{2+}$ 0.3–2.5 | 0.71 | 100 |
| $Mn^{2+}$ 0–2.0 | 0.36 | 85 |
| $Ni^{2+}$ 0–2.0 | 0.34 | 65 |
| $Co^{2+}$ 0–1.0 | 0.20 | 55 |

Note: 100 % are represented by PEPC activity recorded with 2.5 mmol $l^{-1}$ $Mg^{2+}$.

In cases in which seedling shoots were in direct contact with the cultivation medium, the shoots contained higher metal amounts than in cases in which only seedling roots were exposed to metal salt solutions. This difference was

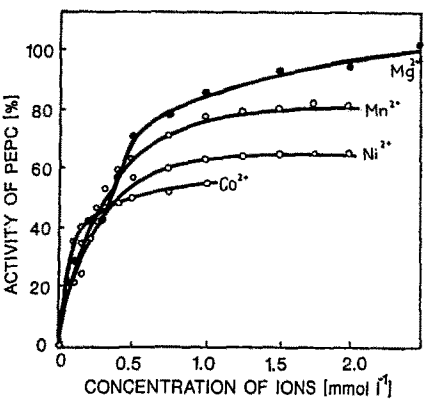


Fig. 2. The dependence of the activity of PEPC isolated from germinating maize seeds on $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$ and $Co^{2+}$ concentrations (100 % = PEPC activity recorded with 2.5 mmol $l^{-1}$ $Mg^{2+}$).

lower in case of $Cd^{2+}$ than in case of $Pb^{2+}$, and for this reason we assume that lead ions were transported to a lesser extent from roots to shoots than cadmium ions. This fact simultaneously indicates that metal ions can also be taken up by above ground plant parts.

Phosphoenolpyruvate carboxylase (PEPC) which catalyzes the transfer of $CO_2$ to phosphoenolpyruvate and the formation of oxalacetate is present in maize seeds at the same level during the first five days of seed germination. The function of this enzyme has not yet been exactly understood, but it has been assumed that it may be involved in the malate pathway, in trans-hydrogenation reactions, or in the regulation of cell pH (Latzko and Kelly 1983).

PEPC isolated from maize seeds germinating for 24 h was inhibited to approximately the same level (approximately 60 % of activity) with 0.001 mmol $l^{-1}$ $Pb^{2+}$, 0.01 mmol $l^{-1}$ $Cd^{2+}$, or 0.005 mmol $l^{-1}$ $Cu^{2+}$ (Table 3). The extent of the inhibition was influenced by the presence of mercaptoethanol in the medium, and during the enzyme preparation preincubation with metal ions by the presence of the substrate (PEP) or of $Mg^{2+}$. The inhibition deepened with the duration of the preincubation (Table 4). Velocity constants of PEPC inactivation and the effects of the substrate (PEP) and $Mg^{2+}$ ions on these constants are presented in Table 5. The calculations of these constants were based on the assumption that the reaction proceeds as a pseudomonomolecular reaction. These preliminary results showed that the protection with the substrate (PEP) was more efficient against maize seed PEPC inhibition with $Pb^{2+}$ and $Cd^{2+}$ than the protection with $Mg^2$. Further, the substrate protected PEPC more efficiently against $Cu^{2+}$ than against $Pb^{2+}$ or $Cd^{2+}$. Differences were also recorded in the extent of the inhibition with respect to the concentration of the metal ions: $Pb^{2+}$ showed higher inhibitory effects than $Cd^{2+}$ and $Cu^{2+}$.

PEPC requires the presence of $Mg^{2+}$ for the catalysis of the reaction. They could be replaced in case of the apoenzyme prepared using the above described procedure with $Mn^{2+}$, $Ni^{2+}$, or $Co^{2+}$ ions (Fig. 2). In the presence of 2 mmol $l^{-1}$ $Mn^{2+}$, PEPC activity reached 85 %, with 2 mmol $l^{-1}$ $Ni^{2+}$ 65 %, and with 1 mmol $l^{-1}$ $Co^{2+}$ 55 % of PEPC activity reached with the natural cofactor $Mg^{2+}$ (2.5 mmol $l^{-1}$, see Fig. 2). Michaelis constants for these ions as cofactors of PEPC were determined according to Lineweaver and Burk (Table 6).

We intend to aim our further experiments at detailed understanding of the effect of heavy metals on PEPC isolated from germinating maize seeds. We tentatively presume that $Pb^{2+}$ and $Cd^{2+}$ may interact with the protein molecule of PEPC present in maize seeds via another mechanism than in case of PEPC present in photosynthesizing maize tissues. PEPC from green maize leaves is not protected against the inactivation with $Pb^{2+}$ and $Cd^{2+}$ by $Mg^{2+}$ ions, some protection against such inhibition was recorded only with PEP (Stiborová and Leblová 1985).

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