Can be reference values of heavy metals useful as soil quality standards? Contributions from assays in representative Mediterranean agricultural soils

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Abstract
This paper presents the results of the research project "Assays of reference concentrations of heavy metals (Cd, Cu, Pb and Zn) in representative agricultural soils of the Mediterranean region under controlled horticultural crops". This project aimed to determine the maximum concentration for Cd, Cu, Pb and Zn related to the reference values for these metals in representative soils of horticultural areas of the Mediterranean region, which do not exceed the maximum concentration established by the legislation for these metals in crops for human consumption. This concentration has been determined for each soil after several assays using reference concentrations of these metals in controlled cultures of lettuce, which represents an important component of the Mediterranean diet and furthermore it is a metal accumulator crop. Reference concentrations are multiples of the reference value established for each metal in horticultural areas of the Valencian Region, a representative area of the European Mediterranean Region. Representative soils were selected each having different properties relevant to regulating the behaviour of heavy metals in soil. The results obtained suggest that the usefulness of criteria based on soil quality standards to define contaminated soils in Mediterranean soils contaminated by heavy metals can vary depending on soil properties.

Key Words
Trace elements, toxicity, root test, biomass test.

Introduction
Heavy metals are toxic elements for the human health when specific concentrations for each element are exceeded. Particularly, cadmium (Cd) and lead (Pb) are non-essential elements for life and highly dangerous, since they cause serious dysfunctions in organisms, including humans. On the other hand, copper (Cu) and zinc (Zn) are essential elements for organisms but can be dangerous if high concentrations in agricultural soils are reached. These elements have generally an anthropic origin, due to the excessive use of agrochemical products (e.g. Cu and Pb), to the wastewater irrigation with an urban-industrial origin (e.g. Cd, and Pb) and to the atmospheric depositions from several emissions (e.g. Cd and Pb). These processes are affecting soils under vegetable crops in the Mediterranean Region (Peris *et al.* 2007), and therefore further research on these metals is needed in this region, where only few studies on heavy metal in soils at a regional level have been carried out (Micó *et al.* 2006). The intake of contaminated food is the main way for introducing these elements to the food web. In this context, the current legislation establishes the maximum concentration of heavy metals allowed in different types of food (including vegetable crops). Specifically, the Regulation EC nº466/2001 of the European Commission fixes the maximum content of Cd and Pb in some crops for human consumption (DOCE 2001). Cu and Zn are not included in this Regulation. However, other international legislations, like the Australian regulation, establish the maximum concentration of Cu and Zn in some vegetable crops (e.g. McLaughlin *et al.* 2000) and, therefore, it can be expected their incorporation in the European regulations in the near future.

It is widely recognized that heavy metal levels in crops are influenced by a number of factors such as soil properties and the type of crop. Leaf crops have a high accumulation capacity of heavy metals in their leaves according to different authors (e.g. Ramos *et al.* 2002). The determination of the maximum concentrations of Cd, Cu, Pb and Zn in different representative Mediterranean agricultural soils, that does not cause the maximum limit allowed in lettuces (*Lactuca sativa L.*) established by the current international legislation, would be of great interest since leaf crops represent an important percentage of the crops cultivated and consumed in the Mediterranean region. Exceeding this concentration in soils would lead to the declaration of such soils as contaminated.

The Spanish Royal Decree 9/2005 (BOE 2005) establishes criteria to declare contaminated soils such as toxicity tests, risk analysis and the comparison of contaminant concentrations in soils with reference values. According to this Royal Decree, a soil will be considered "contaminated" when heavy metal concentrations in soils exceed 100-times the reference values established for them. In this context, this paper presents the framework, methodology and results of the research project "Assays of reference concentrations of heavy metals (Cd, Cu, Pb and Zn) in representative agricultural soils of the Mediterranean region under controlled horticultural crops". This project aimed to determine the maximum concentrations of Cd, Cu, Pb and Zn related to the reference value for each metal in agricultural soils representative of the Mediterranean Region that do not cause the overcoming of the maximum limit for lettuces established by the international legislation. The assay of different doses has enabled to determine these maximum concentrations. The different doses were established taking into account the reference levels for Cd, Cu, Pb and Zn in agricultural soils under vegetable crops of the Valencia Region, a representative area of the European Mediterranean Region, situated in the east of Spain.

Material and methods

Soil samples were collected from the surface horizons (25 cm depth topsoil, approximately) of four agricultural soils (Rojales, Sollana, Nules and Peñíscola) representative of the Mediterranean region (Recatalá *et al.* 2002, Recatalá *et al.* 2004). All the samples were transported to the laboratory where they were air dried at ambient temperature and sieved to pass a 2-mm mesh sieve. Selected soils properties relevant to control the mobility and bioavailability of heavy metals (Adriano 2001) were analyzed according to the official laboratory methods of the Spanish Ministry of Agriculture, Fisheries and Food (MAPA 1994). Soil pH was measured in a 1:2.5 soil:water suspension and electrical conductivity was determined in the saturation paste extract. Organic matter content was determined by the Walkley-Black method and the total carbonate content by the Bernard calcimeter. Particle-size distribution was determined by the Robinson Pipette method to determine the sand, silt and clay percentages. Cation exchange capacity was determined by saturation with sodium acetate solution (pH=8.2), displacement of the absorbed sodium with ammonium acetate (pH=7.0) and determination of displaced sodium by flame atomic absorption spectrometry (FAAS).

Biomass assays based on OECD test 208 (OECD 2006) were performed to assess the toxicity of the different heavy metals considered on the biomass production of lettuce crops (*Lactuca sativa* L.). Heavy metals were added to soils by spraying appropriate volumes of a solution (e.g. $CdCl_2$, $CuCl_2$, $Pb(NO_3)_2$, $ZnSO_4$) diluted with deionised water. This procedure ensured an even distribution of the metal solution in the soil while maintaining its pore structure. The soils were contaminated to obtain a range of six concentrations, the control and five doses that were multiple concentrations of reference values established for horticultural soils in the Valencian Mediterranean region (Sánchez *et al.* 2004). For each metal, the doses were established after considering the results of a rangefinder test. For instance, in the case of Cu, the five doses were concentrations representing 1, 10, 25, 50 and 100-times, respectively, the reference value (65.9 mg Cu kg−1) for this metal. After contamination, each sample was completely mixed in a plastic bag by hand. Control samples were similarly treated using deionised water only. All treatments were equilibrated for seven days before using in the mentioned assays. The crops for each biomass assay were dried in an oven (60 ºC) and sieved to 0.5 mm for analysis of heavy metal contents. Heavy metal contents in crops were determined using the USEPA 3052 method for organic matrices (USEPA 1996). This method was selected because it provided satisfactory results in relation to recovery and precision data (Micó 2005; Peris 2005).

Results and discussion

Soil properties of the four soils used in the assays are shown in Table 1. Soil pH was between 7.8 and 8.5, organic matter between 1.6 and 9.7%, clay content between 20 and 41% and carbonate content between 36 and 52%. The results of the assays show that soil properties greatly influenced metal toxicity in the crop. For instance, the effective concentration of added Cd causing 50% inhibition (EC_{50}) on biomass production increased with the calcium carbonate content of soils, being 839 mg/kg for Rojales, 169 mg/kg for Sollana, 285 mg/kg for Nules and 658 mg/kg for Peñíscola (Recatalá *et al.* 2009a). This means that Cd toxicity in the crop was higher in the soils having less carbonate content because of calcium carbonate has a high affinity for Cd and retains it by adsorption and/or precipitation (which predominates at higher Cd concentrations) reactions (e.g. Papadopoulus and Rowell 1986) Other soil properties (high salinity and a coarse texture) facilitate the Cd availability and mobility in soil. In saline soils, the formation of ion chloride complexes can facilitate the transfer of Cd from soil to plant (e.g. Khoshgoftar *et al.* 2004). Soils having a coarse texture have a low metal sorption capacity (e.g. Hooda and Alloway 1998).

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Table 1. Selected properties of the soils used in the assays.

Soil	pH	ECse dS/m	OM %	$CaCO_3$ %	CEC cmol(+)/kg	Sand %	Silt %	Clay %	Texture
Rojales	7.8	17.9	1.6	50	10.1	31	44	25	Loam
Sollana	8.5	1.5	3.5	36	19.6	28	37	35	Clay loam
Nules	8.2	2.2	9.7	37	31.0	16	43	41	Silty clay
Peñiscola	8.2	1.1	2.1	52	9.9	56	24	20	Sandy clay loam

ECse, electrical conductivity; OM, organic matter content; CEC, cation exchange capacity

In addition, it should be highlighted that for all soils the EC_{50} was greater than 100-times the reference value for Cd (0.74 mg/kg). However, for all the soils, from the second dose, which represents 10-times the reference value for Cd, the crop had Cd contents (Table 2) that exceeded the maximum levels established by the Commission Regulation no. 466/2001 (DOCE 2001). Although Rojales and Peñiscola are the most carbonated soils the lettuces grown in these soils accumulated more Cd that those ones grown in Sollana and Nules due to the effect of salinity and a coarse texture, respectively. Similar results were obtained for Cu (Recatalá *et al.* 2009b), Pb and Zn (Recatalá 2010). However, for these metals the EC_{50} increased with the organic content of soils as soil organic matter has a high affinity for these metals. Lettuces grown in Rojales and Peñiscola also accumulated more Cu, Pb and Zn than those grown in Sollana and Nules because of salinity and texture. As for Cd, for all soils the Pb contents in the crop exceeded the maximum level from the dose equivalent to 10-times the reference value for Pb. For Rojales, Sollana and Peñiscola, the Cu contents in the crop exceeded the maximum level from the dose equivalent to 25-times the reference value for Cu whereas for Nules from the dose equivalent to 50-times. In the case of Zn, for all soils the Zn contents in the crop exceeded the maximum level after the dose equivalent to 100-times the reference value for Zn.

Table 2. Cd content in lettuce crops for the different doses added to soils.

Doses Cd (mg/kg) added to soils	Cd content in crop (mg/kg)			
	Rojales	Sollana	Nules	Peñíscola
1 (control)	<0.5	0.5	<0.5	1
2 (7,4 mg/kg)	47	26	14	32
3 (74 mg/kg)	77	66	66	65
4 (740 mg/kg)	206	147	158	715
5 (1480 mg/kg)	390	342	318	--
6 (2220 mg/kg)	--	500	517	--

-- = no biomass produced.

It can be, therefore, concluded that the criteria established by the Spanish Royal Decree 9/2005 (BOE 2005) to declare a contaminated soil when the concentration of a metal is higher than 100-times the reference value is not valid for the metals studied neither from the point of view of the growth of the crop nor from the point of view of the toxicity due to the accumulation of the metal in the crop that can affect human health through the food web. Giving that reference values are also proposed in other parts of the European Mediterranean region (e.g. Pérez *et al.* 2002) the results achieved in this study can be relevant to proposed criteria to declare contaminated soils in this region.

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References

Adriano DC (2001) 'Trace Elements in Terrestrial Environments. Biochemistry, Bioavailability and Risks of Metals'. (Springer-Verlag: New York).

BOE (2005) 'Real Decreto 9/2005, de 14 de enero, por el que se establece la Relación de Actividades Potencialmente Contaminantes del Suelo y los Criterios y Estándares para la Declaración de Suelos Contaminados'. (Boletín Oficial del Estado BOE nº 15 de 18 de enero de 2005: Madrid).

DOCE (2001) 'Reglamento CE nº466/2001 de la Comisión de 8 de marzo de 2001 por el que se fija el contenido máximo de determinados contaminantes en productos alimenticios'. (Diario Oficial de las Comunidades Europeas: Bruselas).

Hooda PS, Alloway BJ (1998) Cadmium and lead sorption behaviour of selected English and Indian soils. *Geoderma* **84**, 121-134.

Khoshgoftar AH, Shariatmadari H, Karimian N, Kalbasi M, Van der Zee, SEATM, Parker DR (2004) Salinity and zinc application effects on phytoavailability of cadmium and zinc. *Soil Science Society of American Journal* **68**, 1885-1889.

MAPA (1994). 'Métodos oficiales de análisis de suelos y aguas para riegos. Tomo III'. (Servicio de Publicaciones del Ministerio de Agricultura, Pesca y Alimentación: Madrid).

McLaughlin MJ, Hamon RE, McLaren RG, Speir TW, Rogers SL (2000) Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Agricultural Research* **38**, 1037-1086.

Micó C (2005) 'Estudio de metales pesados en suelos con cultivos hortícolas de la provincia de Alicante'. PhD thesis, Universitat de València. (Servei de Publicacions de la Universitat de València: Valencia).

Micó C, Recatalá L, Peris M, Sánchez J (2006) Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. *Chemosphere* **65**, 863-872

OECD (2006). 'OECD Guidelines for the Testing of Chemicals. Section 2: Effects on Biotic Systems Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test'. (Organisation for Economic Cooperation and Development: Paris),

Papadopoulos P, Rowell DL (1986) The reactions of cadmium with calcium carbonate surfaces. *European Journal of Soil Science* **39**, 23-36.

Pérez, C.; Martínez, M.J.; Vidal, J.; Navarro, C. (2002). Proposed reference values for heavy metals in Calcaric Fluvisols of the Huerta de Murcia (SE Spain). In 'Proceedings of the International Symposium on Sustainable Use and Management of Soils in Arid and Semiarid Regions' (Eds A Faz, R Ortiz, AR Mermut) pp. 495-496. Cartagena, Murcia, Spain.

Peris M (2005) 'Estudio de metales pesados en suelos bajo cultivos hortícolas de la provincia de Castellón'. PhD thesis, Universitat de València. (Servei de Publicacions de la Universitat de València: Valencia).

Peris M, Micó C, Recatalá L, Sánchez R, Sánchez J **(**2007) Heavy metal contents in horticultural crops of a representative area of the European Mediterranean region**.** *Science of the Total Environment* **378**, 42-48.

Ramos I, Esteban E, Lucena J J, Garate A (2002) Cadmium uptake and subcellullar distribution in plants of *Lactuca sp*. Cd-Mn interaction. *Plant Science* **162**, 761-767.

Recatalá L (2010) Ensayo de concentraciones de referencia para los metales pesados (Cd, Cu, Pb y Zn) en suelos agrícolas mediterráneos representativos bajo cultivos hortícolas controlados (CORESMED). Informe Final. CIDE–Centro de Investigaciones sobre Desertificación (CSIC, Universitat de València, Generalitat Valenciana). Valencia, Spain .

Recatalá L, Micó C, Peris M, Sáez E, Sánchez J (2002) Analysis of Heavy Metals in Mediterranean Soils (Alicante, Spain) for Soil Quality Policy Making. In 'Proceedings of the International Symposium on Sustainable Use and Management of Soils in Arid and Semiarid Regions' (Eds A Faz, R Ortiz, AR Mermut) pp. 318-320. Cartagena, Murcia, Spain.

Recatalá L, Peris M, Micó C, Sánchez R, Sánchez J (2004) Analysis of Heavy Metals in Agricultural Soils (Castellón, Spain). In 'Proceedings of the Fourth International Conference on Land Degradation' (Eds A Faz, R Ortiz, G García), pp. 325-326. Cartagena, Murcia, Spain.

Recatalá L, Arbelo CD, Sánchez J (2009a) Transferencia de cadmio de suelo a planta en ensayos controlados de concentraciones de referencia en suelos agrícolas Mediterráneos representativos. In 'Actas del IV Simposio Nacional sobre Control de la degradación de los Suelos y Cambio Global'. (Eds J Sánchez Díaz, S Asins Velis) pp.127-129. Valencia, Spain.

Recatalá L, Arbelo CD, Sánchez J (2009b) Determinación de cobre en planta en ensayos controlados de concentraciones de referencia en suelos agrícolas Mediterráneos representativos. In 'Actas del IV Simposio Nacional sobre Control de la degradación de los suelos y cambio global'. (Eds J Sánchez Díaz, S Asins Velis) pp.189-191. Valencia, Spain.

Sánchez J, Recatalá L, Micó C, Peris M (2004) Contenido de metales pesados en suelos agrícolas y su influencia en cultivos hortícolas. Informe Final. CIDE-Centro de Investigaciones sobre Desertificación (CSIC, Universitat de València, Generalitat Valenciana). Valencia, Spain.

USEPA (1996) Method 3052. Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. Revision 0. U.S. Environmental Protection Agency (USEPA), Washington D.C.