

Seasonal Variation in Metal Accumulation in Pepper (*Capsicum annuum*) and Soils

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Abstract: Metals and metalloids are naturally present in the soil. The development of industrial activity (metallurgical and chemical industries) and farming (pesticides, fertilizers,...) since the last century has led to some intensive inputs of these elements become pollutants to the environment. Even at low levels, they can be a serious public health problem because of their toxicity and bio-accumulative nature. The objective of this study was an assessment of soil pollution oriented agricultural and natural vegetation (pepper) due to irrigation water loaded with heavy metals from the dam Hammam Bouhrara, the river Tafna and wastewater sewers and thus air emissions from roads in the town of Hammam Bouhrara located 50km from the city of Tlemcen in different seasons during the year 2011. The results showed a contamination of agricultural land by nickel, cadmium and lead. As for the plants grown, the study indicates a potential ecological risk pepper contaminated particularly by Fe, to a lesser extent by Cd, Pb and Ni, causing potential health risks, against this by Mn deficiency in agricultural soil and the aerial parts of the pepper.

Key words: Pepper • Heavy metals • Soil contamination • Sequential extraction

INTRODUCTION

Metal contamination of soils, due to local atmospheric deposition (industrial and municipal) and various contributions (sludge from sewage treatment plants, compost, fertilizers...) past or present, due mainly to the surface horizons, current levels trace metals (ETM) such as cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb) [1]. However, despite the obvious effect of accumulation, transfer of metal to the depth is also noted, most often derived from comparisons of total metal concentrations in different horizons of the soil [2]. Among the mentioned metals, some trace elements such as Zn and Cu, but become toxic at high concentrations [3]. Others, such as Pb and Cd are toxic dice trace. Their accumulation and transfer is therefore a risk to health humin via contamination of the food chain, but also to the environment as a whole [4].

Thus the aim of this work is to evaluate both the contamination and the risk of metal contamination by trace metals (Cd, Pb, Ni, Mn and Fe) in agricultural soil

rated S1 near urban areas, road and river Tafna city of Hammam Bouhrara and plant (pepper) (*Capsicum annuum*) in 2011 compared to the reference values. Whether these soils will they play a capital role of heavy metals or they will transfer through food chain (bioavailability) to the man. And secondly to highlight the role of vegetation in the interception, distribution and accumulation of heavy metals and finally confirm or deny the negative impact of irrigated land in the region by such wastewater.

MATERIALS AND METHODS

Soil and Plant Samples

Soil Samples: Surface (0-30 cm) soil (S1) were collected in triplicate two sampling stations in Hammam-Bouhrara, Tlemcen (Algeria) 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 were determined.

Wheat Samples: Plant samples (pepper) were taken in triplicate from the same field simultaneously. Replicate samples were washed using distilled 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) were analysed. The pH was measured in water (1:2.5 w/v) and 1M KCl solution (2.5:1), using a pH meter [5] (HM-50V, Toadkk, Tokyo, Japan). The organic carbon has been determined using the Allison (1986) method [5], cation exchange capacity by cobaltihexamine chloride the Orsini and Remy's method (1976) [6]. The total concentration of CaCO_3 was measured in an acidic medium using a Bernard calcimeter and the water-holding capacity has been determined gravimetrically, after draining the water-saturated soil.

Metal Analysis: The concentrations of Cd, Pb, Ni, Mn and Fe in both soil (S1) and plant (pepper) were determined by using the atomic absorption spectrometry (Aurora Instruments Ltd-AI 1200).

The total concentrations of Cd, Ni, Pb, Mn and Fe in the soil were determined after digestion of the samples with aqua regia according to ISO 11466 (1995) [7] as described in Pueyo *et al.* (2003) [8]. The samples were digested with a mixture of HCl (37%) and HNO_3 (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} HNO_3 and stored at 4°C until analysis. Three independent replicates were performed for each sample and blank were measured in parallel.

Sequential Extraction of Metal: The sequential extraction described previously (Tessier *et al.*, 1979) [9] was followed. This method is based on the partitioning of particular metal traces of samples with 8mL 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 were solubilised using 8 mL of 1M NaOAc adjusted to pH 5 with acetic acid (HOAc) (Step 2). The residue from step 2 was extracted with 20 mL 0.04 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25% (v/v) HOAc in order to release the

metals bound to Fe-Mn oxides phases (Step 3). The residue from step 3 was added to 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3 to liberate the metals associated with organic matter phases (Step 4). Finally, the contaminants were released by $\text{HF}\text{-HClO}_4$ mixture (Step 5). The extracts were analysed to assess the metal (Cd, Pb, Ni, Mn and Fe) concentrations. Analyses were performed on independent triplicates samples of the soils and blanks were measured in parallel for each set.

Pepper Samples Weighing Approximately 10G Were Carbonized on an Electrothermal: plate and ashed at 500°C in a furnace for 3 h. A 0.1 g of ash was digested with 2 mL of a mixture of concentrated HCl (37.5%) and HNO_3 (65%) in 3:1 ratio at 80°C until a transparent solution was obtained [10]. The solution was filtered through Whatman No. 42 filter paper and diluted to 50 mL with distilled water to estimate the amount of metals absorbed from the soil.

RESULTS AND DISCUSSION

Chemical Characterization: Table 1 summarizes the results of the different physico-chemical parameters of the soil (S1).

We note from these results that all soils were alkaline and there were no significant changes in pH across seasons.

The soil showed an average CEC (soil sandy loam) and would transfer more heavy metals to plants than the heavier soils (with more clay). The absorption of Cu and Ni into the roots is mainly controlled by CEC. The study area has high humidity in winter and average humidity in fall, spring and summer.

This explains the high rate of levy of heavy metals in the winter season.

The uptake of heavy metals by plants tends to increase when the sunlight increases, in our case in the seasons of spring and summer. The results show that soils of pepper are calcareous soils.

The organic matter content of soils was relatively stable over time and variations were possibly due to contributions from manure or compost, but were noticeable after 10 or 20 years of regular additions. The ideal range is from 3.34 to 3.60% (a soil well supplied will have a rate around 5% organic matter as the case of muck).

Table 1: Physico-chemical properties of soil supporting pepper at different seasons of the year 2011

Seasons Properties	Autumn	Spring	Summer
Moisture (%)	16.55±0.4	12.30±0.4	10.95±0.4
Water pH(H ₂ O)	8.30±0.2	8.66±0.2	8.70±0.2
pH (KCl)	7.28±0.2	7.63±0.2	7.72±0.2
Cation exchange capacity(CEC)	9.67	10.85	11.50
Total amount of calcium	38.50±2	38.70±2	38.90±2
Organics Matter (%)	3.60	3.48	3.34
Total carbon	2.09	2.02	1.94

Table 2: Concentrations of heavy metals (mg Kg⁻¹ dry weight) in the soil supporting pepper at different seasons of the year 2011

Seasons ETM	Autumn	Spring	Summer
Cd	4.55±0.45	4.78±0.48	4.94±0.50
Pb	132.15±1.90	150.24±1.98	138.33±1.98
Ni	52.11±1.49	65.90±1.50	73.55±1.52
Fe	13400.17±12.14	13788.33±12.20	13577.55±12.11
Mn	215.44±3.13	269.22±3.48	250.66±3.31

Table 3: Maximum authorized concentrations of heavy metals in sludges used in agriculture and in soils amended by sludges [9]

ETM	Content limits of heavy metals in soils (mg kg ⁻¹ dry weight)
Cadmium	2
Lead	100
Nickel	50
Iron	40000
Manganese	270

Table 4: The total concentrations of heavy metals (mg Kg⁻¹ dry weight) in soil after fractionation by sequential extraction in different seasons of the year 2011

Seasons ETM	Autumn	Spring	Summer
Cd	8.14	6.04	7.23
Pb	135.66	167.28	178.11
Ni	117.90	138.44	126.33
Fe	23703.41	23888.59	23800.90
Mn	178.77	281.63	228.11

Metal Contents in Soils

Total Metal Contents: Metal concentrations in soil are given in Table 2. In the absence of standards relating to Algerian reference levels in the soil, we adopted a comparative approach identified by references to reports in the literature and supplemented by European standards [11] (Table 3). The results of the extraction by aqua regia showed that levels of cadmium and lead exceed the maximum limits set by EU Directive.

Cadmium: The total contents of Cd ranged from 4.55±0.45 to 4.94±0.50mg/kg, being slightly above threshold limits allowed for soil specified in Table 3. This shows that soil contamination is substantial, which presents a major problem.

Lead: The total contents of Pb ranged from 132.15±1.90 to 150.24±1.98mg/kg, being slightly above threshold limits allowed for soil specified in Table 3. It may indicate a lead contamination.

Nickel: The total contents ranging between 52.11±1.49 and 73.55±1.52mg/kg and were within acceptable standards.

Manganese and Iron: Total Mn concentrations ranged between 215.44±3.13 and 269.22±3.48mg/kg, while Fe ranged between 13400.17±12.14 and 13788.33±12.20mg/kg.

These values are below the normal threshold defined in Table 3, therefore Fe and Mn are not a problem of contamination of soil, but there is a problem of deficiency.

As a conclusion, we can deduce from these results that significant changes were found for different soils with an annual growth marked by seasonal variations.

Indeed, higher levels of Cd and Ni are found in summer, while contents of Pb, Fe and Mn were higher in spring than in summer. Knowledge of the total contents of heavy metals is necessary but not sufficient to assess the potential mobility and environmental risks arising. Prediction of these risks is closely related to physico-chemical forms in which the metals are present, that is to say, their speciation. The study of the speciation of metal contaminants provides information on their interactions with the solid phase and their binding forces with the latter, thus their mobility.

Sequential Extraction: The results of the analysis of total concentrations of Cd, Pb, Ni, Mn and Fe by the method of sequential extraction of soil in the different seasons of 2011 are listed in Tables 4 and are illustrated also in the form of histograms in Figures (1-3):

Cadmium: Cd was mainly bound to carbonates with a maximum of 36.44%. These results indicate that a significant proportion of cadmium is determined by the residual phase and exchangeable, which represents more than 50% in phases exchangeable + acid-soluble cadmium potentially mobilizable.

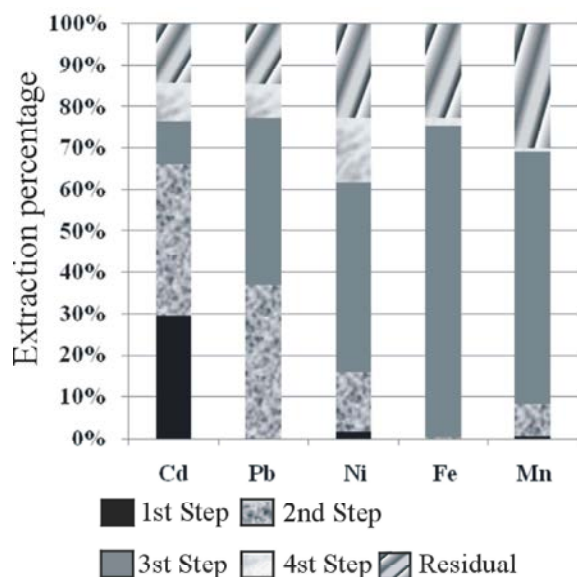


Fig. 1: Metals as a percent of total content after fractionation by sequential extraction (sum of all five fractions) in autumn Season.

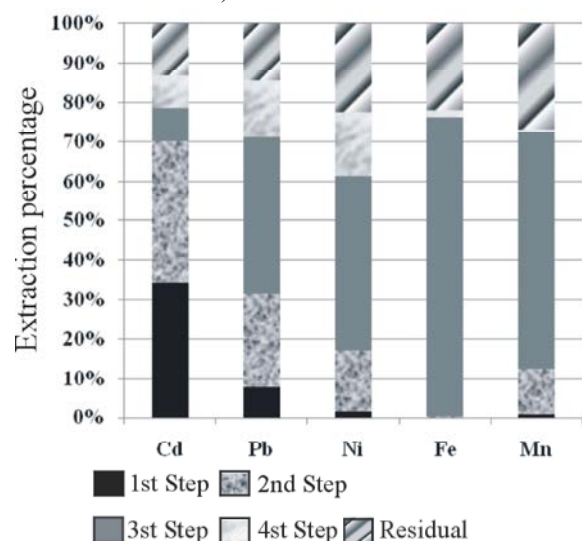


Fig. 2: Metals as a percent of total content after fractionation by sequential extraction (sum of all five fractions) in spring season.

Numerous reports in the literature associate preferentially to Cadmium carbonate fraction [10], the exchangeable fraction and residual phase [11].

The total cadmium content in the different phases ranged between 6.04 and 8.14mg/kg slightly exceeded the limits defined in Table 3, so there is possibility of Cd contamination and it appears that a significant proportion of this pollution is theoretically releasable because it is not associated with the residual phase.

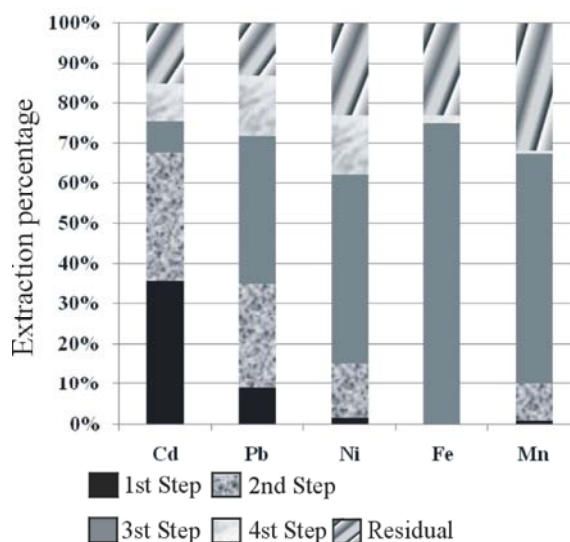


Fig. 3: Metals as a percent of total content after fractionation by sequential extraction (sum of all five fractions) in summer season.

Lead: The analysis showed firstly that Pb was preferentially bound to oxides of Fe and Mn (with a maximum of 40.42%, followed by carbonates 23.75% to 36.65%. On the other hand, it is very weakly adsorbed to the exchangeable fraction. Thus, lead is potentially very mobile.

The classification of phases in order of importance can be presented as:

Oxides of iron and manganese> carbonates> Residual> Organics> exchangeable.

This distribution is consistent with numerous studies found in the literature that associate preferentially lead in the reducible fraction [12] and the acid-soluble fraction.

The total contents of Pb in the various phases between 135.66 and 178.11mg/kg slightly exceeded the limits given in Table 3. Observed soil contamination by Pb.

Nickel: Ni is mainly present in the reducible phase to a maximum of 47.15%; it is also present in the residual phase. Nickel is therefore regarded as a very mobile element and is weakly exchangeable.

The classification of phases in order of importance is as follows:

Oxides of iron and manganese> Residual> Organics> carbonates> exchangeable.

Similar associations have been highlighted by several studies, including those by Aboubaker and Algan *et al.* [13,14].

The total Ni content contained in the various phases was between 117.90 and 138.44mg/kg, exceeding the limits of the standards defined in Table 3. Therefore, we consider the soil to be slightly contaminated.

Iron: Much of Fe (74.95 to 76.20%) was in the reducible fraction, the rest being in the residual phase.

The ratio of Fe in exchangeable and acid-soluble oxidizable fractions was negligible. Fe was found in less available fractions, which makes it very mobile. The total Fe content in the different phases varied between 23703.41 and 23888.59mg/kg, being below standard limits defined in Table 3. These values would not cause major environmental problem. The ranking in descending order of the phases was: Oxides of iron and manganese> Residual> Organics> carbonates> exchangeable.

Similar associations have been highlighted by several studies, including those of Aboubaker and Algan *et al.* [13,14].

Manganese: Mn is not exchangeable and to a lesser extent Fe and Mn oxides represent the dominant complexation phase (59.50 to 61%) for soil, the residual phase was second (26.90 to 31.73%) and the remaining acid-soluble and oxidizable fractions were generally very low in Mn. From these results, we consider that Mn was of low mobility. The ranking in descending order of the phases was as follows:

Oxides of iron and manganese > Residual > carbonates > organic matter > exchangeable.

Similar associations have been highlighted by several studies which have include those of Aboubaker and Algan *et al.* [13,14].

The total Mn content in the various phases (178.77 and 281.63mg/kg) were well below the standard limits (Table 3) and therefore poses no soil manganese pollution but rather present deficiencies.

Thus, following these results, the soil may be contaminated by Cd, followed by that of Ni and Pb. This contamination varied with season.

The causes of deficiencies are probably due to nature of soils (sandy loam and calcareous).

Metal Content in the Plant: To verify the accumulation of metals in the pepper, extraction of heavy metals by aqua regia in the roots and aerial parts was performed extraction of heavy metals by aqua regia in the roots and aerial parts was performed. This assay was performed on these samples of plants collected in 2011. The results of these extractions are listed in Tables (5-8).

Roots: Iron had the highest level in roots but values for Cd and Pb were within the range of critical levels [15] for heavy metals in plants defined in Table 9. This shows contamination by Cd and Pb in the roots of pepper. The Fe content was well above the critical threshold of plants.

Table 5: Concentrations of heavy metals (mg Kg⁻¹ dry weight) in the roots of pepper during the different seasons of the year 2011

Seasons ETM	Autumn	Spring	Summer
Cd	5.33±0.50	5.90±0.45	5.50±0.30
Pb	30.11±1.60	52.42±1.75	40.88±1.60
Ni	18.55±0.80	22.11±1.16	20.50±1.30
Fe	2455.77±7.40	25010.25±7.30	2488.12±7.20
Mn	36.22±1.75	45.44±1.60	40.90±1.55

Table 6: Concentrations of heavy metals (mg Kg⁻¹ dry weight) in the stems of pepper during the different seasons of the year 2011

Seasons ETM	Autumn	Spring	Summer
Cd	3.61±0.64	5.33±0.56	3.41±0.57
Pb	17.04±1.51	34.11±1.49	28.33±1.50
Ni	14.66±1.19	28.55±0.90	24.77±0.90
Fe	80.22±3.85	121.44±1.97	100.50±2
Mn	34.11±1.70	44.43±1.51	35.50±1.53

Table 7: Concentrations of heavy metals (mg Kg⁻¹ dry weight) in leaves of pepper wheat in the year 2011

Seasons ETM	Autumn	Spring	Summer
Cd	2.10±0.09	2.58±0.10	2.30±0.30
Pb	47.22±0.56	85.40±0.56	70.01±0.57
Ni	18.77±0.97	32.90±0.67	30.50±1.15
Fe	108.85±2.25	147.31±3.44	122±3.58
Mn	19.22±1.16	37.54±1.30	35.90±1.50

Table 8: Concentrations of heavy metals (mg Kg⁻¹ dry weight) in fruits of pepper in the year 2011

Seasons ETM	Autumn	Spring	Summer
Cd	3.11±0.48	7.02±0.63	4.80±0.56
Pb	20.21±1.19	49.66±1.55	40.55±1.50
Ni	5.95±0.58	7.33±0.64	6.87±0.63
Fe	1900.11±6.65	1994.77±6.78	1971.22±6.70
Mn	133.21±2.30	188.41±2.76	160.02±2.46

Table 9: Critical and normal levels (mg Kg⁻¹ dry weight) of heavy metals in plants [15]

ETM	Deficiency	Toxicity	Normal Content
Cd	-	5	0,05
Pb	50	12-300	1,0
Ni	1-5	10-100	1,5
Fe	50	1000	150
Mn	10-20	300-500	200

The iron was a significant contaminant in the roots of durum wheat. Moreover, the Ni content was slightly above the threshold toxic to plants in the seasons of spring and summer.

Unlike other heavy metals, Mn concentrations were below normal levels [16] in plants (Table 9), suggesting manganese deficiency in roots of durum wheat.

Shoot: Cadmium, nickel and manganese concentrated more in shoots than roots of pepper. In contrast, iron levels were higher in roots than the aerial part of pepper.

Levels of Cd, Pb, Ni, Mn and Fe measured in spring in the shoots were higher than in autumn and summer. There was a seasonal effect on the levels of Cd, Pb, Ni, Mn and Fe.

The levels of Cd, Fe and Mn were higher in fruits than in stems and leaves, with a maximum respectively range of (5.33±0.56) mg/kg, (121.44±1.97) mg/kg and (44.43±1.51) mg/kg in stems, (2.58±0.10) mg/kg, (147.31±3.44) mg/kg and (37.54±1.30) mg/kg in leaves and (7.02±0.63) mg/kg, (1994.77±6.78) mg/kg and (188.41±2.76) mg/kg in fruits. Thus, Cd and Fe concentrations were slightly above the threshold of toxicity in plant fruits.

Thus, Mn contents were within acceptable standards.

However, the Pb and Ni content were higher in leaves than stems and fruits.

The maximum values of Pb and Ni in the stems, leaves and fruits of were (34.11±1.49) mg/kg and (28.55±0.90) mg/kg, (85.40±0.56) mg/kg and (32.90±0.67) mg/kg, (49.66±1.55) mg/kg and (7.33±0.64) mg/kg respectively.

Ni content exceeded the minimum threshold of toxicity in plants defined in Table 9.

There was a slight contamination by nickel in the stems, leaves and fruits and the iron was a significant contaminant in the fruits of pepper.

Comparing the results between roots and aerial parts, it seems that cadmium, lead nickel and lead were absorbed by the roots, then translocated to enrich the aerial parts.

By contrast, iron was first absorbed into the roots of pepper where it remained stuck in the roots, no transferred to the stems and leaves and transferred to fruits.

There is thus an effect "body" very marked.

However, some chemical elements showed a similarity in behavior either at the plant or in their distribution and migration into tissues. In our case, this similarity in behavior was observed for Cd, Pb and Ni (are translocated in different plant organs); it was noticed to a lesser extent for Fe. Several studies have shown similar Ni accumulation in roots and aerial parts for different species such as clover, ryegrass, wheat and pea [17, 18].

This result confirms that the type of plant is a key determinant of the transfer and accumulation of heavy metals.

CONCLUSION

This research has allowed access to detailed knowledge on the fate of metal pollutants (Cd, Pb, Ni, Mn and Fe) in agricultural soils.

The sequential extraction performed on soil showed a source of contamination by Cd and Pb. Ni and Pb are not a potential threat to the environment despite high levels because they are very mobile.

From eco toxicological point of view, the transfer of metals to the aerial parts of a plant is not desirable since heavy metals can accumulate in the food chain passing. The overall results constitute a first step towards understanding the effects of irrigating soils in Hammam Boughrara.

This study of the real-fate of heavy metals in agricultural soils and pepper in Hammam Boughrara can be useful in managing possible environmental risks in the long term.

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