Cadmium Content and Accumulation in Lettuce Grown on Different Types of Soil

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This study was conducted on a pseudogley (pH 5.0) and a vertisol (pH 6.9) to analyze their total and available Cd levels and Cd accumulation by lettuce for risk assessment of potential human health impacts. Total Cd levels in both soil types were below maximum allowable concentrations, and showed a high dependence on soil pH (R=-0.974). Monoammonium phosphate (MAP) fertilizers containing various amounts of cadmium led to an increase in cadmium uptake, with higher concentrations accumulated in lettuce roots than in leaves. The transfer factor showed that Cd uptake from the pseudogley was much higher than from the vertisol, and its values indicated that lettuce is a cadmium hyperaccumulator. Lettuce head weight decreased with increasing cadmium rates and was highly negatively correlated with Cd concentration in the leaves (R=-0.818).

Keywords: cadmium, monoammonium phosphate, lettuce, transfer factor

Vegetable production necessitates much higher fertilization rates compared to the production of other agricultural crops, thus causing danger of accumulation of deleterious elements found in fertilizers. In lettuce (*Lactuca sativa* L.) as a leafy vegetable crop used fresh and characterized by a short growing season and intense growth, there is the highest risk of uptake of these elements [1].

The presence of high Cd concentrations in mineral fertilizers, phosphorus ones in particular, is a serious problem due to Cd toxicity and availability to plants [2, 3]. Its content is 3.4-232 mg/kg in phosphorus fertilizers [4], 2.08-7.50 mg/kg in superphosphate [5], and 144 mg/kg in MAP [6].

There are different opinions in the literature regarding the effect of phosphorus fertilizers on Cd availability in soils. Many authors have reported an increase in Cd content in soils as affected by the use of phosphorus fertilizers containing Cd [3, 7, 7]. Other authors found no significant difference in soil Cd levels after phosphorus fertilizer treatment [8, 9]. Cd availability in soils is substantially affected by soil type [2, 10, 11], primarily pH and organic matter content [12, 13].

Generally, the uptake of Cd by plants is closely related to its concentration in soils. Cadmium concentration in vegetable crops increases with increasing Cd concentration in the soil [14, 15, 3]. Most commonly, the increase in Cd levels in edible plant parts causes no toxicity symptoms, but can have deleterious effects on human health [16], and adverse effects on crop yield [17, 18]. Significantly higher amounts of Cd accumulate in roots than in aboveground portions [6, 18, 19].

Potential human exposure to toxic effects of soil Cd through the food chain is best indicated by the transfer factor defined as the ratio of total Cd concentration in the soil to that in the plant [2, 20].

The objective of this study was to determine the effect of monoammonium phosphate containing different amounts of cadmium on Cd content in two soil types, Cd availability to plants and potential soil pollution with this metal.

Experimental part
Plant material and field trial

The experiment was conducted in pots under greenhouse conditions at the trial field of the Faculty of Agronomy at Trbušani near Èaeak, Western Serbia (43°55'N, latitude; 20°19'E longitude; 255 m a.s.l.) (fig. 1).

The research involved two soil types, a pseudogley and a vertisol (table 1). Each pot was filled with 5 kg air-dry soil sampled from a 0-30 cm layer and sieved through a 4 mm sieve. Then, one young lettuce cv. 'Sun Star' plant was planted per container. During planting, the plants were watered until field moisture capacity was reached, with subsequent watering treatments employed at a soil moisture below 70% of the field capacity.

The experiment involved mineral fertilization with two MAP (monoammonium phosphate-11%N, 52% P_2O_5) products, one manufactured in Serbia (MAP-S), the other in Russia (MAP-R). The content of Cd in MAP-S and MAP-R fertilizers was 12.32 mg/kg and 0.34 mg/kg, respectively. In order to improve the fertility of the soils used for lettuce production, nitrogen (0.15 g/kg soil) as CAN (calcium ammonium nitrate, containing 27% of N_{TOT}), and potassium (0.1 g/kg soil) as KCl (potassium

Table 1
CHEMICAL PROPERTIES OF SOILS

Type of soil	pH/H₂O	Humus (%)	Available P (mg/kg)	Available K (mg/kg)	Total Cd concentration (mg/kg)	Available Cd concentration (mg/kg)
Pseudogley	5.0	2.52	86	174	0.34	0.12
Vertisol	6.9	3.95	189	251	0.08	0.05

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Fig. 1 Map of Cacak with sampling location

CITY OF ČAČAK
Western Serbia,
Western Balkan
P 1 : 50 000

chloride, containing 60% of K_2O) were incorporated into the soils.

The experiment consisted of an untreated control and four treatments in three replications: 1. Control -without Cd; 2. MAP-S -15 mg/kg soil; 3. MAP-S -30 mg/kg soil; 4. MAP-R -15 mg/kg soil; 5. MAP-R -30 mg/kg soil.

Sample collection and preparation

Soil and plant material samples were collected for analysis at the end of the lettuce growing season. Soil samples were air-dried, ground and sieved through a < 2 mm stainless steel sieve. All plant samples were separated into edible and inedible parts, and then stored in polyethylene bags in the field and transferred to the laboratory. In the laboratory, the samples were carefully washed with tap water and then with deionized water to remove soil and dust particles. Then, they were weighed, dried at 65°C for 72 h, reweighed, ground and stored in plastic bottles for chemical analysis.

Soil and plant chemical analysis

Soil samples were analyzed using standard methods: the soil pH was measured at a 1:2.5 ratio of soil to distilled water, using a pH meter; the humus content was determined by oxidation with $KMnO_4$ solution (according to Kotzman); available phosphorus and potassium-extraction with 0.1M NH_4-lactate and 0.4M CH_3COOH, according to Egner-Riehm (P was analyzed spectro-photometrically; K was determined by flame photometry.

Total Cd content in soil was determined by the aqua regia (HCl/HNO_3, 3/1) extraction method described in [21]. Available Cd content in soil was determined by extraction with 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA solution, pH=7.3 [22].

Cd concentration in lettuce was determined by digestion with $HNO_3/HClO_4$, 5/1, described in [23]. Cd concentration was determined by the atomic absorption spectrophotometer Perkin-Elmer 3300/96, model MHS-10 (Perkin-Elmer, Santa Clara, California, USA).

Data analysis

Results were subjected to analysis of variance (ANOVA), and statistical significance of differences was determined by the LSD-test (P<0.05), (Michigan State University, East Lansing, MI, USA).

Transfer factor

In order to evaluate the ratio of heavy metal concentration in plants to that in soil, the transfer factor (TF) for the heavy metal was computed based on the method described by [24], according to the formula

$$TF = \frac{P_s}{S_t} \qquad (1)$$

where: P_s - heavy metal concentration in plant (mg/kg, dry weight); S_t - total metal concentration in the soil in which the plant was grown (mg/kg).

Results and discussions

Cd concentrations in soils

Total Cd concentration before trial establishment was 0.34 mg/kg in the pseudogley and 0.08 mg/kg in the vertisol, whereas available Cd concentration was 0.12 mg/kg and 0.05 mg/kg, respectively (table 1). The differences in total and available cadmium levels were expected, given the different properties of the two soil types. The pseudogley is acid in reaction, with a poor supply of humus and a moderate supply of available phosphorus and potassium. The vertisol is neutral in reaction and well supplied with humus and available phosphorus and potassium.

Cd dynamics in the soil is dependent on a number of parameters (pH, clay content, organic matter content), which may lead to poor Cd mobility in the soil and decreased adsorption by the plant. Nevertheless, the highest effect on Cd concentration in the soil is exhibited by *p*H, as *p*H can modify the transfer of elements from the bonded phase to the soil solution [25, 11]. In this study, the acid reaction of the pseudogley (*p*H 5.0) resulted in increased Cd concentration, while in the vertisol (*p*H 6.9) Cd mobility decreased, due to the deposition of hydroxides and oxides or due to the formation of insoluble organic compounds [26]. The relationship between soil *p*H and total Cd concentration in the soils is presented in figure 2 and shows a significant negative correlation (R= -0.974).

Overall, the analysis of total average Cd concentration in the soil shows no contamination of these soils with cadmium, since total Cd concentration was below maximum allowable concentration (2 mg/kg), i.e. 0.159 mg/kg in the pseudogley and 0.053 mg/kg in the vertisol (table 2). Potential toxicity of heavy metals is more related to their available concentrations than to their total
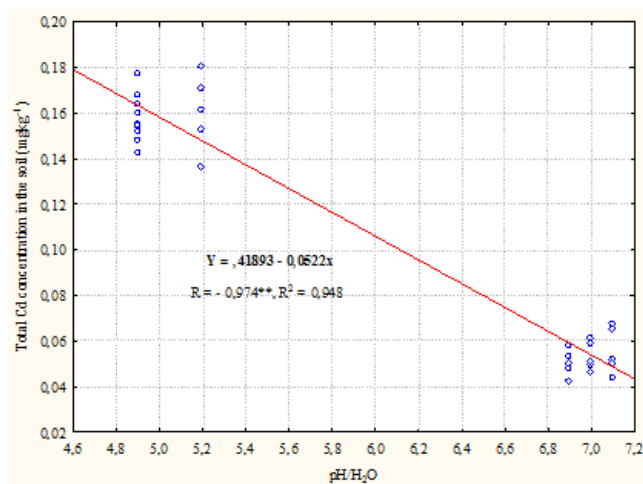
Fig. 2 Relationships between pH/H$_2$O and total Cd concentration in the soil

Table 2
TOTAL AND AVAILABLE Cd CONCENTRATION IN THE SOIL, Cd CONCENTRATION IN THE ROOT AND LEAF OF LETTUCE AND LETTUCE HEAD WEIGHT

Treatments		Total Cd in the soil	Available Cd in the soil	Cd concentration in the root	Cd concentration in the leaf of lettuce	Lettuce head weight
Type of soil (A)	1	0.159a	0.0244	79.62a	1.186a	172b
	2	0.053b	0.0266	29.34b	0.412b	238a
Fertilizer (B)	1	0.106	0.0250	7.10d	0.590d	246.5a
	2	0.106	0.2450	63.65b	0.935b	179.0c
	3	0.106	0.0275	89.20a	1.765a	154.0d
	4	0.106	0.0245	45.10c	0.640d	218.5b
	5	0.106	0.0260	67.35b	0.815c	227.0b
Type of soil x Fertilizer (AxB)	1	0.159	0.023	12.6gh	0.88c	201cd
	2	0.159	0.024	94.8b	1.39a	143f
	3	0.159	0.026	125.3a	1.50a	139f
	4	0.159	0.024	68.4c	0.96c	185de
	5	0.159	0.025	97.0b	1.20b	192d
	1	0.053	0.027	1.6h	0.30e	292a
	2	0.053	0.025	32.5ef	0.48d	215c
	3	0.053	0.029	53.1d	0.53d	169e
	4	0.053	0.025	21.8fg	0.32e	252b
	5	0.053	0.027	37.7e	0.43de	262b
ANOVA	A	**	ns	**	**	**
	B	Ns	ns	**	**	**
	AxB	Ns	ns	**	**	**

Data for Cd are expressed as mg/kg of dry matter.
Data for lettuce head weight are expressed as g.
The same letters in columns indicate non-significant differences between means at $P \leq 0.05$ by LSD test.
ns: non significant

concentration in the soil [27, 28]. In this study, before trial establishment, available Cd level in the pseudogley and vertisol was 0.12 mg/kg, and 0.05 mg/kg, respectively. After the lettuce growing cycle, available Cd concentration in both soil types decreased and exhibited a slight variation, with no statistical significance, both between soil types and between treatments with MAP from different sources and its application rates.

These findings indicate increased Cd mobility in the pseudogley, primarily due to the acid soil reaction and low organic matter content, resulting in increased uptake by the lettuce plant and increased accumulation in the leaves (table 2) compared to the plants grown on the vertisol, and hence the available Cd concentration in the pseudogley was lower than in the vertisol. Similar findings were reported by [9] who studied the effect of increasing rates of phosphorus fertilizers and obtained an average available cadmium concentration of 0.23 mg/kg. As for the percent contribution of DTPA-Cd to the total soil concentration (table 3), %DTPA-Cd ranged from 47.2 to 54.7% in the vertisol, and from 14.5 to 16.4% in the pseudogley. Similar results on clay soils were obtained by [4]

and [29] who determined that DTPA-Cd accounted for 14.5-83.3% and about 38%, respectively, of the total Cd concentration.

Cd concentration in lettuce
Cd concentration in plant tissues normally ranges from 0.05 to 0.2 mg/kg dry matter [30], with more Cd accumulated in the root than in aboveground plant parts [6, 19, 31].

In this study (table 2), Cd concentration in the root of lettuce grown on the pseudogley was between 12.6 mg/kg (control) and 125.3 mg/kg (increased rates of MAP-S). Cd concentration in the root of lettuce grown on the vertisol was in the range of 1.6 mg/kg (control) to 53.1 mg/kg (increased rates of MAP-S). Results show that increased rates of Cd incorporated into the soil through phosphorus fertilizer led to an increase in Cd concentration in the lettuce root, on both types of soil. Moreover, the root of lettuce grown on the pseudogley accumulated statistically significantly higher amounts of Cd compared to that on the vertisol, which is associated with the increased Cd

Table 3

PERCENTAGE VALUES OF DTPA-Cd IN A TOTAL Cd CONTENT IN SOIL AND TRANSFER FACTOR

Type of soil	Fertilizer	%DTPA-Cd	Transfer factor (TF)
Pseudogley	Control	14.5	5.53
	MAP-S (15 mg/kg soil)	15.1	8.74
	MAP-S (30 mg/kg soil)	16.4	9.43
	MAP-R (15 mg/kg soil)	15.1	6.04
	MAP-R (30 mg/kg soil)	15.7	7.54
Vertisol	Control	50.9	5.66
	MAP-S (15 mg/kg soil)	47.2	9.06
	MAP-S (30 mg/kg soil)	54.7	10.00
	MAP-R (15 mg/kg soil)	47.2	6.04
	MAP-R (30 mg/kg soil)	50.9	8.11

content in the pseudogley and the higher mobility of this metal in the acid soil. The same pattern of increase in Cd concentration was observed in lettuce leaves, with the lowest concentration of Cd (0.30 mg/kg) found in the untreated control in the vertisol, and the highest (1.50 mg/kg) in the pseudogley, at high rates of MAP-S. Cd levels in lettuce leaves exceeded maximum allowable concentrations for vegetable crops (Rulebook on the Amount of Pesticides, Metals, Metalloids and Other Toxic Substances, Chemotherapeutics, Anabolics and Other Substances Found in Food Products, Official Gazette of the FRY, issues 5/92, 11/92 and 32/2002). The results of the present study confirm the findings of [3] and [32] who found that lettuce has a high affinity to accumulate heavy metals, which coupled with its short growing season contributes to increased cadmium levels in edible parts. Another confirmation is that cadmium is a highly mobile element readily absorbed and translocated to aboveground parts by the plant. Also, a direct dependence between Cd level in the root system zone and that in the plant was confirmed. There are different mechanisms that explain the ability of the root to accumulate increased amounts of Cd and thus reduce its translocation to aboveground parts, including anatomical, biological and physiological factors.

Cadmium ions are uptaken from the soil through root cells, following which they pass through plasma membrane and enter the cell via plant transporters. In tolerant crops, Cd is immobilized in the root cells by the formation of complexes with malate- and oxalate-type organic acids [33]. Immobilization can also occur by binding Cd to metabolically inactive complexes with proteins containing sulfur and accumulating in the root [34] or by blocking via epidermal cells [35]. The most important mechanism involves capture of cadmium ions in vacuoles and their consequential exclusion from cell sites of cell division and respiration, resulting in reduced translocation to aboveground parts [36, 37]. These internal detoxification systems can reduce Cd translocation, thus making cadmium less toxic to plants.

The correlation analysis of the dependence between total Cd concentration in the soil and its concentration in lettuce leaves (fig. 3) shows a highly significant positive correlation ($R=0.885$), as previously determined by [11].

No significant correlation was observed between available Cd in the soil and Cd concentration in lettuce leaves ($R=-0.082$, fig. 4), which suggests that Cd concentration in lettuce is also dependent on other factors (cultivar, plant physiology).

Nevertheless, potential human exposure to toxic effects of soil cadmium through the food chain is best indicated through the transfer factor (TF) which is directly dependent on soil Cd concentration. In this study, the values of the transfer factor significantly differed between the tested soils (table 3) and showed that the uptake of Cd from the pseudogley soil was much higher than from the vertisol. This is a very important indicator showing a lower risk of Cd uptake by lettuce grown on the vertisol, which reduces the potential risk of contamination of edible lettuce parts. When growing lettuce in uncontaminated soils, [32] obtained a TF value higher than 0.5, which is considered by [38] to pose a risk of metal contamination of plants by anthropogenic activities. These results were confirmed in our study, since although lettuce was grown in an uncontaminated soil, Cd concentration in the leaves was above allowable levels, TF was high, which confirmed that lettuce is a Cd hyperaccumulator with a high transfer factor, as also determined by [2].

Lettuce head weight

Lettuce head weight ranged from 139-201 g (pseudogley) and from 169-292 g (vertisol), showing a decreasing tendency with increasing Cd amount
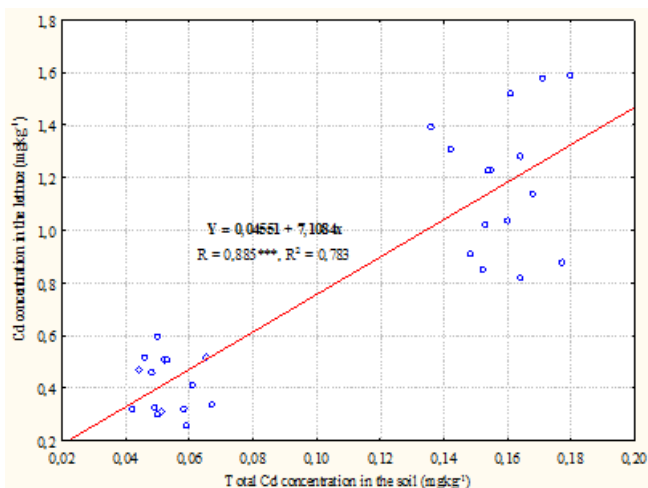

Fig. 3 Relationships between total Cd concentration in the soil and Cd concentration in lettuce
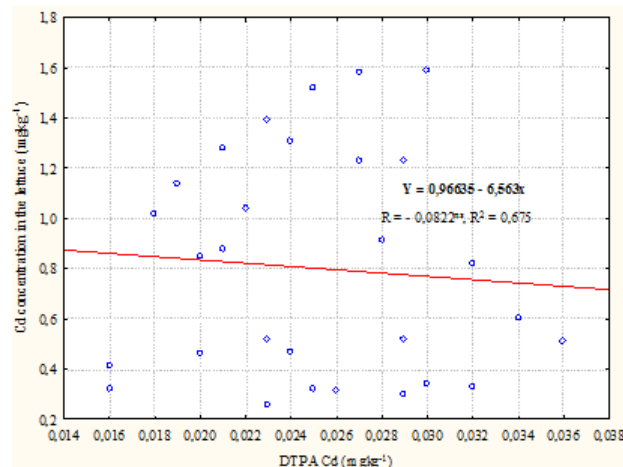

Fig. 4 Relationships between available Cd concentration in the soil and Cd concentration in lettuce

incorporated via MAP to the soil (table 2). [39] used increasing rates of Cd for lettuce and obtained up to 80% reduction in lettuce yield. A significant negative correlation (R=-0.818, fig. 5) was determined between Cd concentration in lettuce and head weight.
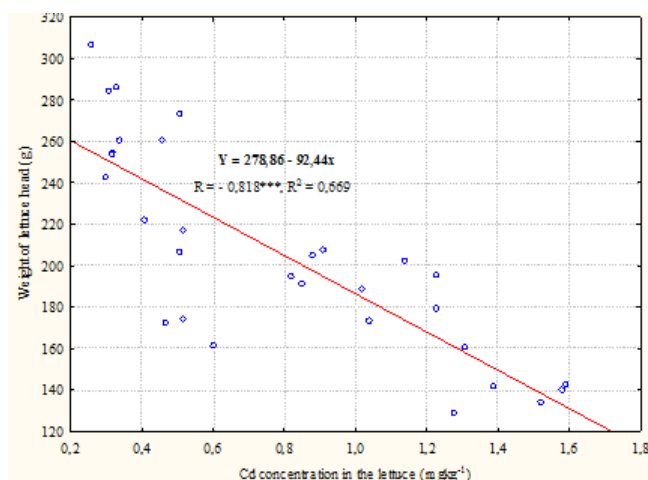

Fig. 5. Relationships between Cd concentration in lettuce and weight of lettuce head
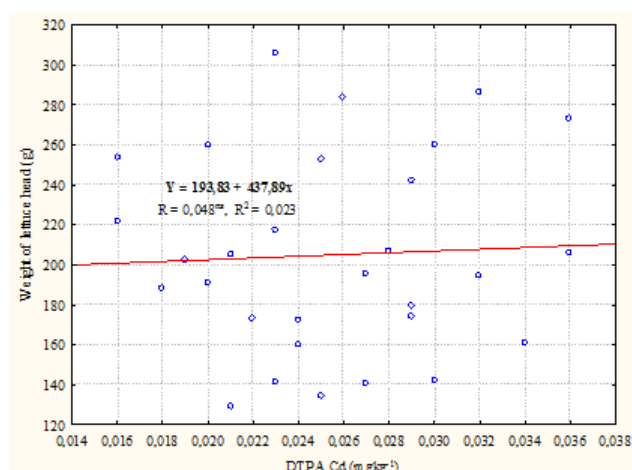

Fig. 6. Relationships between available Cd concentration in the soil and weight of lettuce head
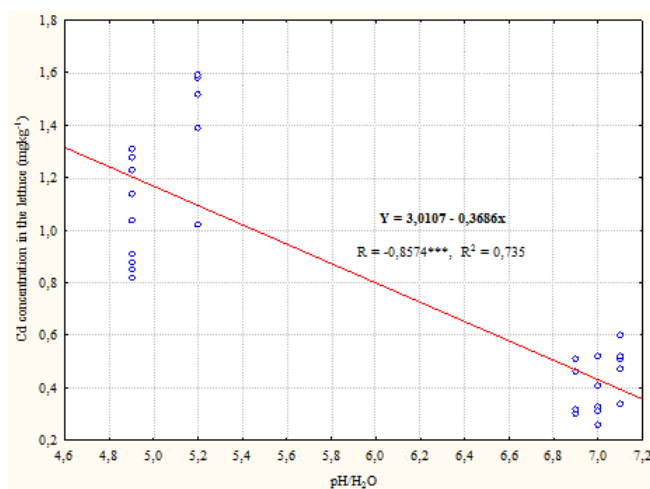

Fig. 7. Relationships between pH/H$_2$O and Cd concentration in letuce

No correlation was observed between available Cd in the soil and head weight (R=0.048, fig. 6).

These results indicate that Cd concentration in lettuce is dependent not only on available Cd levels in the soil, but also on pH, as confirmed by the significant negative correlation (R$_{pH/Cd\ leaf}$=-0.857, fig. 7).

Lettuce yield as dependent on soil available Cd was evaluated by [3] who found no dependence, while [39] determined a weak negative correlation.

Conclusions

Total Cd levels in both pseudogley and vertisol soils were below maximum allowable concentrations, which indicates the absence of contamination in these soils. Available Cd content after lettuce cultivation did not significantly differ between the tested soils, which suggested a higher Cd uptake by plants grown on the pseudogley than on the vertisol. Cd concentration in lettuce grown on the pseudogley was higher than on the vertisol, with higher amounts of Cd accumulated in the root than in the leaves. The values of the transfer factor as the most important indicator of potential human exposure to toxic effects of soil cadmium were high, thus suggesting that lettuce is a Cd hyperaccumulator.

The uptake and accumulation of cadmium by lettuce is mostly affected by soil pH, followed by Cd availability and a high affinity of lettuce for Cd.

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