

ASSESSING CHANGES IN Cd PHYTOAVAILABILITY TO TOMATO IN AMENDED CALCAREOUS SOILS

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ABSTRACT

A plot study was conducted to assess changes in Cd phytoavailability to a tomato cultivar in an agricultural soil in Southeastern Spain amended in two different ways (A and B), under controlled conditions. The experimental soil corresponded to a fine-loamy carbonatic thermic Calcic Haploxeroll (Soil Survey Staff, 1998).

A) Soil was amended with a single application of sewage sludge from a municipal source that had a total Cd concentration of 0.5 mg kg⁻¹ at a rate that represented a final average concentration in the mixture of soil and sludge of less than 50 µg Cd kg⁻¹.

B) The amendment consisted of the addition of a mineral fertiliser with the same amount of NPK as in the sewage sludge application. The final levels of Cd were supposed to be negligible.

A plot series without amendments was also performed (C).

DTPA plus triethanolamine, and ammonium acetate extractable fractions in soils were analysed for all the plots. The time-dependent Cd accumulation in different parts of the tomato plants was studied by means of a Cd salt treatment. For each block (A, B, and C) four

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levels of Cd (0, 3, 30, 100 mg kg⁻¹) were added as CdCl₂. There was a significant increase in plant Cd after the initial cropping.

Tomato stems, leaves and fruits were analysed separately for Cd determination. Differential Cd distribution and accumulation in tomato parts was detected.

Keywords: cadmium bioavailability, cadmium bioaccumulation, cadmium phytoavailability, sewage sludge, calcareous soil, tomato.

INTRODUCTION

During the 90s, there has been in the Mediterranean coastal areas an increasing interest in reuse of organic by-products either for agricultural purposes or for degraded land restoration (mostly at mining areas). Among those waste materials, sludges from the increasing number of wastewater treatment plants **has biend** the main interest.

However, one of the main problems that condition sludge use is the potential effect derived from the presence of heavy metals. In the same way, uncontrolled mineral fertiliser application could be another important input for such metal contaminants of agricultural soils as cadmium occurs in ores used in the production of P fertilizers.

Among other metals cadmium is nowadays of great concern. There is sufficient evidence in humans for the carcinogenicity of cadmium and cadmium compounds, and for genotoxic effects of ionic forms in a variety of types of eukaryotic cells, including human ones. Cadmium enters the body mainly by inhalation and by ingestion. Intestinal absorption is influenced by dietary factors, and increases with dietary cadmium concentration.

Cd is an element that is neither regulated nor essential for plants. Cd content in agricultural soils is generally low, about 0.1 to 5 µg/g. However there are estimations that

foresee a 6% yearly increase due to man's action (Tjell et al., 1981). Since the early century, Cd has been produced and used in a variety of applications in alloys and in different compounds which explains its widespread distribution.

The phytoavailability of Cd largely depends on its speciation in soils. Distribution of Cd forms in soils is extremely variable. Chang et al. (1984) found that in soils treated with sewage sludge the most significant fractions that could explain chemical changes were the carbonate and the organic fractions.

Bioavailable forms are crucial in assessing the toxic impact in a soil-plant system (Shuman, 1991). Plant uptake is closely related to element concentration in soil solution; with the exchangeable fraction (Gerritse et al., 1983). For this reason, there are a lot of methods and extraction solutions for metal determination in soils (Beckett, 1989) and much work has been done in order to establish a correlation between plant sorption and metal concentrations in soils (Legret et al., 1988; He and Singh, 1993; Cabral and Lefebvre, 1998). Different factors have to be considered when considering soil-plant transfer. The transport of Cd in the soil-water systems seems to be a fast process. Soil sorption capacity for Cd has been found to be correlated with pH at the stable adsorption stage ($ZPC < pH < 6.0$). At $pH > 6.0$ strong adsorption is always observed. The bioavailability of soil sorbed Cd increased with pH when $pH < 6$, while it decreased with pH when $pH > 6$. At pH 7.5, large percentages of Cd remain bound, so reducing its toxicity (Liao et al., 1999). Calcite and dolomite in silt and clay fractions seem to be responsible for strong associations with cadmium (Vigil de la Villa et al., 1997).

Uptake of metals by plants growing in sewage sludge-amended soils frequently exhibits a plateau response at high sludge loading rates associated with high total concentrations of metals in the soil. This type of response has generally been attributed to decrease of metal bioavailability by increased sorption at sites provided by the sludge constituents at the high sludge loading rates (Logan, 1997). Metal transport from roots to

shoots is a long distance transport controlled by a certain number of physiological processes including metal unloading into root xylem cells, long distance transport within the xylem to the shoots, and metal reabsorption from the xylem stream by leaf mesophyll cells (Raskin and Ensley, 2000).

In the present work two simplified methods for studying metal mobility and phytoavailability in soils are proposed, and their usefulness in establishing correlations with cadmium concentrations in tomato plants, at fixed soil moisture content, is checked. The final aims were to evaluate the transfer of Cd from agricultural soils to cultivated plants, Cd concentrations in edible parts, and factors affecting cadmium phytoavailability.

METHODS

A number (144) of pots (20 kg capacity) were prepared with the top layer (0-30cm) of a calcareous soil (Calcidic Haploxeroll, according S.S.S. 1998). Three treatment blocks were carried out (addition of sewage sludge (SS), inorganic equivalent fertilisation (IN), and no fertilisation W) and for each block four levels of Cd (0, 3, 30, 100 mg kg⁻¹) were added as CdCl₂. One tomato plant was planted in each pot. All pots were located in greenhouse under controlled climatic conditions (temperature, day leng, cuando se cultivaroin).

Soil and sewage sludge analyses were performed according to conventional methods (Navarro-Pedreño et al., 1996) and results are shown in Table 1.

The amount of sludge added to the soil (30 Ton ha⁻¹) was calculated according to standard requirements of tomato fertilisation (300 N, 200 P₂O₅ and 300 K₂O kg ha⁻¹). The N content of sludge was equivalent to an actual addition of N of 300 kg ha⁻¹ following the recommendation of Cogger et al., (1987).

The inorganic NPK fertilisation, equivalent to the dose applied with the sewage sludge, was done using monoammonium phosphate, potassium nitrate and ammonium nitrate.

Bioavailable Cd at pot soils was obtained by two different extraction procedures:

- a) dissolution in 0,005M DTPA, 0,01N CaCl₂ and 0,1N Triethanolamine at pH 7. The so-called Lindsay-Norwell solution is supposed to extract available metals, organic matter-bound metals, but is also able to dissolve some precipitated forms (Schalscha et al., 1980).
- b) ammonium acetate (1N) at pH 7 (Knudsen et al., 1982), that extracts exchangeable forms (Förstner et al., 1981; Iyengar et al., 1981; Soon and Baltes, 1982).

In all cases, extracted Cd was measured by atomic absorption spectroscopy (AAS Perkin Elmer 2100-graphite furnace HGA 700). Each analytic determination was carried out in quadruplicate and average values are given in Results and Discussion.

Stems, leaves and fruits were analysed separately. Plant samples were obtained at the beginning of the flowering period (S1), at the beginning of ripening stage of fruits (S2), and at the full production stage (S3). Plant tissue (previously cleaned with water) was dried at 60° C in a forced-air oven. Dried samples were mineralised by microwave acid digestion, using HNO₃/H₂O₂ in ratio 4:1 (v/v) (White and Douthit, 1985; Kalra et al., 1988; Moral et al., 1996). Cadmium content was determined using atomic absorption spectrophotometry (AAS Perkin Elmer 2100-graphite furnace HGA 700), with a detection limit about 25 µg Cd.

A statistical ANOVA F test was applied to the results and double way ANOVA test was used considering the combined effect of the fertilisation (F) and the Cd pollution (P).

RESULTS AND DISCUSSION

Soil distribution of cadmium

Ammonium acetate- and DTPA-extractable Cd increased with total soil Cd. The contents of Cd for each soil extract are shown in Table 2. A higher extractability of Cd with DTPA was observed in all experiments.

Cd extracted by ammonium acetate was very similar in organic- and inorganic-fertilised soils and in the non-fertilised ones, except for the highest Cd soil contents (100 ppm). Very little variation of ammonium acetate Cd extractability was observed in the experiments with 3 and 30 mg Cd/kg treatments in all the fertiliser additions. This fact could indicate that the pollutant extracted from soil solution by plants is replaced quickly by the exchange mechanisms of the soil and/or by slight dissolution of some precipitated forms because of the ammonium acetate pH.

The interaction between the type of fertilisation and Cd extracted was higher with DTPA extract, with significant differences between fertilisation blocks and vegetative periods.

DTPA cadmium extractability decreased with time for low Cd levels in the same treatments. Considering that the DTPA extract also includes the Cd fraction associated with the organic matter, we can conclude that Cd sorbed to organic compounds could be progressively readsorbed by plants in calcareous soils.

A different behaviour was detected for the highest treatment of Cd (100 ppm), with a significant increase of Cd-DTPA or a plateau formation with time. The highest increment of Cd-DTPA was detected in the sewage sludge amended soils, richer than others in organic compounds.

This evolution can be explained by the initial formation of insoluble compounds of Cd, which can be transferred gradually to the active surfaces of the soil, probably because of soil pH reduction, and by the lability of the organic complexes of this metal, which can enhance their bioavailability (Hanafi and Salwa, 1998).

Plant cadmium

As we can see in Table 3, leaf tissue is the vegetative aerial part that has the highest concentration of Cd, for all the fertilisation and pollutant treatments. This behaviour has been reported by other researchers with tomato plants (Wolterbeek et al, 1988; Moral et al, 1994). Cd content in stem and branches did not vary significantly with time of exposure for different treatments at low soil Cd concentrations. Fruit Cd accumulation was low compared with the other parts analysed, and apparently under less influence of the different treatments. Cd presence in tomato fruit was one order of magnitude lower than Cd in leaves. Kim et al (1988) found that the ratio between leaf and fruit concentration of Cd in tomato plants grown in polluted soils was about 30-60, depending on the type of soil. Sewage sludge amended soils showed a higher presence of Cd in the aerial part of the tomato plants compared to inorganic fertilisation. However, in similar experiments with several types of plant, Logan and Miller (1985) reported no statistical differences between organic and inorganic fertilisation on Cd in the plant. Cd accumulations in stem, leaves and fruit seem to be affected directly by the presence of Cd in the soils and by the fertilising treatments, organic fertilisation causing the highest concentrations, especially with increasing time of contact.

There was a Cd accumulation in fruit close to $9 \text{ mg kg}^{-1} \text{ d.w.}$ in plants grown in the sewage sludge amended soils with the highest treatment of Cd (100 ppm); this fact indicates a very high transfer rate from soil to fruit. A fruit accumulation coefficient (fruit/soil Cd concentration quotient) was found close to 1/10.

Effects of Cd on biomass production and plant bioavailability

The production of aerial biomass was significantly higher in the fertiliser treatments compared to the control, but no statistical differences between organic and inorganic

fertilisation. The yield of tomato plants followed the sequence: control<inorganic fertilisation<organic fertilisation.

The Cd added to soil seemed to reduce the biomass production in some fertilizing systems, but this effect was low and not correlated with the increasing dose of pollutant. A very high negative effect of Cd was observed on yield in the non-fertilised treatments (W), probably due to the higher impact of the contamination in a non-fertilised scenario.

The plant uptake and accumulation of Cd seem to be better described by the Cd-DTPA extract than with the ammonium acetate extract. The former extraction technique could be recommended to evaluate Cd phytoavailability in calcareous soils. Several authors have found a positive correlation between phosphorus application as monoammonium phosphate and Cd accumulation in plants (Grant and Bailey, 1997), but we could not establish such a correlation.

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Table 1. Composition of the soil and sewage sludge used

	Soil	sewage sludge
Clay (< 0.002 mm)(%)	28	--
Silt (0.05 - 0.002 mm) (%)	30	--
Sand (2 - 0.05 mm) (%)	42	--
Total CaCO₃ equivalent (%)	62	--
Active lime (%)	15.3	--
pH (1:5)_{water}	8.7	6.2
E.C. (1:5)_{water} (dS/m)	0.45	5.51
Oxidable organic matter (g kg⁻¹)	24.5	570
N Kjeldahl (g kg⁻¹)	1.40	30.1
P (mg kg⁻¹)	0.036	17.1
K (mg kg⁻¹)	0.29	1.7
Ca (mg kg⁻¹)	5.31	66
Mg (mg kg⁻¹)	0.64	7.0
Na (mg kg⁻¹)	0.53	0.04
Fe (mg kg⁻¹)	1.8	2300
Cu (mg kg⁻¹)	3.5	270
Mn (mg kg⁻¹)	3.0	123
Zn (mg kg⁻¹)	7.8	235
Cd (mg kg⁻¹)	nd ^a	0.5

^a nd: Cd content under AAS-graphite furnace detection level.

Table 2. Cd-Ammonium acetate and DTPA-Cd soil extracts (mg/kg d.w.) determined in the combined treatments (Fertilisation (F) x Pollution (P))

	Cd-Ammonium acetate extract			Cd-DTPA extract		
	S1	S2	S3	S1	S2	S3
W/0	nd	nd	nd	nd	nd	nd
W/3	0.2 a	0.3 a	0.3 a	1.5 a	0.7 a	1.0 a
W/30	4.6 b	4.6 b	3.6 b	12.7 b	8.5 b	8.4 b
W/100	14.7 c	15.7 c	12.8 c	18.8 c	24.2 c	22.0 c
F-Anova	***	***	***	***	***	***
SS/0	nd	nd	nd	nd	nd	nd
SS/3	0.4 a	0.2 a	0.2 a	1.4 a	0.9 a	1.0 a
SS/30	4.3 b	3.9 b	4.1 b	14.4 b	11.2 b	11.5 b
SS/100	17.1 c	15.6 c	14.5 c	19.7 c	23.7 c	28.7 c
F-Anova	***	***	***	***	***	***
IN/0	nd	nd	nd	nd	nd	nd
IN/3	0.4 a	0.4 a	0.4 a	0.8 a	0.8 a	1.0 a
IN/30	4.2 b	4.1 b	3.3 b	11.8 b	9.0 b	8.1 b
IN/100	15.0 c	16.4 c	10.3 c	19.3 c	22.3 c	21.1 c
F-Anova	***	***	***	***	***	***
F	ns	ns	***	*	ns	***
P	***	***	***	***	***	***
F x P	ns	ns	***	*	ns	***

*,** and *** indicate significant differences at p= 0.05, 0.01, 0.001 respectively and ns = non significant. Figures within vertical columns followed by the same letter are not different statistically (p= 0.05).

See Methods for meaning-fertiliser and Cd additions

Table 3. Cd content in stem and branches, leaf and tomato fruit (mg/kg) determined in the combined treatments (Fertilisation (F) x Pollution (P))

	Stem and branches			Leaf			Fruit	
	S1	S2	S3	S1	S2	S3	S2	S3
W/0	nd	nd	nd	nd	nd	nd	nd	nd
W/3	2.2a	2.8a	3.0	4.2a	4.3a	6,0a	0.4a	0.9a
W/30	10.1b	10.5b	7.2	20.8b	20.4b	14,1b	1.4b	3.4b
W/100	15.2c	15.9c	14.8	42.2c	29.9c	28,8c	4.6c	4.6c
F-Anova	***	***	***	***	***	***	***	***
SS/0	nd	nd	nd	nd	nd	nd	nd	nd
SS/3	3.1a	1.3a	2.5	5.3a	4.6a	11,1a	0.8a	1.0a
SS/30	8.7b	6.8b	12.5	16.7b	19.0b	22,0b	3.2b	5.3b
SS/100	15.4c	15.0c	24.6	33.1c	39.0c	69,5c	4.9c	8.9c
F-Anova	***	***	***	***	***	***	***	***
IN/0	nd	nd	nd	nd	nd	nd	nd	nd
IN/3	3.1 a	2.2 a	3.0	8.1 a	3.3 a	6,0 a	0.6 a	0.7 a
IN/30	6.6 b	4.9 b	5.8	13.3 b	8.7 b	10,0 b	1.6 b	2.3 b
IN/100	9.4 c	6.6 c	10.9	21.2 c	20.1 c	28,9 c	3.2 c	5.2 c
F-Anova	***	***	***	***	***	***	***	***
F	***	***	***	***	***	***	***	***
P	***	***	***	***	***	***	***	***
F x P	***	***	***	***	***	***	***	***

*,** and *** indicate significant differences at $p = 0.05$, 0.01 , 0.001 respectively and ns = non significant. Figures within vertical columns followed by the same letter are not different statistically ($p = 0.05$).

Table 4. Aerial biomass production and yield in the combined treatments (Fertilisation (F) x Pollution (P)).

	Aerial biomass (g/plant d.w.)			Yield
	S1	S2	S3	(g/plant f.w.)
W/0	8,3ab	26,0a	24,8a	459a
W/3	10,8c	14,8b	11,8b	429a
W/30	9,3a	12,0c	14,5b	411a
W/100	7,7b	17,3b	25,5a	336b
F-Anova	**	***	***	*
SS/0	23,0a	47,0a	48,7a	1035a
SS/3	22,0a	42,3b	45,8a	1089a
SS/30	22,5a	43,0b	48,5a	960a
SS/100	18,5b	47,5a	39,0b	972a
F-Anova	*	**	***	ns
IN/0	24,0a	54,1ab	44,6a	711ab
IN/3	25,1a	59,3a	39,5b	639a
IN/30	19,2b	49,8b	36,5c	606a
IN/100	21,3ab	45,0c	40,5b	801b
F-Anova	*	**	**	*
F	***	***	***	***
P	**	**	***	ns
F x P	***	***	***	**

*,** and *** indicate significant differences at p= 0.05, 0.01, 0.001 respectively and ns = non significant. Figures within vertical columns followed by the same letter are not different statistically (p= 0.05).

Table 5. Linear correlation between the different Cd soil pools and Cd in plant parts.

Cd soil pool	Cd in plant			
	Stem + branches	leaf	fruit	
Cd added	W	0.141Cd+2.172 r=0.932	0.321Cd+3.872 r=0.958	0.043Cd+0.151 r=0.997
	SS	0.165Cd+1.741 r = 0.956	0.424Cd+3.674 r=0.981	0.046Cd+0.897 r=0.945
	IN	0.081Cd+1.845 r= 0.897	0.211Cd+2.787 r=0.922	0.036Cd+0.391 r=0.979
Cd in NH₄AcO extract	W	0.984Cd+2.233 r= 0.940	2.256Cd+3.897 r=0.966	0.301Cd+0.159 r=0.922
	SS	1.233Cd+2.055 r=0.967	2.899Cd+4.564 r=0.978	0.421Cd+0.958 r=0.934
	IN	0.623Cd+2.012 r= 0.911	1.876Cd+2.452 r= 0.942	0.316Cd+0.397 r=0.978
Cd in DTPA extract	W	0.677Cd+1.264 r= 0.987	1.454Cd+3.018 r=0.967	0.189Cd+0.256 r=0.955
	SS	0.725Cd+0.752 r=0.989	1.897Cd+1.897 r=0.988	0.254Cd+0.597 r=0.975
	IN	0.389Cd+1.387 r=0.954	0.978Cd+2.124 r=966	0.189Cd+0.265 r=0.995