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# Copper Contamination in Mediterranean Agricultural Soils: Soil Quality Standards and Adequate Soil Management Practices for Horticultural Crops

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Additional information is available at the end of the chapter

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## Abstract

This chapter increases the knowledge on the management of Cu-contaminated Mediterranean agricultural soils, by analysing the current soil quality standards for different Mediterranean regions and proposing new criteria for their establishment based on the influence of soil properties and type of crop. We evaluate the effect of Cu and its interaction with soil properties on biomass production of lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.), by establishing the effective concentrations EC<sub>50</sub> and EC<sub>10</sub> (effective concentrations of Cu in soil that reduces biomass production by 50 and 10%, respectively), and its absorption, translocation and accumulation in the different parts of the plant. Two different biomass assays were carried out in seven types of Mediterranean agricultural soils (four from Europe and three from Australia) contaminated with different Cu concentrations. When lettuce was grown, similar toxic effects and accumulation values were obtained for both of the agricultural areas under analysis. In both cases, the maximum threshold value was obtained for the soil having the highest pH and clay content, independently of the soil type. When comparing both crops in the European Mediterranean soils, toxicity values calculated for tomato were higher, and translocation of Cu to the fruit was constantly low, independently of the Cu dose. Moreover, tomato showed an important phytoremediation potential, extracting Cu from not only low-medium but also from highly (>1700 mg/kg) Cu-contaminated basic agricultural soils, and having low translocation rates to fruits. The analysis of the influence of soil properties on the effect of Cu on plant biomass production led to similar conclusions in both assays. SOM, clay content and CEC are the most relevant properties affecting the dynamic of Cu in soil. Considering this, for the type of crops and soils considered, the effect of Cu on plant biomass production was the most relevant of those analysed, and pH, clay content, SOM and CEC the most relevant soil properties. Therefore, these aspects should be considered when establishing adequate soil quality standards and proposing adequate soil management practices.

**Keywords:** agricultural soils, Cu toxicity, soil quality standards, European Mediterranean region, Australian Mediterranean region

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## 1. Introduction

The contamination of soils, especially agricultural ones, with heavy metals is an extended soil degradation process that affects vast areas of the planet [1–7].

In a world with a productive model based on extensive areas with intensive inputs, some of which are sometimes hazardous and destructive, direct (solid waste disposals, mine residues, etc.) and indirect (inadequate agricultural practices) soil contamination processes are very likely to continue happening, especially in agricultural areas. These can lead to serious environmental problems, linked to soil degradation processes due to excessive accumulation of these toxic substances and can affect different ecosystems. Furthermore, this excessive accumulation of heavy metals in agricultural soils may not only result in environmental contamination but can also cause an increase on the heavy metal uptake by crops, affecting this way food quality and safety. According to [8], soil plays a central role in food safety as it determines the possible composition of food and feed at the root of the food chain.

The heavy metal contamination of soil is one of the most pressing concerns in the debate about food security and food safety in Europe [9] and globally [10]. However, the quality of the resource soil, defining this as the potential impact on human health derived from the propagation of harmful elements through the food chain, has not been properly studied in Europe due to the lack of adequate data, in terms of detail and reliability.

Of these harmful elements, those heavy metals considered micronutrients are particularly relevant, since plants tend to behave differently towards them, being more tolerant, and enhancing their absorption and accumulation in different plant tissues. Of special concern is Cu, since this heavy metal is extensively used as a fungicide; it is the main component of different chemical fertilisers and is present at high concentrations in sewage sludge and pig manure. Komárek et al. [11] carried out an extensive bibliographical research on the use of Cu as fungicide around the world and determined concentrations of Cu in agricultural soils of up to 3216 mg<sub>Cu</sub>/kg.

In order to characterise contaminated soils, commonly, two different approaches have been developed: (i) establishment of soil quality standards and (ii) risk assessment [12]. The approaches based on soil quality standards have a great advantage, as the characterisation can be quick and cheap in many cases. However, difficulties arise if one considers the complexity of soils [13]. On the other hand, the approaches based on direct risk assessment are undoubtedly more realistic, but they require a degree of soil information that is not always available. Moreover, the costs associated with the application of these latter can be hardly undertaken in many cases [14].

Concerning the establishment of the soil quality standards, it is well known that different soil properties affect the dynamics of heavy metals in soils [15] and that different plants/crops

behave differently in relation to toxicity problems and accumulation limits of heavy metals. However, these two aspects are not usually considered in the establishment of these values. Furthermore, high concentrations of elements such as Cu in soil can lead to toxicity problems to plants and the consequent reduction in plant biomass production [16] and/or to potential animal and human health risk because of the accumulation of Cu in vegetables, since, as commented previously, plant uptake from soil is the main way for Cu to enter the food chain [8, 17]. According to [18], some vegetables can accumulate relatively high levels of Cu from soil without any toxic effect. Therefore, both aspects (plant biomass production and Cu accumulation in the plant) are relevant when analysing Cu contamination of agricultural soils and toxicity in crops and necessary to establish/define adequate soil conservation and management strategies.

Regarding the accumulation of Cu in the edible part of the plant, some national and international legislations (e.g. [19, 20]) clearly establish the maximum Cu content in the edible part of the plant, which is 10 mg/kg in fresh weight basis. However, this is not so for the effect on biomass production.

Considering all the above, it arises the need to carry out better and more detailed analysis in order to define adequate soils quality standards taking into account these two factors. The consequences of not considering these two factors are that soil quality standards are commonly too indulgent, not reflecting the complexity of agricultural ecosystems and jeopardising the health of both ecosystems and humans.

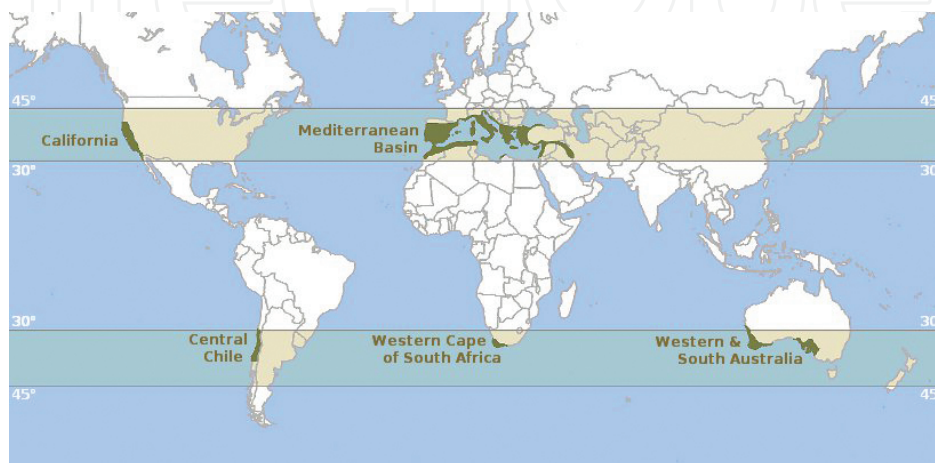
The definition of adequate soils quality standards for different climatic areas, such as the Mediterranean region, and for different crops, such as the horticultural ones, will enable to suggest adequate agricultural practices to manage and preserve the resource soil under Cu contamination problems in the Mediterranean agricultural soils.

## 2. Study area and objectives

The study area selected was the Mediterranean Region. This area includes different parts of the world and covers all the countries with Mediterranean climate, in all or some part of it (**Figure 1**). This region is of special concern since it is said or considered to include the “orchards of the world” [21].

Within this region, one of the areas studied was the European Mediterranean region. The representative soils of this region were sampled from the Valencian Region, an area located in the south-east of Spain. This area can be considered as representative since climatic conditions and soil properties of this area are typical of the European Mediterranean Region. Furthermore, this area has undergone, over the recent decades, the same land use pattern changes as the one occurred in most of the European Mediterranean Region, where there has been an intensification of agricultural development, characterised by high consumption of agrochemicals, and an expansion of industrial-urban uses [23–25].

The other Mediterranean region considered was the Mediterranean area of Australia. Climatic conditions are similar to the ones describe previously, although the properties of the soils present in this area differ slightly, and include, for example, soils with lower pH values. Adequate representative soils were sampled from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Ginninderra Experiment Station (Australian Capital Territory –ACT), sampling those representative of the Mediterranean region [26] and that were dedicated to agriculture.



**Figure 1.** Distribution of the Mediterranean climate in the world [22].

Regarding the protection and conservation of soils, it is important to consider that many Mediterranean countries, including Spain (representative of the European Mediterranean) and Australia, use soil quality standards to characterise contaminated soils.

More specifically, in Spain, and according to the Spanish Royal Decree 9/2005 [27], any soil must be considered as potentially contaminated (or contaminated) when concentrations (or concentrations 100 times) above the corresponding baseline value are determined in them. In agricultural soils, the baseline value for the different elements is established taking into account the upper limit of the normal range of concentrations, which covers the natural variability of the metal in soil associated with background levels at regional level. This normal range of concentrations considers diffuse or nonpoint pollution (e.g. fertilisation and atmospheric deposition) but does not include point pollution due to local human activities (e.g. industries) [17, 28–30]. These values are useful to identify the current contents of heavy metals and to assess the degree of contamination by human activities [30]. Regarding the establishment of these values, Micó et al. [30] and Sánchez et al. [31] established the baseline values for different heavy metals in agricultural soils under vegetable crops of the Valencian Mediterranean region. The baseline for Cu was 65.9 mg/kg, and it is similar to those established in other Spanish Mediterranean regions [32, 33] and in other European Mediterranean regions [34, 35].

On the other hand, Australian guidelines for metal contaminant concentrations in soil and soil amendments are established at a state level (e.g. [36–38]) and are based on European regula-

tions and research [20], which do not reflect the influence of both the soil and the climate of Australia.

Therefore, taking into account all of the above, the objective of the chapter is to analyse and discuss the results obtained by [39–41] concerning the definition of adequate soils quality standards for the Mediterranean region and the approach made to define adequate soil management practices, after considering the different soil properties of different representative soils of the Mediterranean region (European and Australian) and two horticultural crops representative of two different accumulation strategies: accumulator and non-accumulator. This will enable to suggest adequate agricultural practices to manage and preserve the resource soil under Cu contamination problems.

### **3. Materials and methods**

#### **3.1. Sampling and soil characterisation**

Four agricultural plots from the Spanish Mediterranean region and three agricultural plots from the Australian Mediterranean region, all having different soil properties, were selected and sampled.

On one hand, the selection of the Spanish soils was performed considering the information and databases of previous studies [42, 43]. These classify them as representative of the European Mediterranean agricultural area. More specifically, the types of soils represented were two Calcaric Fluvisols with different soil properties (Sollana and Peníscola), a Gleyic Fluvisol (Nules) and a Salic Fluvisol (Rojales), according to the World Reference Base for Soil Resources [44]. The soils selected covered a wide range of the different types of soils devoted to vegetable crops in the European Mediterranean region [45].

On the other hand, the selection of the Australian soils was carried out considering the information of the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The types of soils represented were a Chromic Luvisol (Soil 1), an Eutric Planosol (Soil 2) and a Pellic Vertisol (Soil 3), according to the World Reference Base for Soil Resources [44].

Soil properties were determined according to the official laboratory methods of the Spanish Ministry of Agriculture, Fishery and Food [46] for the soils of the Spanish Mediterranean Region, and to the official soil chemical methods for Australasia [47] for the soils of the Australian Mediterranean Region.

#### **3.2. Experimental design**

Three different sets of experiments were carried out and compared, each one including two different ecotoxicological assays (described later): one set of experiments with European Mediterranean soils and lettuce; another set with Australian Mediterranean soils and lettuce; the last set with European Mediterranean soils and tomato.



The sampled agricultural soils indicated previously were spiked with a Cu contaminant solution to achieve six different total Cu concentrations, the control (no Cu addition) and five different doses (65.9, 659.0, 1977.0, 3295.0 and 6590.0 mg<sub>Cu</sub>/kg). These ranges of doses were selected and established after considering previous studies also carried out in Mediterranean agricultural soils [39, 48, 49].

Two different ecotoxicological assays were conducted in the contaminated soils: one to evaluate the effect of Cu over biomass production (28 days); and the other to analyse the absorption and accumulation of Cu in roots and stem and leaves for lettuce, or in roots, stem and leaves and fruit for tomato (3 months).

For the first assay, biomass production was assessed following the OECD test 208 [50], where 300 g of contaminated soils was placed in pots (10 cm in diameter) and ten lettuce or five tomato seeds were then seeded to 1 cm soil depth. Each treatment was replicated three times (three pots per Cu dose and three per control), and all pots were placed in a glasshouse. Experimental conditions were controlled and maintained according to the requirements specified in the biomass assay procedure [50].

For the accumulation assay, 1.2 kg of contaminated soils were placed in 25 cm diameter pots and ten lettuce or five tomato seeds were seeded to 1 cm soil depth, although only one of the germinated seeds was selected to grow until maturity. As for the biomass assay, each treatment was replicated three times (three pots per Cu dose and three per control) and all pots were placed in a glasshouse. Again, experimental conditions were controlled and maintained according to the requirements specified in the biomass assay procedure [50].

### 3.3. Biomass data analysis

Weight values obtained in the biomass assay were used to establish the EC<sub>50</sub> and EC<sub>10</sub> effective concentrations. Previous to this, homogeneity of variance and normality of weight data was checked using the Kolmogorov-Smirnov test and these were log-transformed when appropriate in order to stabilise variances. Dose-response data were fitted to a log-logistic curve according to Eq. (1) [51] for each of the soils tested in order to establish the EC<sub>50</sub> and EC<sub>10</sub>. TRAP<sup>®</sup> version 1.22 (Toxicity Relationship Analysis Program, United States Environmental Protection Agency) was used for this purpose [52–54].

$$y = \frac{y_0}{1 + e^{b(x-M)}} \quad (1)$$

where  $y$  = biomass (lettuce/tomato shoot weight of plants) produced (mg),  $x = \log_{10}(\text{added Cu})$  (mg/kg),  $y_0$  = biomass produced with non-added Cu (control) (mg), and  $M$  and  $b$  are parameters to be fitted, where  $M = \log_{10}(\text{EC}_{50})$  and  $b$  is a slope parameter that indicates the inhibition rate. The concentration of Cu considered in the control dose was the initial Cu content of the soil assayed. The distribution of residuals, relationship between these and the fitted values and the adjusted coefficient of determination ( $R^2_{\text{adj}}$ ) were examined in order to determine the model's adequacy. The EC<sub>10</sub> was also calculated as described above.

### 3.4. Cu content in soils and plants

Stem and leaves, and root samples of the accumulation assay were grounded and 0.5 sieved prior to their analysis. Total Cu concentration in soils, stem and leaves, and roots was determined using the USEPA 3052 method [55]. Copper content in soils and plants was analysed by a Microwave Plasma Atomic Emission Spectrometer (MP-AES). The precision and the accuracy of the analysis were evaluated calculating the relative standard deviation (RSD) and the recovery of metal of external standards provided by the commercial house (Agilent) and different Certified Reference Materials (CRM). RSD values (from 4 to 9%) were smaller than 10% and were considered satisfactory [56]. Recoveries ranged from 83 to 111% and were within 80–120% interval proposed as satisfactory by [56].

In order to compare the Cu concentrations obtained in stem and leaves with the maximum Cu content in foodstuffs (10 mg/kg in fresh weight basis for lettuce) established by the identified legislation [57], different conversion factors were applied. These were calculated by assessing their moisture content through a gravimetric method [47]. Furthermore, and considering this maximum value, the critical limit that refers to the concentration of Cu in soil that results in the maximum concentration allowed in vegetable crops was defined when possible.

Moreover, to assess the accumulation and distribution of Cu in lettuce and tomato plants, and therefore their phytoremediation potential, three different concentration factors (CFs) were calculated. In this case study, the ratio between the heavy metal concentrations in root (mg/kg dry weight) and in soil; the ratio between the concentrations in stem and leaves and in root; and the ratio between the concentrations in fruits and in stem and leaves were calculated for each soil and dose.

It is important to point out that, in this study, the total Cu in soil that is bioavailable has been considered to be very similar to the total Cu concentration in soil. Although not realistic for aged contaminated soils, spiked soils realistically reflect the conditions in terms of contamination that can take place in agricultural soils as a result of different contamination processes. More specifically, they realistically reflect contamination processes and conditions associated with an excessive Cu-based pesticide and fungicide application, or due to spills [58] or intensive extractive activities nearby [59], where Cu is artificially added and is very bioavailable. In such cases, the values of total and bioavailable Cu content are very similar, so both concentrations can be used to analyse this type of contamination [39, 60].

### 3.5. Statistical analysis

After checking the distribution and homogeneity of variance, mean biomass produced for the different doses and soils was compared applying two-way ANOVAs and Turkey test, in order to elucidate differences amongst soils and doses. The influence of soil properties on biomass production and in the accumulation of Cu in the edible part of the plant was assessed by correlation analyses. Correlations were derived between each of the effective concentrations ( $EC_{50}$  and  $EC_{10}$ ) calculated and the soil properties of the different soils sampled, and between the soil properties and the concentrations in plants at the different doses assayed. The corre-



lation coefficients considered were Pearson’s since the data had a normal distribution. All these statistical analyses were conducted using SPSS© version 19.3.

#### 4. Results

**Table 1** summarises the main properties of the seven soils assayed (Rojales, Sollana, Nules, Peníscola, Soil 1, Soil 2 and Soil 3). As it can be observed, a wide range of different soil properties was covered with the selected soils, enabling this way to analyse the influence of the different properties over the dynamics of Cu in soils and its transference to the plant.

Soil	pH	EC (dS/m)	SOM (%)	CCE (%)	CEC (cmol(+)/kg)	Sand (%)	Silt (%)	Clay (%)	Initial Cu (mg/kg)
Rojales	7.66	0.90	1.6	52	14.5	28	38	33	12.4
Sollana	7.48	2.38	3.8	53	27.6	12	41	47	30.9
Nules	7.72	3.26	8.7	39	37.1	19	34	48	58.5
Peníscola	7.72	1.86	2.7	45	16.8	49	25	25	17.4
Soil 1	5.36	1.10	3.7	0	4.2	10	10	80	7.6
Soil 2	5.67	1.34	4.6	0	13.1	26	36	38	17.6
Soil 3	7.41	2.05	3.5	0	36.5	42	43	15	15.5

EC, electrical conductivity; SOM, soil organic matter content; CCE, calcium carbonate equivalent content; CEC, cation exchange capacity

**Table 1.** Properties of the seven soils assayed [39–41].

soil	EC <sub>10</sub> <sup>a</sup>		EC <sub>50</sub> <sup>b</sup>		R <sup>2</sup> adj. (%) <sup>c</sup>	
	Lettuce	Tomato	Lettuce	Tomato	Lettuce	Tomato
Rojales	8.8 ± 0.9	32.9 ± 0.3	177 ± 2.1	500.7 ± 0.1	89	93
Sollana	46.2 ± 1.3	393.5 ± 0.2	680 ± 3.4	1223.8 ± 0.2	88	81
Nules	159 ± 3.4	491.4 ± 0.6	753 ± 2.9	1696.5 ± 0.4	97	50
Peníscola	–	358.4 ± 0.2	–	663.8 ± 0.2	–	98
Soil 1	49.0 ± 1.7	–	104.0 ± 2.0	–	90	–
Soil 2	106.9 ± 2.0	–	236.4 ± 2.4	–	94	–
Soil 3	443.1 ± 2.6	–	728.9 ± 2.9	–	93	–

– not assayed.

<sup>a</sup>Effective concentrations of added Cu that caused a 10% reduction in the biomass produced.

<sup>b</sup>Effective concentrations of added Cu that caused a 50% reduction in the biomass produced.

<sup>c</sup>Percentage of variance accounted for by the log-logistic model.

**Table 2.** Toxicity threshold values (EC<sub>10</sub> and EC<sub>50</sub>, mg/kg) for Cu added to soil derived from the lettuce and tomato biomass tests in the seven soils assayed [39–41].

**Table 2** shows and sums up toxicity threshold values (EC<sub>10</sub> and EC<sub>50</sub>) calculated for each soil and crop.

Dose Cu (mg/kg)	Rojales			Sollana			Nules		
	Total content of Cu (mg/kg) in soil	Total content of Cu in crop <sup>a</sup> (mg/kg)	CF <sup>b</sup>	Total content of Cu (mg/kg) in soil	Total content of Cu in crop <sup>a</sup> (mg/kg)	CF <sup>b</sup>	Total content of Cu (mg/kg) in soil	Total content of Cu in crop <sup>a</sup> (mg/kg)	CF <sup>b</sup>
0.01 (control)	8.9 ± 1.0	18 ± 3.0	2.03	22.1 ± 3.0	10.1 ± 2.0	0.46	44.4 ± 5.0	12.6 ± 2.0	0.28
65.9	77.0 ± 9.0	16.5 ± 3.0	0.21	70.0 ± 8.0	12.4 ± 2.0	0.18	83.4 ± 10.0	10.5 ± 2.0	0.13
659.0	365.1 ± 44.0	20.1 ± 3.0	0.06	403.2 ± 49.0	14.9 ± 3.0	0.04	359.4 ± 44.0	16.2 ± 3.0	0.05
1977.0	1549.7 ± 188.0	50.4 ± 9.0	0.03	1607.7 ± 195.0	24.0 ± 4.0	0.01	1622.9 ± 197.0	23.0 ± 4.0	0.01
3295.0	3271.2 ± 397.0	212.5 ± 36.0	0.06	3382.5 ± 410.0	74.6 ± 13.0	0.05	2738.7 ± 332.0	29.2 ± 5.0	0.01
6590.0	5831.0 ± 707.0	–	–	5853.0 ± 710.0	–	–	5850.0 ± 710.0	–	–

All the results are expressed in mg/kg in dry weight basis [39].

– no biomass produced.

<sup>a</sup>The conversion factors that have to be applied in order to calculate the content of metal in crop in fresh weight basis are the following: 11.2 for Rojales, 17.3 for Sollana and 17.6 for Nules.

<sup>b</sup>Concentration factor.

**Table 3.** Mean copper content in the edible parts of lettuces (mg/kg in dry weight basis), and mean total contents of copper in the European Mediterranean soils assayed.

Dose Cu	Soil 1			Soil 2			Soil 3								
(mg/kg)	Total	Total	Total	CF <sub>s-r</sub>	CF <sub>r-l</sub>	Total	Total	Total	CF <sub>s-r</sub>	CF <sub>r-l</sub>	Total	Total	Total	CF <sub>s-r</sub>	CF <sub>r-l</sub>
	content	content	content			content	content	content			content	content	content		
	of Cu	of Cu	of Cu			of Cu	of Cu	of Cu			of Cu	of Cu	of Cu		
	(mg/kg)	(mg/kg)	(mg/kg)			(mg/kg)	(mg/kg)	(mg/kg)			(mg/kg)	(mg/kg)	(mg/kg)		
	in soil	in root	in leaves <sup>a</sup>			in soil	in root	in leaves <sup>a</sup>			in soil	in root	in leaves <sup>a</sup>		
0.01 (control)	7.6 ± 4.5	10.6 ± 5.0	1.8 ± 1.0	1.3	0.2	17.6 ± 4.4	8.6 ± 2.4	1.4 ± 0.9	0.5	0.2	15.5 ± 1.0	7.9 ± 6.5	1.5 ± 0.2	0.5	0.2
65.9	53.7 ± 15.0	53.6 ± 17.9	3.0 ± 0.1	0.9	0.1	74.5 ± 20.7	96.3 ± 9.2	1.6 ± 0.2	1.2	0.1	39.6 ± 12.7	14.0 ± 6.9	3.5 ± 0.6	0.4	0.3
659.0	658.0 ± 72.0	–	–	–	–	670.6 ± 48.1	199.9 ± 21.3	10.1 ± 0.8	0.3	0.1	434.9 ± 57.5	60.5 ± 9.3	9.2 ± 0.1	0.1	0.2
1977.0	2281.7 ± 58.1	–	–	–	–	1808.5 ± 136.2	–	–	–	–	1985.0 ± 143.9	183.2 ± 15.9	35.5 ± 5.5	0.1	0.2
3295.0	3197.7 ± 498.2	–	–	–	–	3096.2 ± 540.0	–	–	–	–	3064.1 ± 146.5	–	–	–	–
6590.0	7227.8 ± 995.2	–	–	–	–	5338.0 ± 900.9	–	–	–	–	5604.5 ± 167.4	–	–	–	–

All the results are expressed in mg/kg in dry weight basis [41].

– no biomass produced.

CF<sub>s-r</sub>: concentration factor, between soil and root; CF<sub>r-l</sub>: concentration factor, between root and leaf.

<sup>a</sup>The conversion factors that have to be applied in order to calculate the content of metal in plant in fresh weight basis are the following: 8.2 for Soil 1, 8.8 for Soil 2, 9.9 for Soil 3.

**Table 4.** Mean copper content in the Australian Mediterranean soils assayed and mean copper content in roots and the edible part of lettuce.

**Tables 3–5** show the results obtained in terms of Cu concentration in soils and in the different parts of the plants analysed, indicated previously.

Total content of Cu (mg/kg)										
Dose Cu (mg/kg)	Rojales					Sollana				
	In soil	In plant <sup>a</sup>	In fruit <sup>b</sup>	CF <sub>s-p</sub>	CF <sub>p-f</sub>	In soil	In plant <sup>a</sup>	In fruit <sup>b</sup>	CF <sub>s-p</sub>	CF <sub>p-f</sub>
0.01 (control)	12.4 ± 1.7	23.8 ± 2.5	7.8 ± 1.9	1.92	0.33	30.9 ± 4.3	21.7 ± 2.2	8.2 ± 2.0	0.70	0.38
65.9	64.1 ± 8.9	28.8 ± 3.0	7.3 ± 1.7	0.45	0.25	79.1 ± 11.0	27.6 ± 2.9	8.1 ± 2.0	0.35	0.29
659.0	612.5 ± 84.9	31.9 ± 3.3	–	0.05	–	673.8 ± 93.4	26.3 ± 2.6	8.6 ± 2.0	0.04	0.33
1977.0	1879.9 ± 260.7	63.5 ± 6.6	–	0.03	–	2003.7 ± 277.8	27.8 ± 2.8	7.6 ± 1.8	0.01	0.27
3295.0	3670.0 ± 480.7	242.5 ± 25.1	–	0.07	–	2915.8 ± 404.3	28.5 ± 2.5	9.1 ± 2.2	0.01	0.32
6590.0	6404.5 ± 888.1	641.3 ± 66.4	–	0.10	–	7080.0 ± 922.8	688.5 ± 71.2	–	0.10	–
Dose Cu (mg/kg)	Nules					Peníscola				
	In soil	In plant <sup>a</sup>	In fruit <sup>b</sup>	CF <sub>s-p</sub>	CF <sub>p-f</sub>	In soil	In plant <sup>a</sup>	In fruit <sup>b</sup>	CF <sub>s-p</sub>	CF <sub>p-f</sub>
0.01 (control)	58.1 ± 8.0	17.6 ± 1.8	6.6 ± 1.6	0.30	0.38	17.4 ± 2.4	20.4 ± 2.2	7.6 ± 1.8	1.17	0.37
65.9	108.5 ± 15.0	18.9 ± 2.0	8.8 ± 2.1	0.17	0.46	76.2 ± 10.6	23.7 ± 2.4	7.7 ± 1.9	0.31	0.32
659.0	683.2 ± 94.7	22.0 ± 2.3	6.8 ± 1.6	0.03	0.31	538.3 ± 74.6	31.4 ± 2.8	7.4 ± 1.8	0.06	0.24
1977.0	2023.1 ± 280.5	26.8 ± 2.8	7.8 ± 1.8	0.01	0.29	1658.2 ± 229.9	394.1 ± 40.8	8.3 ± 2.0	0.24	0.02
3295.0	2856.6 ± 396.1	44.4 ± 4.6	7.7 ± 1.9	0.02	0.17	3185.6 ± 441.7	1187.5 ± 122.9	9.9 ± 2.4	0.37	0.01
6590.0	6077.8 ± 842.7	1229.2 ± 127.2	8.7 ± 2.1	0.20	0.01	6476.7 ± 897.9	–	–	–	–

All the results are expressed in mg/kg in dry weight basis [40].

– no biomass produced.

CF<sub>s-p</sub>: concentration factor, between soil and plant; CF<sub>p-f</sub>: concentration factor, between plant and fruit.

<sup>a</sup>The conversion factors that have to be applied in order to calculate the content of metal in plant in fresh weight basis are the following: 11.6 for Rojales, 10.2 for Sollana, 10.5 for Nules and 9.9 for Peníscola.

<sup>b</sup>The conversion factors that have to be applied in order to calculate the content of metal in fruit in fresh weight basis are the following: 16.7 for Rojales, 15.6 for Sollana, 14.8 for Nules and 18.5 for Peníscola.

**Table 5.** Mean copper content in the European Mediterranean soils assayed (mg/kg in dry weight basis), in plant (mg/kg in dry weight basis), and in the edible part of tomato (ripe fruit).

Regarding the definition of the critical limits, these could only be established for the European Mediterranean soils cropped with lettuce. For the Australian agricultural soils cropped with lettuce, the establishment of these limits was not possible due to the important toxic effect observed. On the other hand, for the European Mediterranean soils cropped with tomato, these limits could not be calculated due to the fact the Cu content in fruit kept constant, independently of the Cu dose assayed and type of soil. The results obtained are shown in **Table 6**.

Finally, regarding the statistical analysis, and as explained previously, different correlation analysis were carried out in order to determine which soil properties influence the dynamic of Cu in soil and were more significant in terms of biomass production and of Cu absorption. For further details regarding these analyses, please consult [39–41].

	Equation	Critical limit	R <sup>2</sup> adj. (%)
Rojales	$y = 0.0053x - 0.47$	1975	89
Sollana	$y = 0.0011x + 0.43$	8697	89
Nules	$y = 0.0003x + 0.75$	30817	88

**Table 6.** Critical limit for the soil studied.

#### 4.1. European and Australian agricultural soils cropped with lettuce

As detailed previously, agricultural soils from two different Mediterranean areas of the world were considered. Different biomass assays having the same experimental design and crop were carried out in these areas, enabling to compare the results obtained and to draw different conclusions regarding the behaviour of Cu in soils and plants.

The analysis of the toxicity threshold values obtained for the Spanish and Australian agricultural soils and lettuce showed that biomass production is greatly influenced by Cu and that similar soil properties are relevant when analysing the effect of Cu and its mobility and bioavailability. As it can be observed in **Table 2**, the range of toxicity thresholds established covered similar ranges in both Mediterranean areas, being of 8–753 mg<sub>Cu</sub>/kg in the Spanish Region, and of 49–728 mg<sub>Cu</sub>/kg in the Australian Region. In both cases, the maximum threshold value was obtained for the soil having the highest pH and clay content, independently of the soil type. Therefore, these two soil properties seem to be very relevant when analysing Cu mobility and availability in soils. The difference between the maximum thresholds obtained in each region can be linked to the fact that the soil of the Spanish region had a higher SOM content and a basic pH, which increases the retention capacity of soil.

The comparison of the results obtained in both areas also pointed out the relevance of pH when analysing the mobility and availability of Cu in agricultural soils, even in soils with medium clay contents. For the all soils assayed in the Spanish Mediterranean Region, whose pH values varied slightly and were all between 7 and 8, no biomass was produced after the fifth dose, while no biomass was produced after the second, third and fourth dose in the different soils

of the Australian Region, increasing the toxic effect of Cu as pH decreased. In these latter soils, pH values varied amongst 5–7.5. The most important toxic effect was observed for one of the Australian soils assayed that had a low pH value (5.6) but a medium content of clay (38%).

Therefore, according to the results obtained, two different approaches have to be made when assessing Cu-contaminated agricultural soils, depending on the pH of these. In acidic soils (pH below 7), pH is the most relevant soil property and strongly influences the bioavailability of Cu, in spite of the contents and values obtained for other soils properties. Toxic effect of Cu increases as pH values decreased, and soil properties that we would expect to have some retention capacity are ineffective or have very little effect due to the influence of pH on their reactivity. In fact, at acid pH, the reactivity of SOM and clay is low or even null. Conversely, for basic soils (pH values exceeding 7), other properties have a more relevant effect, being clay/sand content, SOM and salinity the most relevant ones. Clay and SOM retain Cu by adsorption reactions, while salinity and sand content make Cu more bioavailable and increase the toxic effect.

Analysis of the transfer of Cu from soil to plant showed that it varied between these two areas. However, it is important to point out that comparison of results was difficult due to the important toxic effect observed in the Australian agricultural soils. No biomass was produced after earlier doses in the case of these soils, which made it complicated to compare absorption values and rates. In both areas, Cu content in the edible part of the plant increased as Cu concentration in soils also did, but no clear absorption pattern could be identified due to the limited data obtained in the Australian assays. However, the correlation analyses carried out between Cu contents and soils properties showed similarities between them and with the results obtained for biomass production. In this case, pH, salinity and sand content are the most determinant soil properties which enhance Cu transference from soil to lettuce, while SOM and clay content reduce this metals' transference to lettuce.

Concerning the critical limits, as commented previously, these could only be calculated for the European Mediterranean soils. When compared to with the Spanish soil quality standard, the results varied significantly. The critical value calculated for the non-saline soils (Sollana and Nules) was above 100 times the baseline value for Cu, being higher in the soil with the highest organic matter and clay content (Nules), whereas it was below in the soil with high salinity and low organic matter content (Rojales). It is important to point out that these values have to be interpreted carefully and considering they are only theoretical, especially the ones for Sollana and Nules. For these soils, no biomass would be produced if these concentrations were reached, as it has been proved in the assays carried out, where no biomass production was observed when the dose of Cu was 6590 mg/kg.

#### **4.2. Lettuce and tomato cropped in different European Mediterranean**

Within the same region, two different crops in different agricultural soils were assayed in order to analyse their different responses and behaviours to Cu in soil, in terms of biomass production and Cu absorption, and to evaluate the influence of soil properties on the mobility and availability of this metal to plants.

Toxicity threshold values obtained varied significantly between crops for the different soils assayed. For lettuce, as commented previously, effective concentration calculated varied between 8 and 753 mg<sub>Cu</sub>/kg, while for tomato these concentrations varied between 33 and 1697 mg<sub>Cu</sub>/kg. A more detailed analysis of these results indicate that, for EC<sub>10</sub>, the values obtained for tomato are nearly twice the maximum value obtained for lettuce, except for one soil; and for EC<sub>50</sub>, the lowest value obtained for tomato is very similar to the maximum concentration obtained for lettuce. This clearly indicates the different response of these two crops to the different Cu concentrations in soils, showing that tomato is more tolerant than lettuce to Cu-contaminated soils. In fact, according to [61] lettuce can be considered an accumulator crop, while tomato can be considered a non-accumulator crop.

The analysis of the influence of soil properties on the effect of Cu on plant biomass production led to similar results/conclusions in both assays. SOM, clay content and CEC are the most relevant properties affecting Cu soils dynamic [39, 40].

Regarding the metal accumulation in the plant, the concentrations determined both in tomato and lettuce shoots were also very similar, although this latter tends to accumulate slightly higher concentrations. The most important conclusion drawn is that in the case of tomato, low translocation rates to the edible part of the plant are observed, even in soils with high Cu concentrations, while Cu translocation and accumulation in the edible part of lettuce increase as soil Cu concentration increases. The results observed for tomato were particularly interesting, since Cu concentration in fruits kept low and constant, independently of the Cu concentration in soils and shoots. This indicates that these plants tend to accumulate Cu in shoots and roots, with very low translocation of it to fruit, pointing out its phytoremediation potential.

In both cases (lettuce and tomato), the increase in Cu concentration determined in plant was not proportional to the increase in Cu concentrations in soil, due to the fact that Cu accumulation in plant is limited. Since Cu concentration in tomato fruits kept constant, the critical limit of contaminant in soil for this crop could not be calculated and therefore cannot be compared with the critical limits calculated for lettuce.

The analysis of the influence of soil properties on the transfer and bioaccumulation of Cu in these crops also led to similar results/conclusions. Both salinity and sand content arised as soil characteristics that enhance the transfer of Cu from soil to plant; while SOM and clay content have the opposite effect.

Furthermore, it is important to point out that the maximum metal content in the edible part of the plant established by the identified legislations [19, 20] was not exceeded in any of the dose and soils assayed for tomato and by only one soil in the case of lettuce. This soil was the one having the highest salinity content, and therefore, it seems logical to observe this, due to the fact that, as explained previously, this soil property facilitates the transfer of Cu from soil to plant.

Finally, it is important to highlight that for both tomato and lettuce, and considering the results obtained for the effect of Cu and its interaction with soil properties on plant biomass production and metal bioaccumulation in plant, the soil quality standard established by the Spanish legislation is not valid from either approach. Toxicity threshold values calculated for both crops



showed that this soils quality standard was too indulgent, and it indicated this approach as the most restrictive when establishing soil quality standards. Conversely, the critical limit calculated for lettuce (**Table 6**) and the results obtained for the accumulation of Cu in the edible part of the plant show that the soil quality standard established by the Spanish legislation was too restrictive, since this content would not be exceeded in any of the soils assayed. Only one critical limit established showed that this soil quality standard was too permissive and corresponded to the one calculated for the saline soil.

Therefore, the results obtained show that soil quality standards should be established considering the influence of the different soil properties and should be particular for each case and scenario.

Lastly, and since the baseline value considered and used in all the assays carried out is similar to those established in other Spanish Mediterranean regions [32, 33] and in other European Mediterranean regions [34, 35], it is important to highlight that the results obtained in this work could be used as guidance for all the European Mediterranean Region in order to propose adequate soil quality standards; and adequate and valuable phytoremediation strategies that could be applied to Cu-contaminated soils of this region.

## 5. Conclusions

Regarding the effect of Cu on biomass production, the toxicity values established for the different Mediterranean agricultural regions and soils considered cropped with lettuce covered similar ranges. In both cases, the maximum threshold value was obtained for the soil having the highest pH and clay content, independently of the soil type. This indicated that these two soil properties are relevant when analysing Cu mobility and availability in soils.

On the other hand, when analysing the toxicity values established for the Spanish Mediterranean soils but considering the two different crops assayed, significant differences were observed between crops, in terms of tolerance and response. These results indicated that tomato is more tolerant than lettuce to Cu-contaminated soils. However, the analysis of the influence of soil properties on the effect of Cu on plant biomass production led to similar results/conclusions in both assays. SOM, clay content and CEC are the most relevant properties affecting the dynamic of Cu in soil Cu.

Regarding the analysis of the Cu bioaccumulation results, assays carried out with lettuce showed significant differences between the Mediterranean regions considered. However, comparison of results was difficult due to the important toxic effect observed in the Australian agricultural soils.

Significant differences were also observed between crops when comparing the bioaccumulation rates and quantities established for each of them when cultivated in the Spanish Mediterranean Region. The most important result is related to the Cu accumulated in the edible part of the plant. While the concentration of Cu in this part of the plant increased as the concentration in soil also did for lettuce, it was not so for tomato, where the concentration kept constant for all doses and soils assayed.

However, in spite of the results obtained for the bioaccumulation of Cu in the edible part of the plant, the critical limit could only be calculated for lettuce grown in the Spanish Mediterranean agricultural soils. These critical limits showed that the soil quality standard established by the Spanish legislation was too indulgent for the non-saline soils, while it was too permissive for the saline ones.

Furthermore, and taking into account the maximum metal concentration established in the identified legislation [19, 20], this was only exceeded by lettuce grown in the saline soil of the Spanish Mediterranean Region, and only after the fourth dose. Therefore, special attention must be paid to soil with high salinity, since certain crops must not be cultivated in them due to the potential accumulation of Cu in the edible parts of them.

Thus, and taking into account the influence of the soil properties on copper mobility and bioavailability in soil, it can be concluded that the influence of the different soil properties depends mainly on the pH of soils. In basic soils ( $\text{pH} > 7$ ), soil organic matter content and clay content reduce the mobility and bioavailability of Cu through adsorption processes, while salinity and sand content enhance the absorption of this metal by plants. In acidic soils ( $\text{pH} < 7$ ), the effect of low pH, increasing the mobility of Cu, is stronger and more significant than any other soil property.

So, considering the influence of soil properties on copper mobility and bioavailability in soil, soil quality standards for heavy metal contaminated soils should be defined/established considering the soil properties and the interaction of these with the heavy metal under analysis. In the case of Cu, the soil properties that should be considered when establishing these standards are as follows: pH, soil organic matter, clay content, sand content and salinity.

Moreover, for the type of crops considered, the effect of Cu on plant biomass production was the most relevant of those analysed, since it was the one that underwent a more severe impact. Therefore, this effect is one that should be considered when establishing adequate soil quality standards and proposing adequate soil management practices.

Finally, tomato showed an important phytoremediation potential, extracting Cu from not only low-medium but also from highly ( $>1700 \text{ mg/kg}$ ) Cu-contaminated basic agricultural soils, and having low translocation rates to fruits. However, soils with high Cu concentration underwent a noticeable reduction in terms of plant biomass production. Therefore, it is important to find an adequate balance between these two aspects, in order to propose this crop as a phytoremediation alternative in the appropriate soil conditions.

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## References

- [1] Holmgren GGS, Meyer MW, Chaney RL, Daniels RB. Cadmium, lead, zinc, copper and nickel in agricultural soils of the United States of America. *Journal of Environmental Quality*. 1993; 22: 335–348.
- [2] Wong SC, LI XD, Zhang G, Qi SH, Min YS. Heavy metals in agricultural soils of the Pearl River Delta, South China. *Environmental Pollution*. 2002; 119: 33–44.
- [3] Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C, Chambers BJ. An inventory of heavy metals inputs to agricultural soils in England and Wales. *Science of the Total Environment*. 2003; 31: 205–219.
- [4] Romic M, Romic D. Heavy metals distribution in agricultural topsoils in urban area. *Environmental Geology*. 2003; 43: 795–805
- [5] Mapanda F, Mangwayana EN, Nyamangara J, Giller KE. The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agriculture, Ecosystems and Environment*. 2005; 107: 151–165.
- [6] Luo L, Ma Y, Zhang S, Wei D, Zhu YG. An inventory of trace elements inputs to agricultural soils in China. *Journal of Environmental Management*. 2009; 90: 2524–2530.
- [7] Wei B, Yang L. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchemical Journal*. 2010; 94: 99–107.
- [8] Tóth G, Hermann T, Da Silva MR, Montanarella L. Heavy metals in agricultural soils of the European Union with implications for food safety. *Environment International*. 2016; 88: 299–309.
- [9] CEC. Communication from the Commission to the council, the European parliament, the European Economic and Social Committee and the Committee of the regions:

Thematic Strategy for Soil Protection. Commission of the European Communities (CEC), Brussels, Belgium. 2006.

- [10] Kong XB. China must protect high-quality arable land. *Nature*; 2014: 506 (7).
- [11] Komárek M, Cadková E, Chrástný V, Bordas F, Bollinger JC. Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. *Environment International*. 2010; 36: 138–151.
- [12] Recatalá L, Micó C, Sánchez J, Boluda R. Approaches for characterising contaminated sites: an analysis considering the role of soil. In: Villacama Y, Brebbia CA and Usó JL, editors. *Ecosystems and Sustainable Development III, Third International Conference on Ecosystems and Sustainable Development*. Wessex Institute of Technology, Wessex, UK; 2001. p. 347–356.
- [13] Visser WJF. Contaminated Land Policies in Europe, Chemistry and Industry Features. 1995.
- [14] Page GW. Contaminated Sites and Environmental Clean-Up: International Approaches to Prevention, Remediation and Reuse. San Diego, CL, USA: Academic Press; 1997. 212 p.
- [15] Rooney CP, Zhao FJ, McGrath SP (2006). Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils. *Environmental Toxicology and Chemistry*. 2006; 25: 726–732.
- [16] Sheldon AR, Menzies NW. The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant and Soil*. 2005; 278: 341–349.
- [17] Adriano DC. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals, 2nd edition. New York, USA: Springer; 2001. 796 p.
- [18] Kachenko AG, Singh B. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water Air and Soil Pollution*. 2006; 169: 101–123.
- [19] Codex. Codex Alimentarius Commission, Joint FAO/WHO General Standard for Contaminants and Toxins in Foods. Rome, Italy. 1998.
- [20] McLaughlin MJ, Hamon RE, McLaren RG, Speir TW, Rogers SL. Review: a bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research*. 2000; 38(6): 1037–1086.
- [21] Mediterranean Agriculture: Location and Characteristics [Internet]. 2016. Available from: <http://www.yourarticlelibrary.com/agriculture/mediterranean-agriculture-location-and-characteristics-with-diagrams/25443/> (Accessed: 2016-05-28)

- [22] International Cooperative for the Management of Mediterranean-Climate Ecosystems [Internet]. 2013. Available form: <http://www.incomme.org/> (Accessed: 2016-05-28)
- [23] Coccossis HN. Historical land use changes: Mediterranean regions in Europe. In: Brower FW, Thomas A, Chadwick MJ, editors. *Land use Changes in Europe: Processes of Change, Environmental Transformations and Future Patterns*. Dordrecht: Kluwer Academic Publishers; 1991. p. 441–461.
- [24] Groom C, Parker J, Teller A. Agriculture. In: Stanners D, Bourdeau P, editors. *Europe's Environment. The Dobris Assessment*. Copenhagen, Denmark: European Environmental Agency (EEA); 1995. p. 447–463.
- [25] Recatalá L, Segura M, Pastor A, Murillo R, Sánchez J (2009). Cambios de usos del suelo, procesos de desertificación, impactos ambientales e implicaciones en la calidad ambiental a nivel municipal. Capítulo 6. In: Recatalá L, editor and director. *Indicadores e Índices Integrados en la Agenda 21 Local para la Evaluación de la Calidad Ambiental en Áreas Afectadas por Desertificación del Ámbito Mediterráneo*. Valencia (Spain): CIDE-Centro de Investigaciones sobre Desertificación, Universitat de València, Fundación Biodiversidad; 2009. p. 160–196.
- [26] FAO. FAO Soil map of Australia. Food and Agriculture Organisation of the United Nations, Rome, Italy. 1992.
- [27] BOE. Royal Decree 9/2005, 14th January, where it is established the list of soil contaminant activities and criteria and standards to declare soils as contaminated. BOE nº 15 of the 18th of January 2005, Madrid, Spain. 2005.
- [28] McLaughlin MJ, Parker DR, Clarke JM. Metals and micronutrients—food safety issues. *Field Crops Research*. 1999; 60: 143–163.
- [29] ISO/DIS 19258. Soil quality. Guidance on the determination of background values. Geneve, Switzerland: International Standard Organisation (ISO). 2005.
- [30] Micó C, Peris M, Recatalá L, Sánchez J. Baseline values for heavy metals in agricultural soils in an European Mediterranean region. *Science of the Total Environment*. 2007; 378: 13–17.
- [31] Sánchez J, Recatalá L, Micó C, Peris M. Contenido de metales pesados en suelos agrícolas y su influencia en cultivos hortícolas. Valencia: Informe Anual de Proyecto. CIDE-Centro de Investigaciones sobre Desertificación (CSIC, Universitat de València, Generalitat Valenciana), Valencia, Spain; 2004.
- [32] Pérez L, Moreno AM, González J (2000). Valoración de la calidad de un suelo en función del contenido y disponibilidad de metales pesados. *Edafología* 2000; 7(3): 113–120.
- [33] Pérez C, Martínez MJ, Vidal J, Navarro C (2002). Proposed reference values for heavy metals in Calcaric Fluvisols of the Huera de Murcia (SE Spain). In: *Proceedings of the International Symposium on Sustainable Use and Management of Soils in Arid and Semiarid Regions*, 22–26 September 2002; Cartagena, Spain; 2002. p. 495–496.



- [34] Facchinelli A, Sacchi E, Mallen L. Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. *Environmental Pollution*. 2001; 114: 313–324.
- [35] Mitsios IK, Golia EE (2005). Heavy metal concentrations in soils and irrigation waters in Thessaly region, Central Greece. *Communications in Soil Science and Plant Analysis*. 2005; 36: 487–501.
- [36] SA EPA. South Australian biosolids guidelines for the safe handling, reuse or disposal of biosolids. Department of Environment and Natural Resources, South Australian Environment Protection Authority, Adelaide, SA, Australia; 1996.
- [37] NSW EPA Environmental guidelines: Use and disposal of biosolids products. New South Wales Environmental Protection Authority, Sydney, NSW, Australia; 1997.
- [38] Victorian EPA. Environmental guidelines for the management of biosolids (Draft). Environment Protection Authority, Melbourne, VIC, Australia; 1997.
- [39] Recatalá L, Sacristán D, Arbelo C, Sánchez J. Can a single and unique Cu soil quality standard be valid for different Mediterranean agricultural soils under an accumulator crop? *Water, Air and Soil Pollution*. 2012; 223: 1503–1517.
- [40] Sacristán D, Peñarroya B, Recatalá L. Increasing the knowledge on the management of Cu-contaminated agricultural soils by cropping tomato (*Solanum lycopersicum* L.). *Land Degradation and Development*. 2015a; 26: 587–595
- [41] Sacristán D, Recatalá L, Viscarra-Rossel RA. Toxicity and bioaccumulation of Cu in an accumulator crop (*Lactucasativa* L.) in different Australian agricultural soils. *Scientia Horticulturae*. 2015b; 193:346-352.
- [42] 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*, 22–26 September 2002; Cartagena, Murcia, Spain; 2002. p. 318–320.
- [43] Recatalá L, Peris M, Micó C, Sánchez R, Sánchez J. Analysis of heavy metals in agricultural soils (Castellón, Spain). In: *Proceedings of the Fourth International Conference on Land Degradation*, 12–17 September 2004; Cartagena, Murcia, Spain; 2004. p. 325–326.
- [44] WRB. World reference base for soil resources. A framework for international classification, correlation and communication. World soil resources report 103, 2nd edition. IUSS Working Group WRB. Food and Agriculture Organization of the United Nations (FAO), Rome. 2006.
- [45] Tóth G, Montanarella L, Stolbovoy V, Máté F, Bódis K, Jones A et al. Soils of the European Union. European Commission. Joint Research Centre. Institute for Environment and Sustainability. Office for Official Publications of the European Communities. Luxembourg; 2008.



- [46] MAPA. Official methods for soil and water analysis. Volume III. Madrid, Spain: Ministry of Agriculture, Fisheries and Food. 1994.
- [47] Rayment GE, Lyons DJ. Soil Chemical Methods—Australasia. Queensland, Australia: CSIRO Publishing; 2010. 520 p.
- [48] Recatalá L, Micó C, Arbelo CD, Navarro J, Sánchez J (2008). Testing the usefulness of using a generic reference level for Cu for risk assessment in Mediterranean agricultural soils. In: Blum WEH, Gerzabek MH, Vodrazka M, editors. Book of Abstracts of the EUROSOIL 2008. Vienna, Austria; 2008. p. 318–320.
- [49] Peñarroya B. Evaluation of a Cu soil quality standard for different representative Mediterranean agricultural soils. MSc Thesis. Valencia: Universitat de València; 2012.
- [50] OECD. OECD Guidelines for the Testing of Chemicals. Section 2: Effects on Biotic Systems Test No. 208: Terrestrial Plant Test. OECD, Paris, France; 2006.
- [51] Doelman P, Haanstra L. Short-term and long-term effects of heavy-metals on phosphatase-activity in soils—an ecological dose–response model approach. *Biology and Fertility of Soils*. 1989; 8: 235–41.
- [52] Hinojosa MB, Carreira JA, Rodriguez-Maroto JM, Garcia-Ruiz R (2008). Effects of pyrite sludge pollution on soil enzyme activities: Ecological dose–response model. *Science of the Total Environment*. 2008; 396: 89–99.
- [53] Micó C, Li HF, Zhao FJ, McGrath SP (2008). Use of Co speciation and soil properties to explain variation in Co toxicity to root growth of barley (*Hordeum vulgare* L.) in different soils. *Environmental Pollution*. 2008; 156: 883–890.
- [54] Ruyters S, Salaets P, Oorts K, Smoldres E. Copper toxicity in soils under established vineyards in Europe: a survey. *Science of the Total Environment*. 2013; 443: 470–477.
- [55] USEPA. Method 3052. Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. Revision 0. United States Environmental Protection Agency (USEPA), Washington DC, USA; 1996
- [56] Chen M, Ma LQ. Comparison of four USEPA digestion methods for trace metal analysis using certified and Florida soils. *Journal of Environmental Quality*. 1998; 27: 1294–1300.
- [57] ANZFA. Food standards code, Amendment 35. Australia and New Zealand Food Authority, Canberra, ACT, Australia. 1997.
- [58] Strawn DG, Baker LL. Speciation of Cu in a contaminated agricultural soil measured by XAFS, micro-XAFS, and micro-XRF. *Environmental Science and Technology*. 2008; 42: 37–42.

- [59] Abreu MM, Matias MJ, Magalhães MCF, Basto MJ (2008). Impacts on water, soil and plants from the abandoned Miguel Vacas copper mine, Portugal. *Journal of Geochemical Exploration*. 2008; 96: 161–170.
- [60] Micó C. Study of the contents of Heavy metals in soils with horticultural crops of the province of Alicante. PhD Thesis. Valencia (Spain): Universitat de València; 2005.
- [61] Ross SM. Sources and forms of potentially toxic metals in soil-plant systems. In: Ross SM, editor. *Toxic Metals in Soil-Plant Systems*. Chichester: Wiley. 1994.

