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Investigation of effects of cadmium, lead, nickel and vanadium contamination on the uptake and transport processes in cucumber plants by TXRF spectrometry ☆

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Abstract

Uptake and transport processes of some essential nutrient elements (K, Ca, Fe, Mn and Zn) in cucumber plants grown in contamination-free or in contaminated (10^{-5} M Cd, Ni, Pb or V) nutrient solutions containing iron in the chemical form of Fe(III)-citrate, Fe(III)-EDTA or Fe-chloride were studied by total reflection X-ray fluorescence spectrometry (TXRF). The root samples were dissolved using a microwave assisted acidic digestion procedure, while the xylem sap samples were directly analysed after addition of internal standard. It was established that the accumulation in the roots and the transport rate of the four heavy metals investigated increase in order of $V < Ni \ll Cd < Pb$ and $V \ll Pb < Cd < Ni$, respectively. Due to the relatively low accumulation and transport of V in the plant it has the smallest influence on the uptake and transport of the essential elements. Cd hampers the water uptake and thereby the amount of all transported essential elements, and in addition results in higher accumulation of Ca, Fe and Zn in the roots. Pb contamination leads to a drastic reduction of Ca accumulated in the roots and mostly a slight increment in the transport of the essential nutrient elements investigated. Ni contamination hinders the transport of K and Zn, and leads to a higher accumulation of Mn in the roots. The chemical form of iron and hereby the presence of complex forming agents play an important role first of all for the uptake and transport processes of Fe and K. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Metal uptake; Transport processes; Cucumber plants; TXRF

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1. Introduction

Plants can accumulate trace elements, especially heavy metals, in or on their tissues due to their great ability to adapt to variable chemical effects of the environment, thus plants are intermediate reservoirs for trace elements originating from the lithosphere, hydrosphere or the atmosphere. In order to give an overall picture about the effects of different elements on the plants, it is necessary to have a wide knowledge of various factors concerning the soil, the type of the plant, as well as the concentration, the chemical form, the availability, the essentiality and the toxicity of the chemical elements. According to published results these effects depend on the physico-chemical properties of elements, however, each element has different influence on the plants [1–6].

One of the most fruitful ways to obtain valuable information about the interaction of heavy metals with inorganic and organic molecules of plants is the quantitative determination of all chemical compounds of plants by growing them under controlled environmental conditions in the presence and absence of heavy metals. The changes in the chemical composition of plants caused by heavy metals can be established through the analysis of various plant parts (leaves, stems, roots, etc.) and the xylem or phloem fluids.

For elemental analysis of dried plant parts after their acidic dissolution, well-known classical (titrimetry, gravimetry) and instrumental (atomic absorption, emission or fluorescence spectrometry, inductively coupled plasma (ICP) mass spectrometry, anode stripping voltammetry, etc.) analytical techniques have been used [7]. In the case of xylem fluids, however, the volume of xylem sap to be analysed restricts the applicability of these analytical methods. The amount of xylem sap collected for analysis varies generally in the range of 0.1–1 cm^3 per plant [8–10] depending on the species and age of the plants, the frequency and time of sampling, and the nutrition during the bleeding.

In most cases the xylem fluids of soybean, tomato, cucumber, and squash plants were investigated [8–10]. Although the sample volume can be increased by simultaneous collection of xylem fluid from more plants cultivated under the same environmental conditions, the total volume, especially in heavy metal contaminated plants, does not exceed 1 cm^3. Therefore multielemental trace analysis of low-volume xylem fluids or biological tissues requires powerful microanalytical techniques like ICP-mass spectrometry combined with the electrothermal vaporisation system [11] or the total reflection X-ray fluorescence (TXRF) spectrometry [12–15].

To reduce matrix interference it is generally desirable to digest the biological fluid samples with nitric acid before their microanalytical investigation [11–16]. In the case of TXRF, the necessity of acidic digestion of biological fluids depends on the total concentration of organic and inorganic compounds in the sample, which determines the thickness of the dry residue on the carrier surface [17]. Namely, reliable quantification using internal standardisation is possible if the thickness of the dry organic residue does not exceed 12 μm [18]. Therefore, biological fluids with high organic content cannot be analysed without preliminary digestion of the samples [12,14,16]. However, being thin solutions with low dry matter content (1–2 mg/ml), xylem fluids of plants with high exudation rates (cucumber, squash) are directly measurable by TXRF spectrometry [19].

Since the comparison and the evaluation of these literature data are difficult due to the differences in growing circumstances and ages of the plants, chemical form of contaminants, etc. We have decided to study the influence of Cd, Ni, Pb and V on cucumber plants, which were simultaneously grown at the same experimental conditions (lighting, temperature, growing time, concentration of elements in the nutrient solutions). In this study we present the results of TXRF investigation of the xylem fluids and the digested roots of cucumber plants evaluating the effect of these four heavy metals on the uptake and transport processes of essential elements.

2. Experimental

2.1. Growth of the plants and sampling procedures

Cucumber seeds (*Cucumis sativus* L.) were germinated on wet filter paper for 1 day in darkness at 26°C in thermostatically controlled petri dishes. These seedlings were planted in plastic nets placed between polystyrene disks, which were put into PVC cups containing 200 cm^3 5×10^{-4} mol/dm^3 $CaSO_4$ solution. Then they were covered with wet filter paper to keep the plants in a dark, moisture-filled atmosphere at 26°C for 1 more day. After this step the plants were transferred into modified Hoagland nutrient solutions with the following chemical composition: 1.25 mmol/dm^3 KNO_3, 1.25 mmol/dm^3 $Ca(NO_3)_2$, 0.5 mmol/dm^3 $MgSO_4$, 0.25 mmol/dm^3 KH_2PO_4, 11.6 μmol/dm^3 H_3BO_3, 4.5 μmol/dm^3 $MnCl_2\cdot4H_2O$, 10 μmol/dm^3 Fe(III)-EDTA, Fe(III)-citrate or $FeCl_3$, 0.19 μmol/dm^3 $ZnSO_4\cdot7H_2O$, 0.12 μmol/dm^3 $Na_2MoO_4\cdot2H_2O$, 0.08 μmol/dm^3 $CuSO_4\cdot5H_2O$. The nutrient solutions were replaced three times per week. The hydroponic cultures were maintained in a controlled environment at 22–26°C and illuminated by daylight with metal halogen lamps and fluorescent tubes with light intensity of 75 W/m^2 for 12 h/day. At these conditions 45 cucumber plants were simultaneously grown in this 'heavy metal free' modified Hoagland nutrient solution. It should be noted, however, that this nutrient solution also contained a lead concentration of 17 nmol/dm^3, due to the impurities of the chemicals applied for the preparation of the nutrient solutions. By the applied graphite furnace atomic absorption technique Cd, Ni and V were not detectable in these solutions. At the second leaf stage, 3–3 plants were transferred into each heavy metal contaminated nutrient solution containing $Cd(NO_3)_2$, $Pb(NO_3)_2$, $NiSO_4$ or $VOSO_4$ in concentrations of 10 μmol/dm^3. The control plants remained in the 'heavy metal free' solutions.

After a growing period of 32 days, the stems of the plants were cut 5 mm above the root collar and the xylem saps were collected for 60 min from the freshly cut surfaces into polyethylene vials using micropipettes. After determination of their weights the xylem sap samples were stored at −22°C. Subsequently the root samples were centrifuged to remove the rest of the nutrient solutions from the surface and their fresh weight was measured. Then the roots were dried at 80°C until they reached the weight constancy.

2.2. Sample preparation

For the TXRF investigations of xylem fluids, 190 μl of each sample was spiked with a standard solution of Ga or Ni to a final concentration of 1 μg/cm^3. From each of these spiked and mixed solutions 25 μl was dropped onto a quartz glass carrier and dried in a clean box applying a ceramic-coated hot plate at 80°C for 30 min. From each solution three parallel samples were prepared and analysed.

The dried root samples were dissolved using a microwave assisted digestion procedure. Depending on the mass of the dried roots, 8 cm^3 concentrated nitric acid (p. a. grade, Merck) was added to the samples having mass less than 500 mg. If the mass of the roots exceeded this value, the samples were divided into two parts and digested separately applying a three-step procedure with power of 250, 300, 500 W for 3, 10 and 20 min at a pressure of 20, 80 and 100 psi, respectively. During the digestion nine vessels were heated simultaneously. After digestion the solutions were filled up to 25 cm^3 with deionized water. The internal standard elements were added to the samples in all cases before the TXRF measurements.

2.3. Instrumentation

The root samples were dissolved in a pressure-controlled microwave digestion system (MDS-2100 CEM Corporation, Matthews, USA).

The analysis of xylem saps and the digested root samples were carried out by an EXTRA IIA total reflection X-ray fluorescence spectrometer (produced by Atomika Instruments GmbH, Oberschleissheim, Germany). Specific analytical parameters are as follows: Mo and W micro focus X-ray tube (50 kV, 38 mA), high energy cut-off filters (quartz-glass mirrors) and attenuation filters (for Mo tube: 200 μm Mo, 240 μm Al; for W(B) tube: 1000 μm Al, 100 μm Ni). The fluorescence photons emitted by the elements of the samples were recorded by a Si(Li) detector of 80-mm^2 area. The integration time was 500 s for each sample. The energy of the applied analytical lines: K Kα 3.312 keV, Ca Kα 3.690 keV, V Kα 4.949 keV, Mn Kα 5.894 keV, Fe Kα 6.398 keV, Ni Kα 7.471 keV, Zn Kα 8.630 keV, Pb Lα 10.267 keV and Cd Kα 23.106 keV.

3. Results and discussion

To investigate the accumulation and transport processes of essential and contaminating elements in cucumber plants it is necessary to determine the volume of the xylem sap collected during a 1-h bleeding and also the weight of the roots after a certain growing period. As Fig. 1 demonstrates the transported amounts of the xylem sap increase in the presence of Pb and V contamination related to the control plants independently from the chemical form of iron. In the case of Ni pollution, however, a relatively high decrease (30–50%) can be observed. Addition of Cd resulted in a drastic reduction of the xylem fluid in the case of Fe(III)-EDTA and Fe(III)-citrate, while in the presence of $FeCl_3$ the bleeding was completely blocked and it was not possible to collect xylem fluid at all. The explanation of this great hindrance is that the cadmium disturbs the water uptake through the roots and influences the transpiration. This phenomena has already been observed in the case of other plants, too [20]. These data are in very good agreement with the mass of the roots (Fig. 1a). It can be seen that in the case of Ni the rate of decreasing is approximately 30–50%. The growth of the roots in the presence of Cd contamination was extremely hampered and both the dry and the fresh weights were only 10–20% of the control plants. The ratio of the dry and fresh weights are very similar for plants growing in the 'pure' and the Pb, Ni or V contaminated nutrient solutions, while in the case of Cd contaminated plants a higher ratio can be observed, independently from the different chemical form of iron. It is obvious that this ratio correlates with the water content of the roots, thus it can be established that, with exception of Cd, the water uptake varied proportionally with the masses of the roots. As cadmium is able to alter the water balance in the plant, with this phenomenon the obtained higher ratio can be explained. The weights of the roots and also of the xylem saps (Fig. 1) decreased in the order of Fe(III)-EDTA > Fe(III)-citrate > Fe(III)-chloride.

In order to understand the effect of the different contaminating elements on the essential ones, the accumulated and transported amounts were measured by the TXRF method after an acidic digestion procedure. Fig. 2a shows the amounts of Pb, Cd, Ni and V in the roots. These analytical

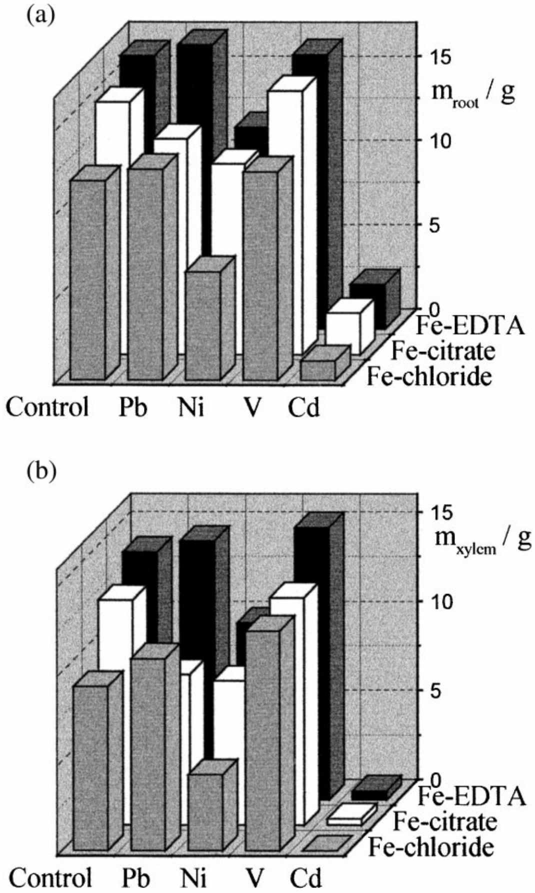

Fig. 1. Fresh mass of the cucumber roots (a) and the amounts of xylem sap (b) collected during 1-h bleeding period from cucumber plants grown in control and heavy metal contaminated nutrient solutions containing Fe(III)-EDTA, Fe(III)-citrate or $FeCl_3$.

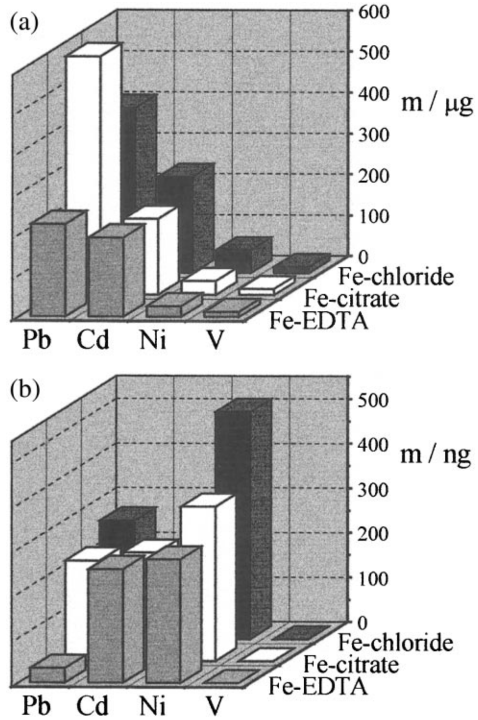

Fig. 2. Amounts of heavy metals accumulated in the cucumber roots (a) and transported in the xylem sap (b) of cucumber plants during a 1-h bleeding period related to 1 g of fresh root.

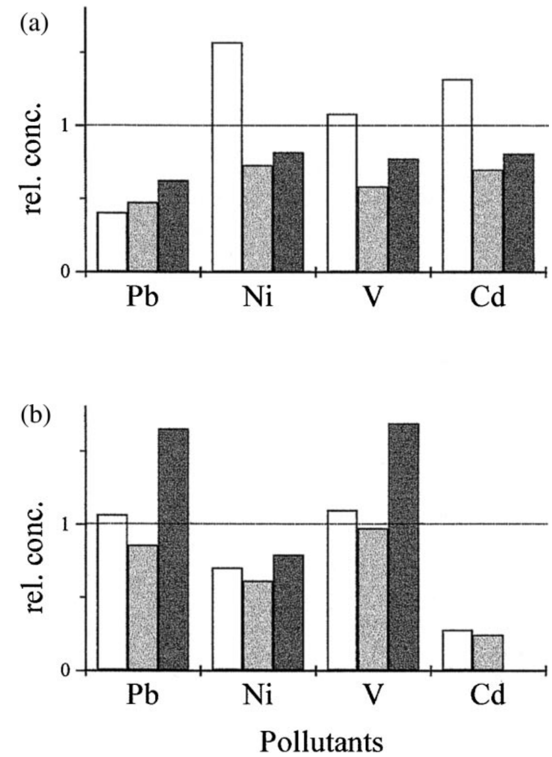

Fig. 3. Relative change of K-concentration in the root (a) and in the xylem sap (b) of cucumber plants grown in heavy metal contaminated nutrient solutions containing Fe(III)-EDTA (white), Fe(III)-citrate (light grey) or $FeCl_3$ (grey).

data can be characterised by RSD values of 1–10%. It can be seen that mainly Pb and Cd accumulated in the roots. In one of our earlier experiments [21] we found the same results: the lead uptake reaches the highest value in the presence of citrate. On the other hand, the addition of Fe(III)-EDTA leads to a lower uptake of Pb compared to the Fe(III)-chloride. These results are in accordance with the observation of Tanton and Crowdy [22] whereas the movement of Pb-EDTA into the transpiration stream is limited. The behaviour of Cd seems to be similar to that of Pb. This metal was also strongly accumulated in the roots, as it was expected from literature data [23]. Comparing the amounts of Pb and Cd in the roots and in the xylem sap (Fig. 2b), it can be seen that in the case of Fe(III)-citrate the trans-
ported mass is similar, while in the presence of EDTA complex-forming agent the Cd was more mobile than Pb. Unfortunately in the case of plants grown in $FeCl_3$ and Cd containing nutrient solution there are no data for the xylem fluids, because the water uptake was practically stopped. Ni shows a different behaviour from the former two elements. Its amount in the roots is much lower than those of Pb and Cd, which means, that Ni does not deposit in the roots, but is transported continuously to the shoots. V could enter the roots only in a very small amount (13–16 μg/g). As usual only a few percent of the uptaken element can be transported in the xylem sap, therefore vanadium was not detectable by the TXRF technique.

The effects of the four heavy metals on the essential elements are shown in Figs. 3–7. The relative concentration values were calculated as a ratio of concentrations measured in contaminated and control plants. It can be established that Pb

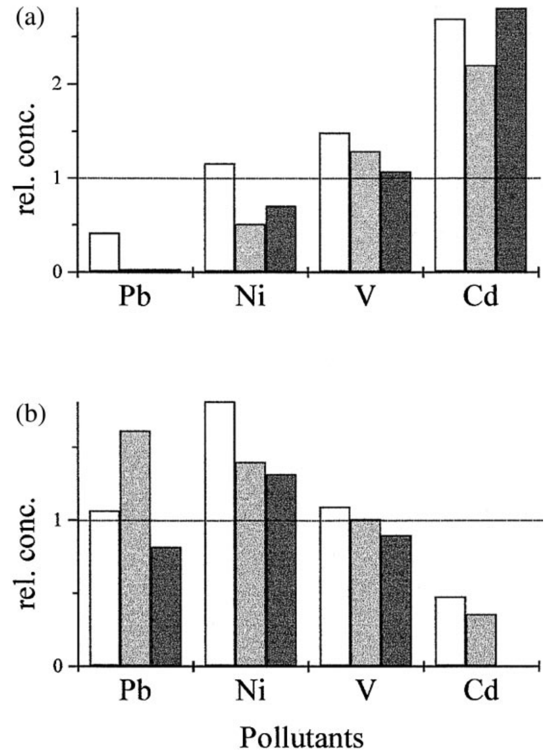

Fig. 4. Relative change of Ca-concentration in the root (a) and in the xylem sap (b) of cucumber plants grown in heavy metal contaminated nutrient solutions containing Fe(III)-EDTA (white), Fe(III)-citrate (light grey) or $FeCl_3$ (grey).

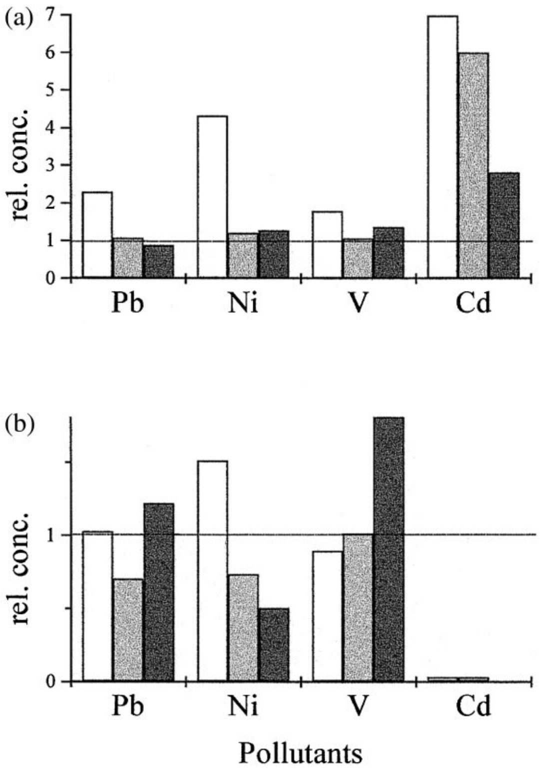

Fig. 5. Relative change of Fe-concentration in the root (a) and in the xylem sap (b) of cucumber plants grown in heavy metal contaminated nutrient solutions containing Fe(III)-EDTA (white), Fe(III)-citrate (light grey) or $FeCl_3$ (grey).

hampers the uptake of K (Fig. 3a) in all cases of different chemical forms of iron. For Ni, V and Cd, in the presence of citrate and chloride, the K uptake decrease, and using EDTA as a complex forming agent, a stimulating effect can be observed. In spite of this, the change of the K-transport (Fig. 3b) shows another trend, namely this process is partly blocked by Ni and there is a relatively strong hindrance caused by Cd. In the case of Ca accumulation and transport processes, Pb and Cd contamination results in contrasting effects (Fig. 4). While Pb hampers, almost totally, the accumulation of Ca in the root and it has some stimulating effect on the transport of Ca, the Cd contamination leads to a higher accumulation rate on the roots and a reduced Ca-transport related to the control plants. On the accumulation of iron also the Cd has the main influence: it increased the uptake rate, but the translocation of iron to the shoots is totally hampered by this metal (Fig. 5). Cd exerts an effect also on the Zn movement (Fig. 6). This contamination results in considerably higher uptake rates of Zn (250–400%), while the transport in the xylem sap is hindered by Ni, V and Cd depending on the presence of complex forming agents. From the results demonstrated on Fig. 7 it follows that Cd considerably hampers the accumulation and the transport processes of Mn. However, Ni contamination results in a higher accumulation rate of Mn in the roots compared to the control plants.

4. Conclusions

The effects of Cd, Pb, Ni and V on the accumulation and transport processes of Ca, K, Fe, Mn

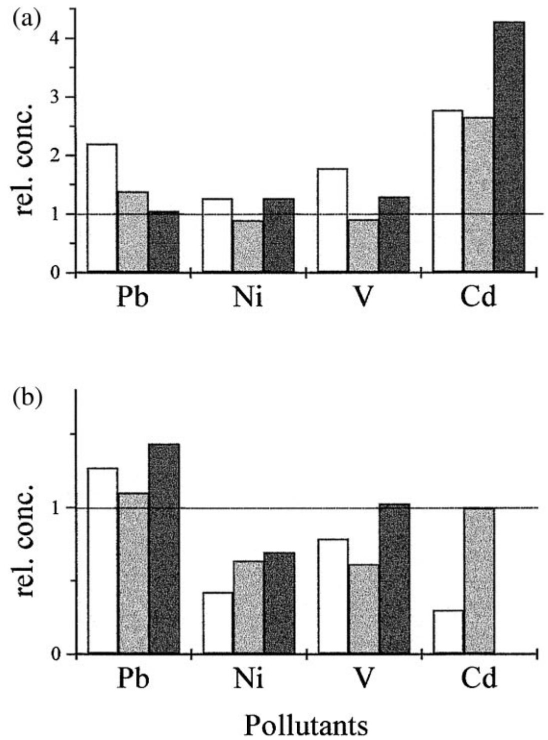

Fig. 6. Relative change of Zn-concentration in the root (a) and in the xylem sap (b) of cucumber plants grown in heavy metal contaminated nutrient solutions containing Fe(III)-EDTA (white), Fe(III)-citrate (light grey) or $FeCl_3$ (grey).

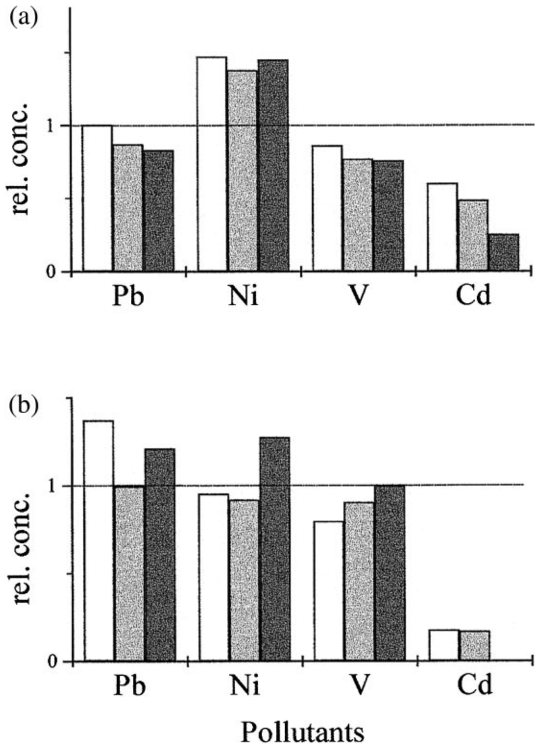

Fig. 7. Relative change of Mn-concentration in the root (a) and in the xylem sap (b) of cucumber plants grown in heavy metal contaminated nutrient solutions containing Fe(III)-EDTA (white), Fe(III)-citrate (light grey) or $FeCl_3$ (grey).

and Zn in cucumber plants can be studied by the TXRF method. Due to the low dry matter content of the xylem fluid (1–2 mg/cm^3) its analysis can be directly carried out by this technique, while analysis of the root samples needs a microwave assisted digestion. Regarding the plant physiological results, it can be stated that the water uptake is strongly hampered by cadmium. Comparing the investigated element contents in the roots and in the xylem fluids it can be concluded that the main part of Pb and Cd accumulates in the root and only a very small amount of these two elements are transported to the shoots. On the other hand the mobility of elements investigated is different and increases in the order of $V \ll Pb < Cd < Ni$. The influence of heavy metals on the accumulation and transport of essential elements depends on all the variable parameters (pollutants, essential elements and the chemical form of iron). From the data obtained it can be concluded that the general effect of heavy metals investigated changes in the following sequences: $Cd > Pb \sim Ni \gg V$. The explanation of these phenomena requires the determination of the transport partners in the xylem (e.g. metalloproteins, complexes formed with malic, citric or fumaric acids).

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