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Absorption and translocation of Cd in bush beans (*Phaseolus vulgaris*)

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A series of experiments was conducted to examine some factors affecting the absorption and translocation of Cd in young bean plants (*Phaseolus vulgaris* L. cv. Bulgarian). Absorption of Cd by roots was reduced in the presence of other cations of increasing valency or ionic radii. Reduced absorption was also found in the presence of EDTA. Concentration of Cd in exudates from excised stems increased with increased passage of Cd solutions and approached the concentration in the external medium (4.5 μM Cd). This was apparently associated with saturation of adsorption sites in the stems. The stem behaved as a cation exchange column resulting in a chromatographic distribution of Cd towards the top of the plant. These experiments indicate that Cd existed in the xylem fluid as a free or weakly complexed cation. Additional experiments showed that the total amount of Cd absorbed by bean plants was elevated by inducing higher transpiration rates. The effect of water flux on Cd transport indicated apoplastic flow to the stele.

Additional key words – Cadmium, root absorption, transpiration rate, xylem exudate.

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Introduction

Cadmium is one of the most toxic heavy metals in the contemporary environment. A 50% decrease in yield was found in some field crops with tissue concentrations as low as 2–9 ppm Cd (Page et al. 1972). Other non-essential heavy metals, such as Pb, may reach ten or twenty times this concentration without affecting yield (Baumhardt and Welch 1972). The high sensitivity of Plants to Cd is thought to be due to inhibitory effects on ATPase activity and subsequent decline in respiratory and photosynthetic processes (Lee et al. 1976), destructive effects upon cell tissue (Lamoreux and Chaney 1977) and reduction of Zn absorption by plants (Chaney et al. 1976, Lagerwerff and Biersdorf 1972).

Toxicity of Cd is accentuated by its relatively high mobility in plants. It was proposed by Tyler and McBride (1982) that Cd may be absorbed by and translocated along calcium pathways; this may be related to their similar ionic radii (Ca 99 pm, Cd 97 pm). The pathway of Cd movement within the root to the stele is less clear. Cutler and Rains (1974) assumed symplastic transport of Cd across the root cortex, although no detailed work has been carried out on the subject.

The manner in which Cd is translocated in the xylem is also unclear. Petit and van de Geijn (1978) described a chromatographic distribution of Cd occurring along negative exchange sites in the xylem. The process is similar to that proposed for the translocation of calcium by Bell and Biddulph (1963) and Jacoby (1967), and would imply that Cd exists in the xylem fluid in a free ionic state or as a cationic complex. However, this is contrary to electrophoretic evidence showing that Cd exists as an anionic complex in the xylem exudate of soybean (Cataldo et al. 1981, White et al. 1981) and tomato (White et al. 1981) and would not therefore behave as a free cation. These differences may however be explained by speciation of Cd complexes that formed at different concentrations in solution.

In the present series of experiments it was intended to trace the movement of Cd through the root to the leaves, and to investigate some of the factors and mechanisms affecting the movement of Cd in bean plants.

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Abbreviations – ASE, artificial sewage effluent; HT, high transpiration; LT, low transpiration.

Materials and methods

Bush beans (*Phaseolus vulgaris* L. cv. Bulgarian) were germinated and grown in vermiculite with photoperiod of 16 h and at 25°C. The plants were illuminated with GRO-LUX F40 GRO florescent tubes with a photon flux density of 150 μmol m^{-2} s^{-1}. The plants were irrigated with 0.25 strength Hoagland solution (Epstein 1972) and after six days the roots were rinsed of vermiculite and transferred for a further four days to an aerated 0.1 strength Hoagland solution or an artificial sewage effluent (ASE). The artificial sewage effluent consisted of a solution composed of the major ions found in local sewage effluent: 10 m*M* NaCl, 3.25 m*M* $CaCl_2$, 2 m*M* $NH_4H_2PO_4$, 0.8 m*M* KNO_3, plus essential micronutrients. During experimentation ^{109}Cd labelled $CdCl_2$ was added to all solutions and the pH of the solutions was adjusted to 7.0.

Plant material was prepared for Cd analysis by wet digestion. Plant parts were weighed (fresh and dry weight), and then digested in 2.0 ml of a 24:1:24 mixture of concentrated $HClO_4$:H_2SO_4:HNO_3 at 80°C for 10 min. The temperature was then raised to 180°C and digestion was continued until dryness. The residue was redissolved in 1.0 ml Na_2EDTA 1.0 *M* at pH 11.0 as described by Jacoby (1967). Aliquots (0.5 ml) were mixed with 3.5 ml of Bray's scintillation liquid (Bray 1960) and the ^{109}Cd activity was determined in a Packard liquid scintillation counter. The results were corrected for quenching by the external standard method.

A pressure apparatus was constructed in order to collect samples of xylem exudate as described by Jacoby (1965). In brief, it consisted of a simple household pressure cooker adapted with five sealed inlets into which the apical end of excised stem segments could be fitted and which were connected to capillary outlet tubes. Bases of the stem segments were immersed into solution and, by application of compressed air to the apparatus, solution was pressed through the stem segments and exudate was collected at the capillary outlets. Samples of exudate were collected in scintillation vials which were changed upon accumulation of approximately 100 μl of exudate. Volume of exudates was determined as the weight difference of vials before and after collection.

Results

Root absorption

It was the purpose of the following experiment to determine the effect of major cations in sewage effluent, and of complexing with EDTA, upon Cd absorption by roots. Seven treatment solutions, each containing 0.5 μ*M* $^{109}Cd^{2+}$, were prepared: (i) deionised water, (ii) 10 m*M* K_2SO_4, (iii) 10 m*M* Na_2SO_4, (iv) 10 m*M* $CaSO_4$, (v) 10 m*M* $MgSO_4$, (vi) 1 m*M* Na_2EDTA, (vii) ASE.

Root systems were excised from six bean plants previously grown in vermiculite and then in 0.1 strength Hoagland solution. The roots were trimmed to 1 g fresh weight, rinsed in deionised water and lightly blotted with a paper towel. Each root system was immersed sequentially into ten 50 ml portions of one of the treatment solutions. The roots were kept in each portion for 5 min with constant stirring. Aliquots were taken from each solution before and after root immersion and analysed for ^{109}Cd. Absorption of Cd by roots was calculated as the difference between Cd concentrations in solutions before and after immersing the roots.

The time course of Cd adsorption by roots was almost linear in all treatments after a short initial period of more rapid binding (Fig. 1). Both mineral cations and EDTA decreased adsorption rates of Cd considerably. The order of interference with Cd absorption by the ions was Na < K ≪ Mg < Ca. At a concentration of 10 m*M*, the monovalent ions, Na^+ and K^+, inhibited root absorption of Cd by 61 and 72% respectively, whilst 10 m*M* of the divalent cations and 1 m*M* EDTA inhibited more than 95% of adsorption and ASE inhibited Cd adsorption by 88%.

Effect of ionic concentration of the xylem fluid upon translocation of Cd in stem segments

Stem segments of six-day-old bean plants grown in vermiculite were cut at the stem base to a length of 5 cm

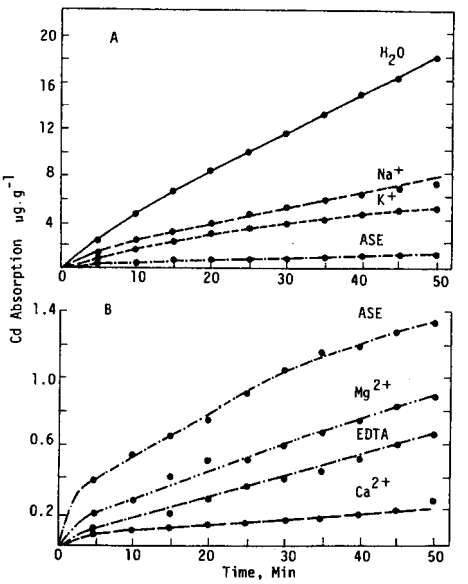

Fig. 1. Cadmium absorption by excised root systems. A, absorption from 0.5 μ*M* Cd only, and in the presence of 10 m*M* NaCl, 10 m*M* KCl or ASE (artificial sewage effluent); B, scale enlarged: absorption in the presence of ASE, 1 m*M* Na_2EDTA, 10 m*M* $MgCl_2$ or 10 m*M* $CaCl_2$.

and assembled in the pressure apparatus. The bases of the stems were immersed in one of the prepared solutions given in Tab. 1. Solutions (b) and (c) represented the respective composition of major cations in xylem exudate obtained by (i) pressing 0.5 m*M* $CaCl_2$ through decapitated root systems as described previously (Jacoby 1967), and (ii) centrifugation of stem segments (Bollard 1960) from plants grown in vermiculite. For each of the three solutions there were at least 6 plant replicates.

Tab. 1. Composition of Cd solutions pressed through stem segments.

Solution	Cd μ*M*	m*M*				pH
		NaCl	$MgCl_2$	$CaCl_2$	KCl	
a	4.5	0.0	0.0	0.0	0.0	6.63
b	4.5	0.65	0.83	0.33	1.8	6.63
c	4.5	0.68	3.96	3.75	17.52	6.63

The effect of the different ionic solutions upon the retention of Cd in the stems is shown in Fig. 2. Cadmium concentrations in exudates are expressed as a function of the number of pore volumes (stem volume occupied by perfused solution) of solution passed through the segments. The pore volume was calculated to be 15% of the fresh weight of the stems, as determined by Barak et al. (1984), using methods described by Van Bel (1978). By using this parameter, variability of stem diameter is taken into account for the passage of Cd through different stem segments. The final equilibrium concentration of Cd in the xylem exudate of plants treated with solution (a) was only 2.2% of that of the external solution, whereas in experiments with solutions which contained other cations in addition to Cd it reached 15.5 and 89% for solutions (b) and (c), respectively. It is evident, particularly for solutions (b) and (c), that with increasing amounts of Cd passed through stem segments, the Cd concentration in exudates increased. It is interesting to note that equilibrium concentrations in exudates from solution (c), which simulated the cationic composition of natural xylem fluid in the stem, approached the concentration of Cd in the external solution.

Stem segments which had absorbed Cd from solutions (a) and (b) were rinsed under deionised water and cut into 1.0 cm sections. These were then weighed and digested for ^{109}Cd determination. The distribution of Cd in stems for the two treatments is shown in Fig. 3. Even though twice the number of pore volumes of solution were perfused through stem segments in treatment (a) as compared to treatment (b), a greater fraction of the total Cd was absorbed at the base of the stems of treatment (a). Subsequent amounts of Cd absorbed further up the stems showed a chromatographic distribution in which concentrations decreased towards the top.

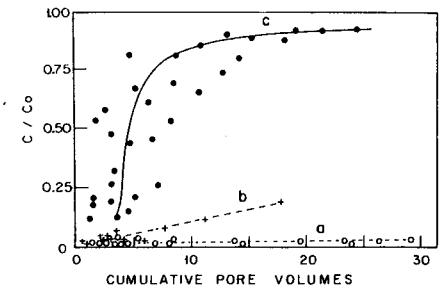

Fig. 2. Relation between relative Cd concentration (concentration in exudate/external concentration, C/C°) in xylem exudate of excised stem segments perfused with various solutions and the number of pore volumes of solution passed through the segments. Solutions contained: a, 4.45 μ*M* $^{109}CdCl_2$; b, 4.5 μ*M* $^{109}CdCl_2$, 0.65 m*M* NaCl, 1.8 m*M* KCl, 0.3 m*M* $CaCl_2$, 0.83 m*M* $MgCl_2$; c, 4.5 μ*M* $^{109}CdCl_2$, 0.68 m*M* NaCl, 17.5 m*M* KCl, 3.75 m*M* $CaCl_2$, 3.96 m*M* $MgCl_2$.

Effect of transpiration upon Cd translocation

Plants were grown for six days in vermiculite. Six uniform plants were then transferred for a further four days to ASE. During the last dark period, prior to injection of Cd into the solutions, one set of 3 plants was enclosed in a transparent plastic tent through which water-saturated air was passed. This procedure induced a high humidity and allowed the plants to attain a low transpiration rate prior to experimentation. The second set of plants was not enclosed. The relative humidities of the high transpiration (HT) and low transpiration (LT) treatments were 68 and 97%, respectively.

At the end of this period the roots of all the plants were immersed in ASE containing 0.5 μ*M* $^{109}CdCl_2$. This solution was renewed after 20 min, thus preventing rapid initial depletion of Cd in the experimental solution. Plants were then allowed to transpire under the

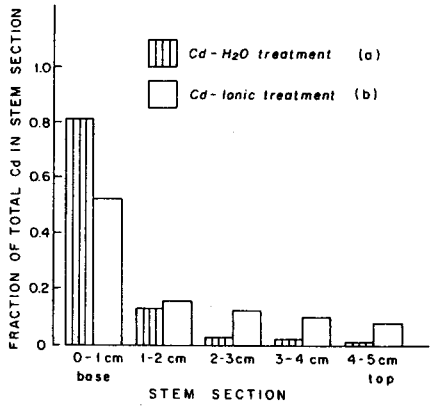

Fig. 3. Cadmium distribution in excised stem segments after acropetal perfusion with 4.5 μ*M* $^{109}CdCl_2$ solutions in absence (a) or presence (b) of 0.65 m*M* NaCl, 1.8 m*M* KCl, 0.33 m*M* $CaCl_2$, 0.83 m*M* $MgCl_2$.

Tab. 2. Effect of transpiration on absorption and distribution of Cd in bean plants during 14 h. Absorption from 0.5 μM $CdCl_2$ in ASE. HT (high transpiration) – 26.3 ml plant^{-1}; LT (low transpiration) – 7.7 ml plant^{-1}.

Plant organ	Cd content, ng ± SE		Cd content ratio HT/LT
	HT	LT	
Roots	13000±800	8800±600	1.5
Hypocotyl	40±2.2	17±2.4	2.4
Internode 1	56±1.9	20±0.9	2.8
Internode 2	75±17	22±3.8	3.4
Petioles	53±9.9	10±0.6	5.3
Leaf blades	41±7.1	5±1.8	8.2
Crown	17±4.4	2±0.6	8.5
Total	13300±820	8900±610	1.5
Tops	282±13.8	76±7.1	3.7

two humidity conditions for 14 h in the light. The transpiration rates were calculated as the difference in weight of Cd-ASE solutions before and after the experiment. At the end of the experimental period roots were rinsed in deionised water and exchangeable Cd was extracted by immersing the roots in a cold 10 mM $CaSO_4$ solution for 10 min. Plants were then dissected into various parts, weighed, digested and ^{109}Cd was determined.

The amount of water transpired by the HT plants was 3.4 times that by the LT plants during the experimental period, namely 26.3 and 7.7 ml per plant, respectively. A similar ratio (3.7) was observed between the average total contents of Cd in the tops for the two treatments (Tab. 2), and a significantly higher concentration of Cd was found in all plant parts of the HT treatment. Most of the Cd was retained in the roots and only 1–2% of the total absorbed Cd was transferred to the plant tops. The concentration of Cd in roots of HT plants was 1.5 times higher than in roots of LT plants, but this ratio was not similar to the transpiration ratio.

A greater proportion of total Cd in the tops was transferred from the stem base to petioles, leaves and apex in HT than in LT treatments. Accordingly, the fraction of Cd remaining at the base of stems was higher in LT than in HT treatments. Thus the ratio of Cd content in the two treatments for different plant parts increased from 2.4 to 8.5 with ascent towards the apex.

Mean concentrations of Cd in the transpiration stream [Cd(tops)/volume transpired] were similar (88 ± 8.2 and 94 ± 4.7 nM) for LT and HT treatments, respectively), suggesting that increased transport to the tops of the plants occurred in response to increased mass flow of solutes in the transpiration stream.

Discussion

In the absence of a solid phase, the rate and amount of Cd adsorbed by roots depended upon the ionic composition of the external solution (Fig. 1). Cadmium uptake by roots was reduced in the presence of other cations in the order $Na^+ < K^+ \ll Mg^{2+} < Ca^{2+}$. This sequence was related to increasing valency and ionic radii of competing cations (Na 97 pm, K 133 pm, Mg 66 pm, Ca 99 pm). Most of the Cd absorbed is bound to roots. This absorption could be explained by exchange equilibria between root binding sites and the solution. In addition, chelation with EDTA also reduced Cd uptake by roots considerably. Similar effects of chelates were reported by Tyler and McBride (1982) for Cd and by Dekock and Mitchell (1957) for Cu and are probably a result of reduced activity of the metal in solution. This contrasts with the situation in soil-water systems where cations are strongly bound to the solid phase and in which chelation of metal ions has been found to increase their effective concentration in the soil solution and hence availability to plants (Lindsay 1974). Reduced uptake of Cd^{2+} in the presence of ASE was mainly attributed to the combined effect of competing cations, although the presence of chloride ions instead of sulphate may also have an effect.

The amounts of Cd translocated in the xylem to leaves depended upon chemical interactions between Cd in xylem sap and absorbing sites in vascular tissue. Chromatographic distribution of Cd absorbed onto stem segments demonstrated that xylem vessels behaved as an exchange column for this cation, as proposed by Petit and van de Geijn (1978). Moreover, by increasing concentrations of competing cations in the perfusing solutions, the amounts of Cd adsorbed decreased and the concentration of Cd in the exudate increased. A similar behaviour was previously noted for Ca by Jacoby (1967). The effect of chromatographic distribution and exchange along xylem vessels would explain the lower Cd concentrations found in leaves compared to those of stems of low transpiring plants in Tab. 2. For high transpiring plants this was not the case. It would appear that, with increasing saturation of absorption sites in root and stem tissue, the amount of Cd translocated to the tops of the plants is increased. This may eventually lead to accumulation of Cd in the leaves. The foregoing results suggest that the behaviour of Cd in the xylem is that of a free cation. Experiments conducted in our laboratory (R. T. Hardiman. 1983. Thesis. Hebrew Univ. of Jerusalem, Israel) supported this observation and showed no evidence of Cd complexation in xylem fluid.

Passage of Cd through stem segments seemed similar to that of solute movement through porous adsorptive media. The presence of competing cations in the xylem fluid reduced adsorption of Cd in stem segments and consequently accentuated the breakthrough curve of Cd so that the Cd concentration in the fluid approached the concentration in the external medium. This was particularly evident when the ionic composition of synthetic xylem fluid passed through stem segments was similar to that measured in natural xylem fluid. The resulting Cd concentrations in xylem exudate demonstrate a typical breakthrough curve (Nielsen and Biggar

1963) in which concentrations of the metal in xylem fluid increases with increasing saturation of adsorption sites of the xylem.

The result of the transpiration experiment (Tab. 2) indicated that the amount of Cd found in the tops was directly proportional to the amount of water transpired and that the concentration of the solution reaching the tops was not affected by the transpiration rate. This could imply that transfer of Cd from roots to the stele was not governed by metabolic processes, and that entry into the stele occurred via the apoplast, by-passing the casparian strip of endodermal cell walls. Entry could occur at places of secondary root emergence (Dumbroff and Peirson 1971, Peterson et al. 1981) or through the apical parts of the roots as suggested by Clarkson and Hanson (1980) for calcium. Such mass flow of Cd would occur in only a fraction of the total water flow through the root into the stele. This would explain the lower Cd concentration that was calculated to be in the solution reaching the tops, compared to that in the external medium.

Most of the Cd in the plants accumulated in the root, as also noted by other authors (Cutler and Rains 1974, Jarvis et al. 1976, Cataldo et al. 1981), and although there was a greater accumulation of Cd in the root of the higher transpiring plants, the concentration ratio between the two treatments was not proportional to the transpiration rate. It is thought that absorption of Cd by roots is governed by exchange equilibria and cation composition of the external medium as shown in Fig. 1. Increased transpiration may accelerate replenishment of Cd at these sites. This should increase absorption, but not in a linear relation to the rate of transpiration, since absorption is not a linear function of concentration (Cutler and Rains 1974, Smeyers-Verbeke et al. 1978). Translocation of Cd to the tops, which is related to water flux, would therefore appear to occur in parallel to binding by the root.

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