

Full Length Research Paper

Assessment of metals accumulated in Durum wheat (*Triticum durum* Desf.), pepper (*Capsicum annuum*) and agricultural soils

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Soil contamination by heavy metals is widely spread and concerns have been raised over the potential risks to humans, animals and agricultural crops. Toxic metals are readily accumulated in some plants and may cause a threat to humans and grazing animals. In this study, an assessment on contaminated edible vegetables by heavy metals is made. Two major sites (denoted S1 and S2) where durum wheat (*Triticum durum* Desf.) and pepper (*Capsicum annuum*) are widely cultivated in Algeria have been considered. S1 and S2 have been subjected to chemical characterisation, total metal quantification (Cd, Cu, Zn, Pb, Ni and Co) and sequential extraction as well metal speciation. Total content of the metals in the soils were found to be below the internationally recommended and permissible limits set for agricultural use for all the heavy metals except for Pb (mostly bound to iron and manganese oxides) in S2. Whereas Cd and Zn concentrations were higher in both S1 and S2; Cd, Cu, Zn, Ni and Co were mainly present in residual form, appearing less bio-available. As a consequence, these soils exhibit significant contamination. In the edible portions of durum wheat and pepper, the respective concentrations of Cd and Cu as well as of Pb, Zn, and Ni are found to exceed the permitted limits of the European Standards. The study indicates a potential ecological risk of the contaminated plants by most heavy metals except in the case of Co, causing potential health risk.

Key words: Heavy metals, soil, contamination, sequential extraction.

INTRODUCTION

Soils are major receptacles for heavy metals released from industrial activities; municipal wastewater sludge, urban composts, road traffics, atmospheric deposits and chemicals used in agriculture (phosphate fertilisers, pesticides) and spread out into the environment (Adriano, 1986). Heavy metals are extremely persistent in the environment; they are non-biodegradable and non-thermodegradable and thus readily accumulate to toxic

levels (Sharma et al., 2007). Many soils especially those found in hazardous waste sites are frequently contaminated with heavy metals, including lead, copper, chromium, and cadmium. In addition, they may move also through soils to reach ground waters or may be taken up by plants. It is known that the transfer of metals to food chain is a recognised risk and in order to reduce it, the European Union (Commission of the European Communities, 2001) has established limits for contaminants in foodstuff that include metals such as Cd and Pb.

Vegetables and particularly leafy ones accumulate high amounts of heavy metals (Parida et al., 2003; Clemente et al., 2007). Cadmium is a metal of great environmental concern (Perronnet et al., 2000). It is not essential for life; it is very mobile in soils and is readily absorbed and accumulated in the plants. Therefore, it may alter human health and grazing animals and functioning of

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Abbreviations: cm, Centimeter; m, meter; mm, millimeter; S1, soil 1; S2, soil 2; EC, electrical conductivity; W, water; V, bulk; °C, degree(s) Celsius; h, hours; ml, milliliter; mol L⁻¹, mole liter⁻¹; M, mole liter⁻¹; %, percentage; g, gram; No, numero; mS cm⁻¹, millisecond centimeter⁻¹; mg Kg⁻¹, milligram kilogram⁻¹.

ecosystems (Bitton, 1999). Lead is well known as a physiological and neurological toxin affecting many biochemical processes and almost every organ and system in the human body (Agency for Toxic Substances and Disease Registry: ATSDR, 1999). Actually, environmental risks are related to the bioavailability of the metals in the soils (Conder et al., 2001). Single and sequential extractions have been designed in order to predict either the retention/release of the metals in the soils, or their bioavailability (Tessier et al., 1979; Pérez-Cid et al., 1996; Kennedy et al., 1997; Pichtel and Anderson, 1997; Rauret et al., 2000; Sahuquillo et al., 2003).

The objective of this study is to quantify and estimate the total concentration and the form/fraction of a specific metal in the two soils S1 and S2 located near the industrial site of a suburb of Hammam Bouhrara city (2800° N latitude and 300°E longitude). This later is 50 Km far away from the Tlemcen's province (west Algeria). In addition, the total amount of the accumulated metals in the two plants: durum wheat and pepper growing in these soils have been evaluated. The results of the respective soils and vegetables have been compared to the established safe limits.

MATERIALS AND METHODS

Soil and plant samples

Soil samples

Surface soils (S1, S2) have been collected in triplicate from the horizon-cultivated soils (0 to 30 cm) at two sampling stations located in Hammam-Bouhrara, Tlemcen (Algeria) and distant at 100 m from each other. The samples were air-dried, crushed, passed through 2 mm sieve and stored at ambient temperature. Afterwards, the soil properties and the concentrations of the heavy metals have been examined.

Vegetable samples

Plant samples (durum wheat, pepper and maize) have been also picked up in triplicate from the same field simultaneously. Replicate samples were washed using clean water and then separately oven dried at 80°C till constant weight was achieved. The samples were then crushed separately through a steel grinder and the crushed material was passed through 2 mm sieve.

Chemical characterization of the soils

Three replicates of each sample (S1, S2) have been analysed: Electrical conductivity (EC) has been measured in a suspension characterized by a residue/water ratio of 1:5 (w/v), after 1 h stirring and using the conductivity meter (Systronics, India). The pH was measured in water (1:2.5 w/v) using the pH meter (HM-50V, TOA DKK, Tokyo, Japan). The organic carbon has been determined using the Walkley and Black's method (Allison, 1986); Cation exchange capacity by cobaltihexamine chloride the Orsini and Remy's method (1976).

The total concentration of CaCO₃ was measured in an acidic

medium by a Bernard calcimeter and the water-holding capacity has been determined gravimetrically, after draining the water-saturated soil.

Metal analysis

The concentrations of Cd, Co, Cu, Ni, Pb and Zn in both soils (S1, S2) and plants (durum wheat and pepper) have been determined by using the atomic absorption spectrometry (Aurora Instruments Ltd-AI 1200).

Soils

Total metal concentrations: The concentrations of Cd, Co, Cu, Ni, Pb and Zn have been evaluated after digestion of the samples (S1, S2) with aqua regia according to ISO 11466 (1995) and as it has been described Pueyo et al. (2003). The samples were digested with a mixture of HCl (37%) and HNO₃ (70%) in a ratio of 3:1 (v/v) first at room temperature for 16 h and then, at 130°C for 2 h under reflux conditions. Each suspension was then filtered, diluted to 100 ml with 0.5 mol L⁻¹ HNO₃ and stored at 4°C until analysis. Three independent replicates were performed for each sample and blanks were measured in parallel.

Metal speciation: The sequential extraction described previously by (Tessier et al., 1979) has been followed. This method is based on the partitioning of particular metal traces of samples with 8 mL magnesium chloride solution (1 mol L⁻¹, pH 7) in order to liberate exchangeable/acid-extractable metals (Step 1). Metals associated and bound to carbonates phases have been solubilised using 8 mL of 1M NaOAc adjusted to pH 5 with acetic acid (HOAc) (Step 2). The residue from step 2 has been extracted with 20 mL 0, 04 M NH₂OH.HCl in 25% (v/v) HOAc in order to release the metals associated with the bound to Fe-Mn oxides phases (Step 3). The residue from step 3 has been added to 3 mL of 0,02 M HNO₃ and 5 mL of 30% H₂O₂ adjusted to pH 2 with HNO₃ to liberate the metals associated with the bound to organic matter phases (Step 4). Finally, the contaminants have been released by HF-HClO₄ mixture (Step 5). The extracts were analysed in order to assess the metal (Cd, Cr, Cu, Ni, Pb and Zn) concentrations. Analyses were performed on independent triplicates samples of the soils (S1 and S2) and blanks were measured in parallel for each set.

Plants

Durum wheat and pepper samples weighing approximately 10 g were carbonized on an electrothermal plate and ashed at 500°C in a furnace for 3 h. The 0.1 g of ash was digested with 2 mL of a mixture of concentrated HCl (37.5%) and, HNO₃ (65%) in 3:1 ratio at 80°C until a transparent solution was obtained (Allen et al., 1986). The solution was filtered through Whatman No. 42 filter paper and diluted to 50 mL with distilled water in order to estimate the amount of metals that can be transferred from the soil.

RESULTS AND DISCUSSION

Chemical characterization

Table 1 summarizes the obtained results and shows the different physico-chemical parameters of the two soils (S1, S2).

S1 and S2 are basic and, the two matrices present low

Table 1. Chemical characterization of both soils considered in this study (mean \pm S.D., n =3).

	S1	S2
pH	8.52 \pm 0.2	8.67 \pm 0.2
EC (mS cm ⁻¹)	0.21	0.21
Moisture (%)	6 \pm 0.3	5.9 \pm 0.4
Organic matter (%)	3.69 \pm 0.8	3,8 \pm 0.4
CEC	7	10,5
Total amount of Calcaire	38.51 \pm 1	36.48 \pm 2
Total C	2.14 \pm 0.8	2.24 \pm 0.6

Table 2. Contents of metal (mg Kg⁻¹ dry weight) in different soils considered in this study (Mean \pm S.D., n = 3).

	Metal concentrations (mg Kg ⁻¹ dry weight)	
	S1	S2
Cd ^(a. b)	3.81 \pm 0.1	3.31 \pm 0.1
Co ^(a. b)	3.02 \pm 0.2	6.91 \pm 1
Cu ^(a. b)	13.60 \pm 0.2	8.85 \pm 0.1
Ni ^(a. b)	26.62 \pm 1	35.37 \pm 1
Pb ^(a. b)	99.4 \pm 3	129.4 \pm 2
Zn ^(a. b)	1300.07 \pm 4	1389 \pm 29

Table 3. Results of the total concentrations of heavy metals in S1 and S2 after fractionation through the sequential extraction.

	Metal concentrations (mg Kg ⁻¹ dry weight)	
	S1	S2
Cd	5,28	4,58
Co	3,88	5,9
Cu	16,15	10,65
Ni	31,77	37,32
Pb	90,83	86,3
Zn	1461,47	1359,7

percentages of organic matter and show a low value of EC.

Metal contents

Metal contents in soils

Total metal contents: Metal concentrations in S1 and S2 are given in Table 2. Index of soil contamination should be determined by its total metal concentrations.

Limits have been fixed by EU rules (Directive 86 / 278 /EEC). They represent maximum permitted concentrations of metals in S2 where the total concentrations of Zn and Cd are quite above the established limits. On the other hand, according to the European Directive 2000, S1 and S2 being basic showed high content of Cd, Pb and Zn.

These different values can indicate a contamination of agricultural soils. Consequently, this contamination could be aggravated if toxic metal are mobilises favouring plant uptake.

Metal speciation: To evaluate the environmental impact of these metals we should note that is not sufficient to determine their total content, since their behaviour in a given medium and capacity for mobilisation are equally important. These factors will depend on their chemical form in the soil. As a consequence, it is important to identify and quantify different species, forms or phases present in a soil. Sequential extraction schemes can be used to differentiate between short and term metal bioavailability. Metal fractionation of Cd, Co, Cu, Ni, Pb and Zn was conducted by using the sequential extraction.

The sequential steps for the extraction procedure assume that the following chemical forms of metals are recovered by each of the steps: exchange, carbonate-bound, Fe/Mn oxide-bound, OM-bound and residual. The sum of the five fraction values are given in Table 3. Relative distribution of metals as a percentage of the total metal (sum of all five fractions), are shown in Figure 1 S1 and 2 S2.

Cadmium was extracted in large quantities during the fifth extraction step and the second extraction step carbonate-bound. This suggests that Cd in these samples is less bioavailable. However, it's important to notice that this is a problem to environment, where Cd content is high, considering the limit value of regulatory documents. Cu was mainly extracted through the fifth and fourth step. It is known that Cu forms specific complexes with organic matter, a fact that was also observed by other workers (Narwal et al, 1998). Soils show lower amount of Cu associated to exchange fraction, as a consequence Cu is less bio-available.

For the two studied soils, Pb is mainly associated to the Fe-Mn oxide and residual fractions and to a lesser extends, bound to organic matter, showing lower values in the exchange fraction. This induces low bioavailability values, but a considerable tendency to become available. In the two tested soils, Zn is mainly in the residual and Fe-Mn oxide fractions showing lower values in the exchange fraction. This means that Zn has lower degree of mobility. In the case of S2, Ni is widely distributed among the bound to organic matter and residual fractions. In the two studied soils, Co is mainly associated to residual and Fe-Mn oxide fractions and, to a lesser extend, bound to organic matter, showing lower values in the exchange fraction.

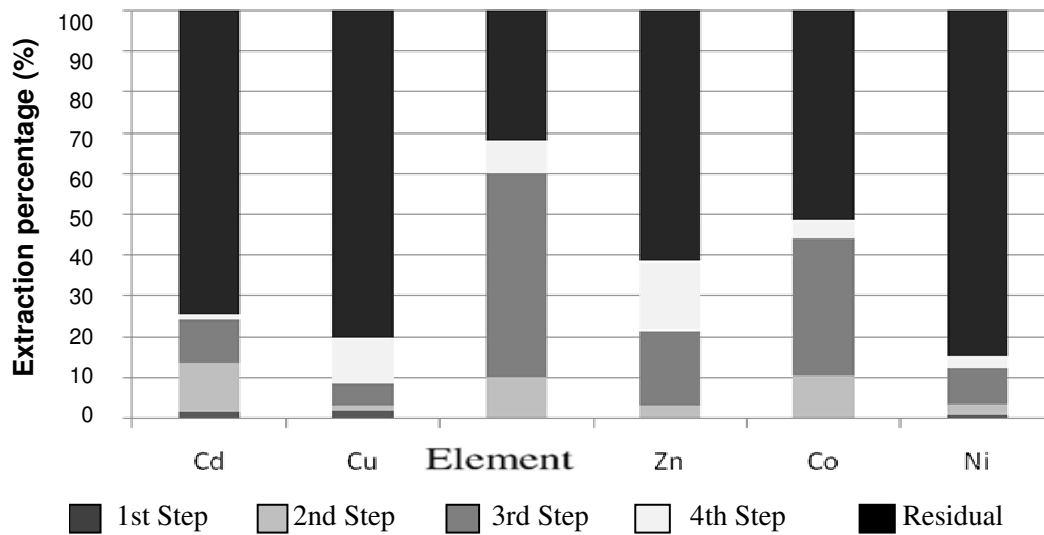


Figure 1. Metals as a percent of the total content after fractionation through the sequential extraction.

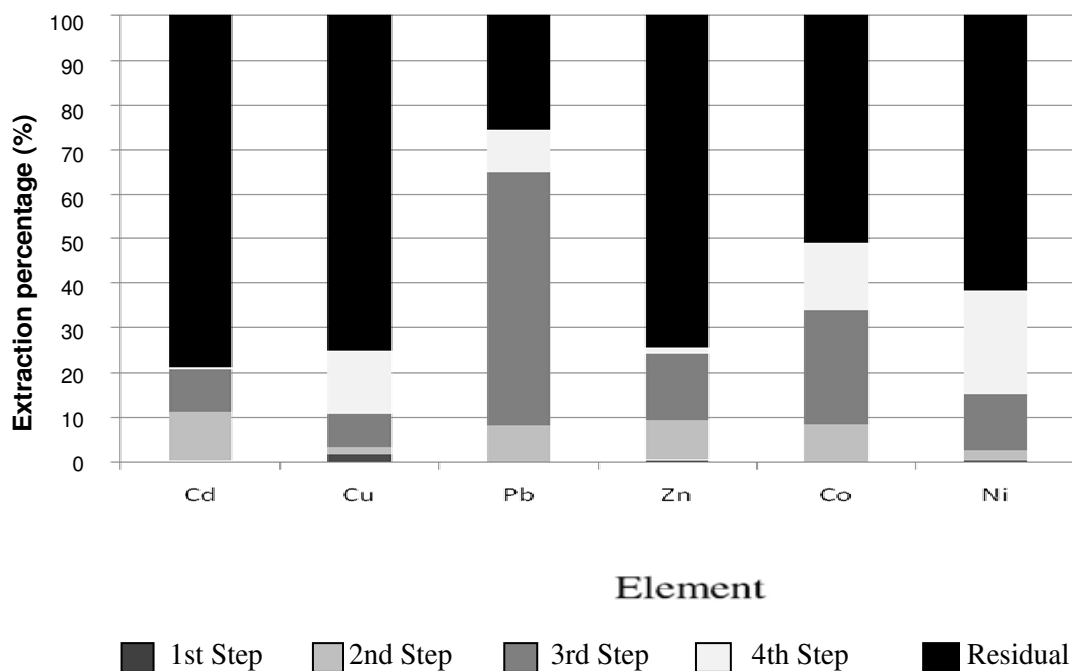


Figure 2. Metals as a percent of the total content after fractionation through the sequential extraction.

Metal content in the plants

The contents of Cd, Cu, Pb, Zn, Co and Ni in different parts of the plants (durum wheat and pepper) are shown in Table 4. The amount of all the elements varied with the different parts of plant. However, the concentrations of

Cd, Cu, Pb, Ni and Zn were higher than those in the plants reported in the literature. The results from this investigation indicate that Cd accumulates primarily in stems and seeds and with much lower concentration in the roots, leaves and nodes of the durum wheat.

It is found that the roots of pepper accumulated Cd

Table 4. Heavy metal concentrations (mg Kg⁻¹ dry weight) in different parts of plants (durum wheat and pepper).

Edible vegetable	<i>Durum wheat L.</i>					Pepper			Normal content in plant (mg/kg)	
	Different parts of the plant	Sheathes	Leaves	Nodes	Seeds	Roots	Roots	Stems		Leaves
Cd		3.58	0.31	0.21	3.10	1.58	2.54	1.61	0.63	0.05
Co		-	-	-	-	-	-	-	-	0.2
Cu		2.63	6.48	3.9	57.12	8.79	1.09	26.56	23.32	10
Ni		7.14	9.44	5.2	6.09	19.29	10.96	15.7	22.32	1.5
Pb		16.65	0.44	-	16.89	39.42	23.73	11.96	52.42	1
Zn		274.95	113.4	198.77	165.67	584.94	169.33	726.45	318.66	50

highest than the stems and leaves. These differences in sheath and seeds and other parts of the durum wheat and also in the root and stems of pepper uptake might be explained by the fact that one of the normal functions of stems and seeds of durum wheat and root of pepper is to selectively acquire ions from the soil solution, whereas the other parts of durum wheat and stems of pepper do not normally play this role (Salt et al., 1997). Costa and morel (1993, 1994) in their studies on lettuce and lupin confirmed that there was a metabolically active component in Cd uptake by roots (Jiang et al., 2001).

Cu is mainly accumulated in stems of durum wheat and pepper. Pb and Ni accumulated in high quantities in roots and leaves of durum wheat and pepper. Zn is widely accumulated by nodes of durum wheat and stems of pepper.

Conclusion

Our investigation provides an indication of the impact of the metal contamination on the soil-plant. From the chemical analysis of the two soils examined in this work, we can conclude that S1 and S2 are contaminated since the total Cd, Pb and Zn concentrations exceed widely the limits established by Directive 86/278/EEC and European Directive. Using sequential methodology given by (Tessier et al., 1979).

Our results show that these metals are mainly present in the residual fraction. This supports the assumption that Cd, Pb and Zn are less bioavailable in the examined soils. However, these results show that the durum wheat and pepper growing respectively in the soils S1 and S2 have ability to remove and accumulate strongly metals ions in their different parts of the plant.

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