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# Effect Of Fertilization On Available Cadmium Level In Soil And Lettuce

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**Abstract**: The objective of this study was to evaluate the effect of organic fertilizer (cattle manure) and monoammonium phosphate (Russian MAP-R and Serbian MAP-S) on available Cd levels in soil and Cd uptake by lettuce plants grown on vertisol and fluvisol. Fertilization treatments were as follows: control - without fertilization, mature cattle manure (20 g kg<sup>-1</sup> soil), MAP-R (0.1 g kg<sup>-1</sup> soil), MAP-S (0.1 g kg<sup>-1</sup> soil). Prior to the experiment, available Cd level was higher in vertisol (0.06 mg kg<sup>-1</sup>) than in fluvisol (0.04 mg kg<sup>-1</sup>). The manure application had no significant effect on increased DTPA-extractable Cd content in both soils, as compared to control. Available Cd level was decreased by MAP-R (vertisol 0.0494 mg kg<sup>-1</sup>, fluvisol 0.0227 mg kg<sup>-1</sup>) and increased by MAP-S application (vertisol 0.0577 mg kg<sup>-1</sup>, fluvisol 0.0288 mg kg<sup>-1</sup>) in both soil types as compared to control. The use of manure and MAP increased Cd concentration in lettuce, in all treatments except in manure treatment on vertisol. Lettuce head weight was highest in manure treatment on both soil types.

Key words: DTPA-extractable Cd, manure, phosphorus fertilizers, fluvisol, vertisol.

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#### Introduction

Soil pollution with heavy metals (Cd, Pb, Zn, Cr, Ni) is a large problem in intensive plant production. Major sources of heavy metals in soils include the parent material and phosphorus fertilizers as one of the most important anthropogenic sources of soil pollution with heavy metals, Cd in particular (Bogdanovic et al. 1999; Jiao et al. 2004; Chen et al. 2009; Williams and David 1973). Williams and David (1973) report the Cd content of superphosphate of 38-40 mg kg<sup>-1</sup>, Bogdanovic et al. (1999) applied superphosphate containing 2.08-7.50 mg kg<sup>-1</sup>, whereas Cd level in MAP was 144 mg kg<sup>-1</sup> (Jiao et al. 2004). The use of phosphorus-based fertilizers leads to increased soil Cd levels, thereby affecting Cd uptake by plants (Fontes et al. 2008). Manure applications increase soil Cd levels, having a long-term effect (Benke et al. 2008). Both the uptake and level of Cd are dependent upon soil type, primarily acidity, organic matter content, soil texture (Golia et al. 2008; Williams and David 1973). Plants have the ability to accumulate different amounts of Cd in edible parts, without causing an unfavorable effect on their growth (Chen et al. 2009). Singh (1990) obtained an increase in Cd levels in test plants with increasing phosphorus rates.

Lettuce (*Lactuca sativa* L.) is a leafy vegetable crop that shows affinity to accumulate Cd. Cadmium uptake from soil is higher in lettuce than in potatoes and tomatoes (Singh 1990), whereas no such differences were detected as compared to spinach (Sani *et al.* 2011).

Results of previous research on the effect of Cd on crop yield show differences. He and Singh (1994) reported that crop yield is generally not dependent upon fertilization and different soil Cd levels. Maclean (1976) found a decrease in lettuce yield as induced by an application rate of 5 mg kg<sup>-1</sup>, whereas Moustakas *et al.* (2001) and Kavvadias *et al.* (2012) observed that Cd rates higher than 10 mg kg<sup>-1</sup> result in decreasing lettuce yield. John *et al.* (2008) determined that the negative effect of increasing Cd rates on the fresh weight of lettuce is manifested in reduced root growth, as confirmed by Zheng *et al.* (2010).

The objective of this study was to evaluate the effect of manure and different types of phosphorus fertilizers applied to two types of soil on soil Cd level and Cd availability to lettuce.

#### Materials and methods

## Plant material and field trial

The experiment was conducted in pots in greenhouses at the trial field of the Faculty of Agronomy at Trbušani near Čačak, Western Serbia (43°55'N, latitude; 20°19'E longitude; 255 m altitude). The research involved two soil types, including fluvisol and vertisol (Table 1). Each pot was filled with 3 kg air-dry soil sampled

from a 0-30 cm layer and sieved through 4 mm sieve. In general, soil conditions were good for normal vegetative growth of lettuce, as previously reported (Fallovo et al., 2009).

Soil type	pH/KCl	Organic matter (%)	Total N (%)	Available phosphorus (mg 100g <sup>-1</sup> )	Available potassium (mg 100g <sup>-1</sup> )	Available Cd (mg kg <sup>-1</sup> )
Fluvisol	6.9	3.03	0.16	35.0	63.2	0.04
Vertisol	6.9	4.56	0.25	51.6	89.5	0.06

Table 1- Chemical properties of soils

The experiment involved fertilization with organic fertilizer- manure and 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) (Table 2). The composition of organic fertilizers-cattle manure, which was used in the experiment was as follows:  $N_{TOT}$ -0.5%,  $P_2O_5$ -0.3%,  $K_2O$ -0.6%, organic matter-25%, C:N ratio-18:1. In order to improve the fertility of the soil used for lettuce production, nitrogen (0.15 g kg<sup>-1</sup> soil) as CAN (calcium ammonium nitrate, containing 27% of  $N_{TOT}$ ), and potassium (0.1 g kg<sup>-1</sup> soil) as KCl (potassium chloride, containing 60% of K<sub>2</sub>O) were incorporated into the soil.

Table 2 - Cd content of fertilizers

Fertilizer	Cd content (mg kg <sup>-1</sup> )	
Mature cattle manure	0.53	
MAP-R	0.34	
MAP-S	12.32	

Fertilization treatments were as follows: control - without fertilization, mature cattle manure - 20 g kg<sup>-1</sup> soil, MAP-R – 0.1 g kg<sup>-1</sup> soil, MAP-S – 0.1 g kg<sup>-1</sup> soil.

The material used in the experiment was lettuce (*Lactuca sativa* L.), dutch butterhead cultivar Sunstar. A single young transplant was planted per container in three replications per treatment. During planting, the plants were watered until field moisture capacity was reached, with subsequent watering treatments being employed at soil moisture below 70% of the field capacity.

## **Chemical analysis**

Soil samples were analyzed using standard methods, as follows: pH in 1M KCl – electrometric method; organic matter was determined by the Walkley–Black's procedure (Nelson and Sommers 1982); total N was determined by

Kjeldahl analysis (Gerhardt Vapodest); available phosphorus and potassium – extraction with 0.1M NH<sub>4</sub>-lactate and 0.4M CH<sub>3</sub>COOH, according to Egner-Riehm (P was analyzed spectrophotometrically by the phospho-vanadate colorimetric method (Hewlett Packard 8452A); K was determined by flame photometry (Flapho 4, Carl Zeiss, Jena); available Cd – diethylenetriamine pentaacetic acid (DTPA) extraction of the Cd involved shaking 5.0 g of soil for 2 h with 25 ml of a solution containing 0.005 mol L–1DTPA 0.01 mol L–1CaCl<sub>2</sub>, and 0.1 mol L–1TEA (triethanolamine) buffered at pH 7.3 (Lindsay and Norvell 1978) and analyzed using atomic absorption spectroscopy (Perkin-Elmer, 3300/96, MHS-10)

After harvest, the plant materials were washed, dried, weighed and ground for analysis. For plant samples, 0.5 g of dried samples was digested with  $HNO_3$  and  $HCIO_4$  in a 5:1 ratio until a transparent solution was obtained (Allen, 1989) and analyzed using atomic absorption spectrophotometry (Perkin-Elmer, 3300/96, MHS-10).

#### Statistical analysis

All data in the present study were subjected to analysis of variance (ANOVA) and means were separated by LSD test at  $P \le 0.05$  using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA).

## **Results and Discussion**

## Available Cd in the soil

Prior to the experiment, the level of available Cd in fluvisol was 0.04 mg kg<sup>-1</sup> (Table 1). After the lettuce growing cycle, the Cd level decreased in all treatments (Figure 1), ranging from 0.0227 mg kg<sup>-1</sup> (MAP-R) to 0.0288 mg kg<sup>-1</sup> (MAP-S and manure). The same decreasing tendency of available Cd levels was also observed in vertisol (Figure 1), falling within the range of 0.06 mg kg<sup>-1</sup> before planting to 0.0494 mg kg<sup>-1</sup> (MAP-R) and 0.0577 mg kg<sup>-1</sup> (MAP-S) after harvest. The measured decrease in available Cd levels in the soil after lettuce harvest suggests the uptake of Cd accumulated in plant tops through the root system during the growing season. The low level of available Cd found in both soil types before the experiment was the result of their low natural supply of this element and neutral soil reaction (pH 6.9) which directly induced a reduction in available Cd levels in the soil (He and Singh 1994). Cd levels showed high significant differences between the test soils (Table 3), with the higher level being determined in vertisol, the type of loamy soils. The adsorption complex of these soils contains mineral clays which have a higher ion adsorption capacity as

compared to fluvisol, thereby contributing to an increased content of available Cd in the soil. Moreover, vertisol is the type of soil richer in organic matter. Maclean (1976) found that the content of available Cd increases in soils having a larger amount of organic matter, as confirmed in this study.



\*The same letters indicate non-significant differences among means at  $P \leq 0.05$  by LSD test

The use of MAP-R resulted in decreased Cd levels in both soil types than in the control (Figure 1). Available Cd levels in the soil increased with the use of MAP-S as compared to both the control and MAP-R, being due to the incorporation of higher amounts of Cd with this fertilizer into the soil. The increase in available Cd levels in the soil can be due to the formation of the soluble phosphate complex CdHPO<sub>4</sub>, as previously reported by Lindsay (1979) and Krishnamurti et al. (1999). Lambert et al. (2007) observed direct dependence of soil Cd level on the degree of fertilizer contamination with Cd, as also confirmed in this study. Similar findings were obtained previously by He and Singh (1995) who gained increased levels of available Cd in the soil after treatment with MAP fertilizer having an increasing content of Cd of 2.7 to 12.52 mg kg<sup>-1</sup>. Mann et al. (2002) evaluated the effect of phosphorus fertilizers containing higher amounts of Cd on Cd levels in a number of soil types and found the concentration of available Cd in sandy loam soil to increase with increasing fertilizer rate, as opposed to sandy soils which exhibited no increase in available Cd levels regardless of the high rates of phosphorus fertilizers applied. Levi-Minzi and Petruzzelli (1984) found that MAP induces a decrease in adsorbed Cd in the soil, particularly in the soil having a lower amount of organic matter, whereas Bogdanovic et al. (1999) suggested that increasing rates

of phosphorus fertilizers do not result in statistically significant changes in Cd concentration in chernozem soil.

The use of manure also increased available Cd levels in the soil as compared to the control. The results suggest that optimal manure application rates pose no risk of soil contamination with cadmium. However, the long-term use of increased rates of manure can cause an increase in available Cd levels for plants (Lipoth and Schoenau 2007; Benke *et al.* 2008). Ju *et al.* (2007) observed that soil Cd concentration increases only after treatment with enormously high rates of manure and phosphorus fertilization.

Effect of soil types on the traits analyzed								
Traits	Mean sqr. Effect	Mean sqr. Error	F	p-level				
Available Cd in the soil	.003971	.000022	178.0862	.000000				
Cd concentration in lettuce	.243613	.002628	92.71243	.000000				
Weight of lettuce head	28981.50	216.3700	133.9442	.000000				
Effect of fertilization on the traits analyzed								
Traits	Mean sqr. Effect	Mean sqr. Error	F	p-level				
Available Cd in the soil	.000073	.000022	3.2783	.048336				
Cd concentration in lettuce	.053684	.002628	20.43081	.000010				
Weight of lettuce head	8200.00	216.3700	37.8980	.000000				
Effect of soil types x fertilization on the traits analyzed								
Traits	Mean sqr. Effect	Mean sqr. Error	F	p-level				
Available Cd in the soil	.000002	.000022	.0854	.967035				
Cd concentration in lettuce	.023212	.002628	8.83402	.001098				
Weight of lettuce head	85.50	216.3700	.3952	.758230				

Table 3 - Analysis of variance of the tested parametres (ANOVA)

Table 3 shows the impact of soil type, fertilization and ineraction of soil type x fertilization on available Cd in the soil, Cd concentration in lettuce and weight of lettuce head. The ANOVA indicated highly significant effects of the soil type and all tested parameters, and fertilization and Cd concentration in lettuce and weight of lettuce head. Highly significant differences in Cd concentration in lettuce were found in relation with ineraction of soil type x fertilization. Furthemore, available Cd in the soil was significantly affected by the fertilization. The interaction of weight of lettuce head and soil type x fertilization did not show statistical significant.

#### Cd concentration in lettuce

Cd levels in lettuce grown on vertisol were between 0.437 mg kg<sup>-1</sup> DM and 0.515 mg kg<sup>-1</sup> DM and those on fluvisol ranged from 0.521 mg kg<sup>-1</sup> DM to 0.824 mg kg<sup>-1</sup> DM (Figure 2). These values of Cd levels in lettuce fall within the values obtained on control agricultural soil by Sterret *et al.* (1996). In the present study, all treatments except manure application on vertisol resulted in increased Cd levels in lettuce as compared to the control (Figure 2). The results obtained can be attributed to the fact that lettuce has the affinity to uptake and accumulate Cd; therefore, the plants accumulated Cd from the phosphorus fertilizers applied in the soil. Manure treatment on vertisol did not lead to an increase in Cd levels in lettuce, whereas that on fluvisol induced a slight increase as compared to the control, which was an expected occurrence, given the low Cd content of manure. Lipoth and Schoenau (2007) found that only long-term manure treatment can sometimes result in increased Cd availability to plants.

Nevertheless, high Cd accumulation was not found in lettuce since the applied phosphorus fertilizers were not significantly contaminated with Cd. The differences observed in Cd levels in lettuce between MAP-S and MAP-R were directly induced by their different Cd content. Namely, the Cd content of MAP-R was lower than that of MAP-S (Table 2) and, hence, lower amounts of this metal were introduced into the soil. Similar findings on increased Cd levels in lettuce resulting from increased Cd content of fertilizers were obtained by (Sterret et al. 1996; He and Singh 1995). Guttormsen et al. (1995) reported that increased rates of Cd applied through fertilizers induced an increase in Cd levels in the vegetable crop, with the differences being non-significant, as in the present study. Jiao et al. (2004) analyzed the effect of increasing Cd content of MAP and found an increase in Cd level in different parts of wheat. Mclaughlin et al. (1995) suggested that Cd concentration in potato tubers is affected by Cd content of phosphorus fertilizers, with residual Cd in the soil being the major source of its uptake. This study also showed significant correlation between Cd levels in lettuce and available Cd in the soil i.e. Cd uptake by the plant increased with increasing levels of available Cd in the soil. Similar results were reported by (Fontes et al. 2008; Chaudri et al. 2001). It is noteworthy that lettuce showed no symptoms of Cd excess in all treatments in this study. This suggests the ability of Cd to accumulate in lettuce without any visual proof of its presence as well as the fact that the Cd amounts introduced into the soil were not phytotoxic.



\*The same letters indicate non-significant differences among means at  $P \leq 0.05$  by LSD test

## Weight of lettuce head

The measured weight of lettuce head was lowest in the control (277 g on vertisol and 198 g on fluvisol, Figure 3). The highest weight of lettuce (356 g and 295 g on vertisol and fluvisol, respectively) was obtained with the use of manure, which suggests that manure as a complete organic fertilizer not only affects the soil nutrient regime but also acts as a soil amendment. Namely, manure improves soil structure, air and water relationships, thermal and microbial regimes of the soil, thereby enhancing the development of the root system and, hence, growth of lettuce head. In addition, the use of manure resulted in reduced Cd accumulation in lettuce and, thus, higher yield. Similar results were obtained by Qiang *et al.* (2009).



\*The same letters indicate non-significant differences among means at  $P \leq 0.05$  by LSD test

Phosphorus fertilization in both soils induced an increase in lettuce head weight relative to the control. A greater weight of lettuce head was obtained with MAP-S treatment as compared to MAP-R. The above results comply with those of Kavvadias et al. (2012) who found that increasing Cd application rates, up to a certain amount (10 mg kg<sup>-1</sup> Cd), lead to an increase in fresh biomass as compared to the control, whereas further Cd additions reduce fresh biomass, although not to a significant level.

## Conclusion

Treatment with MAP-R (Russian MAP) resulted in decreased Cd levels as compared to the control and manure in both soil types. After treatment with MAP-S (Serbian MAP), available Cd levels in vertisol and fluvisol increased non-significantly as compared to the control and manure, but statistically significantly as compared to MAP-R. All treatments, excepting manure on vertisol, gave increased Cd levels in lettuce as compared to the control. The Cd content of lettuce was lower after MAP-R treatment than after MAP-S treatment. The highest weight of lettuce (356 g on vertisol and 295 g on fluvisol) was obtained with the use of manure. A greater weight of lettuce head was obtained with MAP-S treatment as compared to MAP-R.

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# UTICAJ ĐUBRENJA NA SADRŽAJ PRISTUPAČNOG KADMIJUMA U ZEMLJIŠTU I ZELENOJ SALATI

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## Rezime

Cilj ovih istraživanja je bio da se ispita uticaj organskog đubriva (stajnjaka) i monoamonijum fosfata (ruski MAP-R i srpski MAP-S) na sadržaj pristupačnog Cd u zemljištu i usvajanje Cd od strane zelene salate, gajene na vertisolu i fluvisolu. Tretmani đubrenja su bili sledeći: kontrola - bez đubrenja, stajnjak (20 g kg<sup>-1</sup> zemljišta), MAP-R (0,1 g kg<sup>-1</sup> zemljišta), MAP-S (0.1 g kg<sup>-1</sup> zemljišta). Pre eksperimenta, sadržaj pristupačnog Cd je bio veći u vertisolu (0.06 mg kg<sup>-1</sup>) nego u fluvisolu (0.04 mg kg<sup>-1</sup>). Primena stajnjaka nije imala značajan uticaj na povećanje sadržaja DTPA - pristupačnog Cd kod oba tipa zemljišta, u odnosu na kontrolu. Sadržaj pristupačnog Cd je smanjen primenom MAP-R (vertisol 0.0494 mg kg<sup>-1</sup>, fluvisol 0.0227 mg kg<sup>-1</sup>) i povećan primenom MAP-S (vertisol 0.0577 mg kg<sup>-1</sup>, fluvisol 0.0288 mg kg<sup>-1</sup>) kod oba tipa zemljišta, u odnosu na kontrolu. Upotreba stajnjaka i MAP je povećala koncentraciju Cd u salati u svim tretmanima, osim kod primene stajnjaka na vertisolu. Masa glavice salate je bila najveća primenom stajnjaka na oba tipa zemljišta.

Ključne reči: pristupačni Cd, stajnjak, fosforna đubriva, fluvisol, vertisol