

Vojislav M. Mihailović², Novica M. Petrović^{1,2},
Ivana V. Arsenijević-Maksimović^{1,2}, Žarko S. Kevrešan¹

¹ Faculty of Agriculture Novi Sad

Trg D. Obradovića 8

21000 Novi Sad, Yugoslavia

² Institute of Field and Vegetable Crops Novi Sad

M. Gorkog 30

21000 Novi Sad, Yugoslavia

CADMIUM TRANSLOCATION AND RETRANSLOCATION BY PEA (*Pisum sativum* L)

ABSTRACT: Under semi-controlled conditions, we studied cadmium (Cd) translocation and retranslocation in the aphylla pea cultivar Jezero at two stages of growth and development. The applied Cd concentrations were 0 (control), 10^{-7} and 10^{-5} M Cd and the age of plants at the time of treatment was 26 and 63 days. The application of Cd via the root lasted for 48 h. The plants were analyzed immediately after the treatment, 38 days later and at maturity. Dry matter mass and Cd contents were determined in individual plant organs. The results showed that dry mass had decreased noticeably depending on the Cd concentration used and plant age. The decrease of dry matter mass in both vegetative and generative plant organs was more significant when Cd was applied at flowering (63 day-old plants) than when the plants were younger (25 day-old). In all the treatments, the highest Cd content was found in the root. The translocation of Cd from the root to the aboveground vegetative and generative plant parts took place and it was relatively more intensive at the lower Cd concentration than at the higher one. The possibility that a minor amount of Cd was retranslocated from the leaves to the seeds and pods could not be ruled out either.

KEY WORDS: pea, plant age, cadmium, plant organ mass, translocation, retranslocation.

INTRODUCTION

The rates of uptake, accumulation, and translocation of heavy metals (HMs) in plants vary according to the physical-chemical properties of their ions as well as according to plant species and genotype involved. A number of authors have established for many species that HMs accumulate more intensively in the root than in the aboveground plant parts (Florijn and Van Beusichem, 1993; Gárate et al., 1993). HM translocation, and hence, HM distribution typically depend on the plant's capacity to build chelate complexes with the components of xylem sap (Kochian, 1991). In contrast to

the other HMs, cadmium and nickel are rapidly transported to the aboveground plant parts after uptake in many plant species (Verkleij and Schat, 1990; Petrović and Kastori, 1994). Cadmium differs from nickel in that it retranslocates poorly. Because of this, Cd content in seeds and fruits of some plant species is very low, even when these grow on soils highly contaminated by Cd (Leita et al., 1996).

Having in mind all of the above as well as the fact that Cd is transported to the aboveground plant parts primarily by transpiration (Barceló and Poschenrieder, 1990), i.e., that it depends on the morphological and anatomical structure of the aboveground plant parts, we thought it would be of interest to study Cd translocation and retranslocation in an aphylla pea genotype characterized by a small leaf area and, hence, a low transpiration activity.

MATERIALS AND METHODS

The trials with the aphylla pea cultivar Jezero were conducted in a greenhouse under semi-controlled conditions. The seeds were germinated in vermiculite in a thermostat at 25°C. After germination, the young plants were transferred to 2 l pots containing 1/2 strength Hoagland's nutrient solution (Hoagland and Arnon, 1950), in further text: complete nutrient solution. In the greenhouse, day/night temperature was 25/15°C, photoperiod 16 h, and relative humidity 70–75%. The translocation and retranslocation of Cd and its effect on plant organ mass were studied both in the early and late stages of growth and development (Experiments A and B, respectively).

Experiment A

After 25 days on a complete nutrient solution, a portion of plants was treated with 0 (control), 10^{-7} or 10^{-5} M Cd for 48 hours. Cadmium was applied as CdCl₂ dissolved in distilled water. Following the Cd treatment, the roots of intact plants were immersed in 20 mM Na₂-EDTA for 15 minutes to remove Cd adhering to root surfaces. Immediately afterwards, a portion of plants was taken for analysis, i.e., harvested and separated into roots and the aboveground parts (I), while the rest of them were returned onto the complete nutrient solution.

At full flowering, i.e., 38 days after the Cd treatment, another portion of plants was taken for analysis. The plants were separated into roots, aboveground parts (I), and newly-formed aboveground parts (II).

At maturity, the remaining plants were harvested and separated into roots, aboveground parts (I), aboveground parts (II), pods and grains.

Experiment B

Pea plants were grown on the complete nutrient solution until full flowering, i.e., for 63 days. A portion of these plants was then treated with either 0

(control), 10^{-7} or 10^{-5} M Cd (prepared as described above) for 48 hours. As in the experiment A, the roots of intact plants were immersed in 20 mM $\text{Na}_2\text{-EDTA}$ for 15 minutes. After 48 hours, a portion of the plants was taken for analysis having been separated into roots and aboveground parts. The remaining plants were transferred onto the complete nutrient solution and grown till maturity, when they were harvested and separated into roots, aboveground parts, pods, and grains.

The dry matter mass of individual organs was determined after oven drying at 60°C to constant mass. Cadmium content was measured by AAS using a Varian AA-10.

The results were statistically processed by the analysis of variance. Differences between the treatments were calculated using Duncan's multiple range test.

RESULTS

Dry matter mass

The dry matter mass of roots and shoots of plants harvested at flowering (analyzed 38 days after the treatment with Cd) decreased significantly relative

Tab. 1. — Effect of Cd on dry matter mass of pea (g. plant⁻¹). Plant age at the time of treatment with Cd was 25 days (A) and 63 days (B).

Cd concentration [M]	Plant organ				
	Root	Total above ground portion of the plant		Pods	Grain
		I*	II**		
<i>EXPERIMENT (A)</i>					
<i>Flowering</i>					
0 (Control)	0.530	1.413	3.358	—	—
10^{-7}	0.456	1.235	2.990	—	—
10^{-5}	0.408	1.080	2.655	—	—
Duncan 5%	0.071	0.150	0.491	—	—
<i>Maturity</i>					
0	0.587	1.163	4.006	0.502	3.543
10^{-7}	0.501	1.129	3.251	0.435	2.900
10^{-5}	0.439	1.022	3.044	0.377	2.484
Duncan 5%	0.098	0.111	0.507	0.078	0.384
<i>EXPERIMENT (B)</i>					
<i>Maturity</i>					
0	0.586	1.168	4.057	0.545	3.460
10^{-7}	0.450	1.050	3.444	0.400	2.442
10^{-5}	0.390	0.839	3.047	0.300	1.675
Duncan 5%	0.070	0.246	0.547	0.083	0.424

* Above-ground portion of 25 days old plants

** Above-ground plant parts formed after the 25th day

to the control. The decrease depended on the Cd concentration applied as well as on the plant organ studied. Both 10^{-7} and 10^{-5} M Cd significantly reduced the dry matter mass of the plants. The largest drop was recorded in the above-ground plant parts (I) developed during the Cd treatment and the lowest in the newly formed aboveground parts (II) treated with 10^{-7} M Cd (Tab. 1).

At maturity, the dry matter mass of both vegetative and generative organs dropped considerably (Table 1). Contamination of plants with Cd in the earlier stages of growth and development had a particularly large effect on the dry matter mass of the grain and root. At this stage too, 10^{-5} M Cd brought about a larger decrease of total dry matter mass than 10^{-7} M.

At maturity, the dry matter mass of pea plants treated with Cd at flowering (Experiment B) was significantly reduced relative to that of control plants. The effect of 10^{-5} M Cd was particularly unfavorable, especially on the dry matter mass of the grain and root (Tab. 1).

The two Cd concentrations had no significant effect on the ratio: [root dry matter mass] / [shoot dry matter mass] in any phase of the study. The dry matter mass of the root and aboveground parts had been reduced nearly to the same amount (Tab. 1).

Table 1 shows that the phytotoxicity of Cd depends not only on its concentration in the nutrient substrate but also on the age of plant at the time of contamination, in other words, on the rate of its uptake, translocation, and retranslocation by plants, or plant metabolic activity.

Cd content, translocation, and retranslocation

Forty-eight hours after the Cd treatment, the amount of Cd that had accumulated in the root was higher than that in the aboveground parts of the young pea plants. The Cd content in the plants treated with 10^{-5} M Cd was two times higher than the content in the plants treated with 10^{-7} M Cd. However, the translocation of Cd from the root to the aboveground plant parts during the treatment was somewhat greater in the lower Cd concentration treatment (Tab. 2).

At flowering, 38 days after the treatment with Cd, the percentage contribution of root Cd content to total Cd content of the plant was 48% for the 10^{-7} M Cd treatment and 62% for the 10^{-5} M one. This means that the translocation of Cd, i.e., its movement to the newly formed aboveground parts, was significantly higher in the treatment with 10^{-7} M Cd (Tab. 2).

At maturity, the contribution of root Cd content to total Cd content in the plant was 20.4% for the 10^{-7} M Cd treatment and as high as 51.2% for the 10^{-5} one. In the pea genotype under study, the retranslocation of Cd to the generative organs — the pod and grain — was noticeable. It depended significantly on Cd concentration in the nutrient substrate and was considerably more obvious in plants treated with the lower Cd concentration (Tab. 2).

In the 63 day-old plants treated with 10^{-5} M Cd at flowering, the total Cd content per plant after 48 h treatment was considerably higher than in either the plants treated with 10^{-7} M Cd or plants that were 25 days old at the

Tab. 2. — Translocation and retranslocation of Cd in pea at different stages of growth and development ($\mu\text{g Cd. organ}^{-1}$). Plant age at the time of treatment with Cd was 25 days (A) and 63 days (B).

Cd concentration [M]	Plant organ				
	Root	Total above ground portion of the plant		Pods	Grain
		I*	II*		
<i>EXPERIMENT (A)</i>					
<i>After treatment with Cd</i>					
10^{-7}	17.33	6.35	—	—	—
10^{-5}	34.26	10.80	—	—	—
Duncan 5%	5.55	1.21	—	—	—
<i>Flowering</i>					
10^{-7}	10.62	7.74	3.75	—	—
10^{-5}	24.71	10.72	4.30	—	—
Duncan 5%	3.19	1.39	0.76	—	—
<i>Maturity</i>					
10^{-7}	4.22	6.12	7.15	3.07	0.14
10^{-5}	23.27	8.22	7.21	5.57	0.21
Duncan 5%	3.21	1.42	0.53	0.65	0.03
<i>EXPERIMENT (B)</i>					
<i>After treatment with Cd</i>					
10^{-7}	23.56	3.53	2.35	—	—
10^{-5}	108.54	8.47	6.93	—	—
Duncan 5%	7.50	0.67	0.48	—	—
<i>Maturity</i>					
10^{-7}	15.44	3.70	7.37	0.99	2.66
10^{-5}	85.02	7.71	19.50	2.13	11.93
Duncan 5%	7.72	1.07	1.66	0.38	1.83

* Above-ground portion of 25 days old plants

** Above-ground plant parts formed after the 25th day

time of Cd treatment. The transport of Cd to the aboveground plant parts during the treatment was quantitatively higher at 10^{-5} M Cd and relatively higher at 10^{-7} M Cd. The results have shown that the transport of Cd from the root to the aboveground plant parts primarily depended on the Cd concentration in the substrate (Tab. 2).

At maturity, Cd content per plant was either 30.1 or 126.3 $\mu\text{g Cd/plant}$, depending in the first place on the Cd concentration applied. The translocation and retranslocation of Cd were also visibly dependent on Cd concentration in the nutrient medium. The transport of Cd from the root to the vegetative aboveground parts was more pronounced in the treatment with 10^{-7} M Cd. When retranslocation of Cd to the generative organs is considered, more Cd moved to the grain in Experiment B, while in Experiment A larger amounts of Cd were found to have moved to the pod (Tab. 2).

The results of the study suggest that Cd accumulation in pea depends not only on the concentration of the element in the substrate but also on the stage of plant growth and development at the time of Cd application. In the aphylla-type pea, Cd translocation and retranslocation are conspicuous. After comparing the Cd content in pea with those in other plant species, it can be concluded that the aphylla-type pea belongs to the group of plant species in which Cd uptake and accumulation are less pronounced.

DISCUSSION

Contamination of nutrient substrate brings plant roots into direct contact with HMs, which could be one of the reasons why HMs have a greater inhibitory effect on root growth than on the growth of the aboveground parts of the plant (Kastori et al., 1997). Recently, HMs have been thought to affect plant growth and development mostly through phytohormones. Most HMs affect plant metabolism and auxin transport (Vangronsveld and Clijsters, 1994). Excess HM amounts inhibit both the division and elongation of root cells and thereby visibly reduce their growth (Wierzbicka, 1988; Jalil et al., 1994). Factors that inhibit root growth also inhibit biosynthesis and translocation of cytokinins (Marschner, 1995). Excess HMs affect not only the growth of the primary root but also the formation of lateral roots in general and the rate of formation of root hairs in particular, thereby affecting root area as well (Brune and Dietz, 1995). The extent of root mass reduction depends on the nature of HM, its concentration and, especially, the plant species under study (Pettersson, 1976; Yang et al., 1996).

After taking up HMs by the root, many plant species transport them to a greater or lesser extent to the aboveground organs, where they disrupt plant metabolism (Lang et al., 1995). The inhibition of chlorophyll biosynthesis by Cd is specific (Parekh et al., 1990). Excess Cd reduces chlorophyll content in the PSI and PSII as well as in their antennae complexes (Lang et al., 1995). Cd promotes the activity of chlorophyllase in leaves (Jana and Choudhuri, 1984). This enzyme not only inhibits plant growth but also speeds up the aging process in plants (Lagriffoul et al., 1998).

In a number of species, HM accumulation is more intensive in the root than in the aboveground parts of the plant (Woolhouse, 1983). Studies carried out in sugar beet, maize, pea, and lettuce have shown that there is 10 to 20 times more Cd in the roots of these plant species than in their aboveground parts (Petrović et al., 1990; Florijn and Van Beusichem, 1993; Gárate et al., 1993). The high capacity of the root to accumulate HMs may be a way whereby the plant protects its aboveground parts from excess HM levels in the environment. In most cultivated plant, Cd concentrations above 10 mg/kg of dry matter are considered toxic (Page et al., 1981).

Cadmium differs from other HMs in that, after uptake, it is readily transported into the aboveground plant parts (Verkleij and Schat, 1990). Most authors think that the mechanisms of Cd uptake and ascendant transport are similar to those of the Ca^{2+} ion, i.e., that these are passive processes (Jar-

vis et al., 1976). Opinions still vary on the Cd uptake and transport by plants (Girling and Peterson, 1981). According to Leita et al. (1996), most plant species take up and transport Cd by the xylem in the form of Cd²⁺.

The mobility of Cd varies according to the species. For example, Cd is considerably more mobile in oil rape and pumpkin than in tomato, wheat, and oat (Pettersson, 1976). Studying 23 plant species, Jarvis et al. (1976) found notable differences among them not only in terms of the rate of Cd uptake but also in terms of the elements transport from the root to the aboveground parts. Furthermore, Florijn and Van Beusichem (1993) also found significant differences in Cd content and mobility among 19 maize inbred lines. The translocation of Cd from the root to the aboveground parts was about four times greater in *Triticum aestivum* L. than in *Triticum turgidum* L. var. *durum* (Hart et al., 1998).

Cd translocation in plants depends not only on the species but also on the available Cd concentration in the substrate (Cieslinski et al., 1996). In many species grown in the presence of high Cd concentrations, Cd translocation to the aboveground plant parts was found to be reduced as compared to the treatments in which plants were grown on a substrate with a lower Cd concentration. The results of our study support this finding. It is hypothesized that such conditions trigger internal detoxification mechanisms whereby the aboveground plant parts rid themselves of excess Cd (Baker et al., 1990).

Mechanisms that enable Cd accumulation in the fruit and seed have not been sufficiently clarified. Popelka et al. (1996) hold that *Arachis hypogae* L. transports Cd to the generative organs by the phloem, i.e., by retranslocation. There are also researchers who think that Cd retranslocates poorly and that its presence in the fruit and seed is negligible, even on highly contaminated soils (Leita et al., 1996).

Based on what science has learned thus far about the translocation and retranslocation of Cd into the generative organs of plants, it can be concluded that these two processes depend primarily on the plant genotype and the available Cd concentration in the substrate, while the influence of other factors is much less significant. The concentration of Cd in strawberry fruits depends both on the genotype used and the Cd level present in the nutrient medium (Cieslinski et al., 1996). The Cd content in the reproductive organs of 44 maize lines ranged from 0.08 to 3.70 mg/kg of dry matter (Hinsley et al., 1978). The Cd concentration in celery seeds was as much as 10 times higher than that found in the reproductive organs of wheat (Van Lune and Zwart, 1997). In the aboveground parts of *Papaver somniferum* L. plants, the highest Cd accumulation was in the seed, followed by the leaves, stem and, finally, the seed coat (Pavlikova et al., 1996).

Taking note of the results of the present study and the available literature data about Cd content, translocation, and retranslocation in other plant species, we can conclude that the aphylla-type pea belongs to the group of plants with a low capacity for Cd uptake and accumulation. The reason may be that this type of pea has a small leaf area and hence low transpiration. This is suggested by the results of Salt et al. (1995), who found a positive correlation between the rate of transpiration and the rate of Cd transport from the root to the

aboveground plant parts. Given that in our study it was the root Cd content that decreased the most after the treatment, it can be assumed that the larger part of Cd in the pods and seeds came from the root by translocation. While Cd retranslocation from leaves to generative organs could not be completely eliminated, the results showed that it had no significant effect on Cd accumulation in the pods and grains. Our results support the proposition that Cd retranslocates poorly from the leaf to the fruit. The knowledge that Cd is translocated from the root to the generative organs can be of importance for the production of biologically safe food.

CONCLUSIONS

Using the water culture method, we studied the translocation and retranslocation of Cd in the aphylla-type pea (cultivar Jezero) at two stages of growth and development. Cadmium was applied as 0, 10^{-7} and 10^{-5} M CdCl₂. The following conclusions have been reached.

The reductions in plant dry matter mass depended significantly on Cd concentration and plant age. The decrease of dry matter mass of both vegetative and generative plant organs was more significant when Cd was applied at flowering (63 day-old plants) than when plants were young (25 day-old), especially in the treatment with the higher Cd concentration.

In all treatments, Cd content was highest in the root. Based on the Cd content per plant, it can be concluded that the aphylla-type pea belongs to the group of plants with a low capacity for Cd uptake and accumulation.

It was established beyond doubt that translocation of Cd from the root to the aboveground vegetative and generative plant parts did occur. The possibility that a minor amount of Cd was retranslocated from the leaves to the seeds and pods could not be completely eliminated, either. However, the results showed that this had no significant effect on Cd accumulation in the pods and grains.

The results of the study support the proposition that Cd retranslocates poorly from the leaf to the fruit. Nevertheless, the knowledge that Cd is translocated from the root to the generative organs can be important for the production of safe food.

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ТРАНСЛОКАЦИЈА И РЕТРАНСЛОКАЦИЈА КАДМИЈУМА
У ГРАШКУ (*Pisum sativum* L)

Војислав М. Михаиловић², Новица М. Петровић^{1, 2}, Ивана В.
Арсенијевић-Максимовић^{1, 2} и Жарко С. Кеврешан¹

¹ Пољопривредни факултет Нови Сад, Трг Д. Обрадовића 8,
21000 Нови Сад, Југославија

² Научни институт за ратарство и повртарство Нови Сад,
М. Горког 30, 21000 Нови Сад, Југославија

Резиме

Проучавана је транслокација и ретранслокација кадмијума (Cd) у афила типу грашка сорте Језеро, у два стадијума раста и развића, у полуконтролисаним условима. Примењене концентрације Cd биле су 0 (контрола), 10^{-7} и 10^{-5} M Cd, а старост биљака у време третмана била је 26 и 63 дана. Третмани Cd преко корена трајали су по 48 h. Биљке су анализиране непосредно након третмана, 38 дана касније и у фази зрелости. Сува маса биљака и садржај Cd одређени су у појединачним биљним органима. Резултати су показали да се маса суве материје значајно смањила у зависности од примењене концентрације Cd и старости биљака. Смањење масе суве материје и вегетативних и генеративних органа било је значајније када је Cd примењиван у фази цветања (63 дана старе биљке) него када су биљке биле млађе (25 дана старе). Код свих третмана, највиши садржај Cd утврђен је у корену. Такође, дошло је до транслокације Cd из корена у надземне вегетативне и генеративне биљне органе и овај процес је био релативно интензивнији када је примењена концентрација Cd била нижа. Могућност да је дошло до ретранслокације минималних количина Cd из листова у махуне такође не може бити искључена.