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Assessment of Lead, Copper and Cadmium Tolerance by Four Vegetable Species

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Abstract

Heavy metal pollution in agriculture soils has serious negative effects on human health and has become an important issue both in developed and developing countries. This study was conducted in ElOurdanine region (Monastir, Tunisia) and performed at four different sites using treated wastewater for irrigation. The aim is to detect the ability of accumulation of some heavy metals by four vegetable crops: peas (leguminosae), carrot (Apiaceae), lettuce (Asteraceae) and spinach (Chenopodiaceae) grown in contaminated soil. Total of 4 soils samples from the four experimental sites were collected and analyzed before and after vegetables cultivation. The quantitative content of Cadmium (Cd), copper (Cu) and lead (Pb) in the four species revealed differential abilities of accumulation. Significant differences were recorded. The comparison of the four species together showed that lettuce and spinach are the most accumulators of heavy metals, an average concentrations above than 7.50 $mg.kg^{-1}$ DM were recorded, while the pea pods were characterized by the lower concentrations (<0.5 $mg.kg^{-1}$ DM for Cd), carrot expressed a moderate accumulation with an average of four sites not exceeding 3 $mg.kg^{-1}$ DM for Cd. The lower accumulators (pea and carrot) would be most suitable for cultivation on contaminated soils while both spinach and lettuce appear to be high accumulators of Cd and Pb that are considered a higher risk to human health than Cu.

Keywords: ability of accumulation; heavy metals; vegetable crops; Tunisia.

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1. Introduction

Leaf and root vegetables are important crops that are highly consumed by Tunisian people.

These vegetables are grown throughout the year and in several areas of the country to family-scale and industrial culture; areas allocated vary by year and are at approximately 6500 ha. Therefore, the safety of these vegetables is very important since heavy metals are easily accumulated in human vital organs and threaten human health [1]. Heavy metals are natural constituents of the Earth's crust. However, due to human activities and industrial waste input, heavy metal contamination of soil and water resources has become a concern of scientific interest [2;3;4;5].

The increased addition of heavy metal to soil and water has resulted in the widespread occurrence of metal contamination in ecosystems [6;7]. Among the various heavy metals, some are essential for plant growth and development; however, at elevated concentrations they adversely affect the physiology and biochemistry of plants [8;9;10,11;12].

Heavy metals such as Cd and Pb have been shown to have carcinogenic effects; high concentrations of these heavy metals in fruits and vegetables were related to high prevalence of upper gastro-intestinal cancer [13]. Heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) and metalloids such as arsenic (As) and selenium (Se) not only pollute soils in the immediate vicinity within which they are produced but can be easily dispersed via air and water, leading to contamination of soils far from the source of the pollutants [14]. Previous studies have shown that heavy metal causes a significant decrease in growth and biomass accumulation of many crops [2;15;4;16].

Excessive concentration of heavy metals is known to cause deleterious effects on many physiological processes of plants such as photosynthesis, mineral nutrition, and the relationship with water [17;16;12].

It was reported that bioaccumulation of heavy metals in vegetables are influenced by many factors, climate, atmospheric depositions, the concentrations of heavy metals in soil, the nature of soil on which the vegetables are grown and the degree of maturity of the plants at the time of harvest [18;19].

Anthropogenic activities, such as pesticide and herbicide application, mining, or irrigation with wastewater have significantly enhanced heavy metal levels in soils in many areas in the world, which have imposed adverse environmental problems [20;21;22].

The need for more land or at least better use of currently available arable land increases according to Tang and his colleagues [23], indicated that the bioavailability of soil metal to vegetable was controlled by soil properties, soil metal speciation and plant species. According Hao and his colleagues [24] plants growing on contaminated soils will reflect elevated concentrations of heavy metals in the soils to varying extents, depending on the soil total concentrations, soil physico-chemical conditions (especially pH) and the genotype of the plant. Sharma and his colleagues [25] reported the elevated levels of heavy metals in vegetables from the areas having long-term uses of treated or untreated wastewater. Other anthropogenic sources of heavy metals include the addition of manures, sewage sludge, fertilizers and pesticides, which may affect the uptake of heavy metals by modifying the physico-chemical properties of the soil such as pH, organic matter and bioavailability of heavy metals in the soil. Although, the heavy metals can be beneficial to plants at certain levels but can be toxic when exceeding specific thresholds.

The accumulation ability of heavy metals varies considerably with the plant species; it has been reported that lettuce, radish and carrot as being high Cd accumulators while cauliflower and red cabbage were only moderate accumulators [26]. On the other hand, Kuboi and his colleagues [27] classified plant families into three groups with regard to the extent to which they accumulated Cd : low Cd accumulators (Leguminosae), moderate accumulators (Gramineae, Liliaceae, Cucurbitaceae and Umbelliferae) and high accumulators (Chenopodiaceae, Cruciferae, Solanaceae and Compositae).

The study reported here was designed to investigate variations in the accumulation of Cd, Cu and Pb by four commonly grown vegetables (carrot, lettuce, spinach and peas) cultivated on contaminated soil.

2. Material and methods

2.1. Vegetables sowing/planting

Seeds of spinach (cv. Monstrieux de Virofley), carrot (cv. Nantaise améliorée) and pea (cv. Douce de provence) were sown directly on soil at the four experimental sites on 15 September 2013 and were grown simultaneously, while lettuce (cv. Verte maraichère), seedlings were planted later on October. Each vegetable species was grown in different sites (30 m² with 3 replications) using drip irrigation with wastewater commonly used in irrigation of fruit trees in El Ourdanine region (Monastir, Tunisia). All vegetables were grown to completely maturity and harvested according to the maturity stage of each species.

2.2. Soil sampling and analysis

Four surface soil samples (0–20 cm in depth) were collected from each site before and after vegetable planting. For the soil metals, samples (2 g) were weighed into duplicate 100 ml digestion tubes, with HNO_3 (3 ml) added to HCl (10 ml) and allowed to stand overnight. They were heated for 1 h at 50°C followed by 3 h at 140°C. After cooling and filtration, solutions were diluted with 2M HNO_3. Heavy metal concentrations were determined using atomic absorption spectrophotometer (Model 2380 Perkins Elmer Inc., Norwalk, CT, USA).

2.3. Vegetables sampling, pre- treatment and analysis

Vegetable samples comprising of leafy (lettuce, spinach), pods (peas) and root (carrot) vegetables were sampled from the same locations simultaneously (about 4 kg).The samples of vegetables were collected, cleaned of soil and divided into two parts. The first part has been extensive washed with natural water (twice) followed by two washings with distilled water to assess the effects of washing on the removal of heavy metals from the vegetable surfaces and left to dry in the open air (laboratory), and stored at 4 ° C before analyzing heavy metals. The second part unwashed was kept under the same conditions of temperature prior to analysis in the laboratory. The non-edible parts of each vegetable were removed according to our common practice. Then the samples were oven dried at 80°C till the constant weight was achieved. Samples of plant material (1 g) were weighed into 100 ml block digestion tubes, concentrated nitric acid (10 ml) added and allowed to stand overnight. They were then heated for 3 h at 60°C, followed by 6 h at 110°C. After cooling, the digests were passed through a pre-washed filter (Whatman No. 540), the digestion tubes were rinsed three times, passing the washings through the filter and the filtrates made up to 100 ml volume using distilled water.

For the heavy metal analysis of dry vegetable, 1 g sample was taken into a 100 ml acid washed beaker and 15 ml of tri-acid mixture was added [25]. The mixture was then digested at 80°C till the transparent solution was achieved. After cooling, the digested samples were filtered using Whatman No.

42 filter paper and the filtrate was diluted to 50 ml with deionizer water. Determination of the three heavy metals Cu, Cd and Pb in the filtrate of vegetables was achieved by atomic absorption spectrophotometer (Model 2380 Perkins Elmer Inc., Norwalk, CT, USA).

The instrument was calibrated using manually prepared standard solution of respective heavy metals. General purpose reagent cadmium nitrate of a minimum purity of 99% was used in the preparation of the solution used to spike the samples for Cd. An analytical grade of a nitrate salt of Pb and granules of Cu were used in the preparation of solutions used in the spiking of samples for Pb and Cu.

2.4. Statistical analysis

The experiment was arranged as a randomized split- plot design with species as the main plot factor and heavy metals as the second factor (sub-plot). Data analysis was performed using SAS v. 6.0; one way analysis of variance (ANOVA) was used to separate the means which were compared by Duncan's multiple range test (DMRT) at P = 0.05.

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2.6. Results

Figure 1 shows the mean total heavy metals concentrations in the soil before and after vegetable production.

After growing of vegetable species, an average reduction of heavy metals in the soil was recorded and varied from 35 to 43%, explaining that these plants have accumulated in their roots or leaves a fair amount of heavy metals. Cu and Pb concentrations were more important than Cd both before and after vegetable planting. This indicates that local anthropogenic activities may pose a threat on vegetable production and safety.

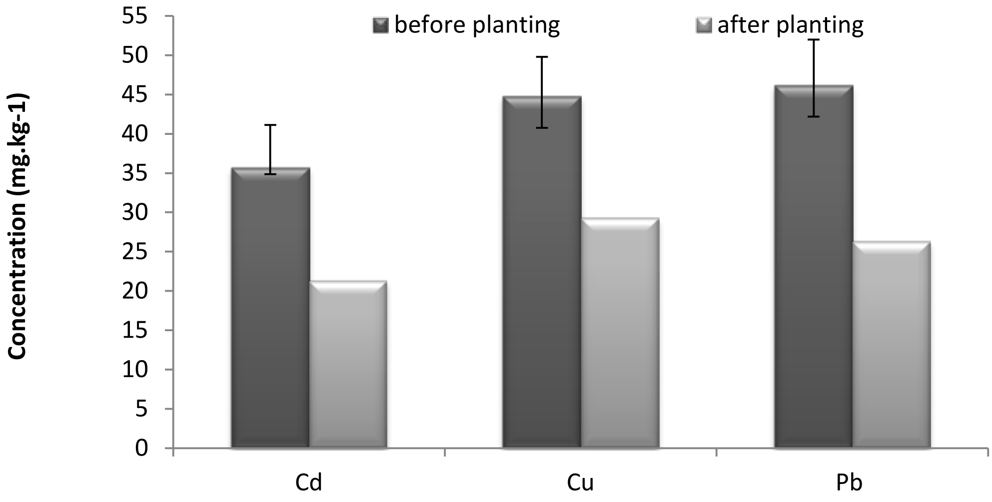

Figure 1: Mean total heavy metal concentration (mg.kg^{-1}) before and after vegetable cultivation in the soil

Leafy vegetables, lettuce and spinach accumulate higher amounts of three heavy metal compared to root (carrot) and pods (pea) vegetables; Pb accumulation is more important than Cd and Cu. This concentration varied from 8, 58 to 10, 31 mg. Kg^{-1} D.M on lettuce and spinach, respectively.

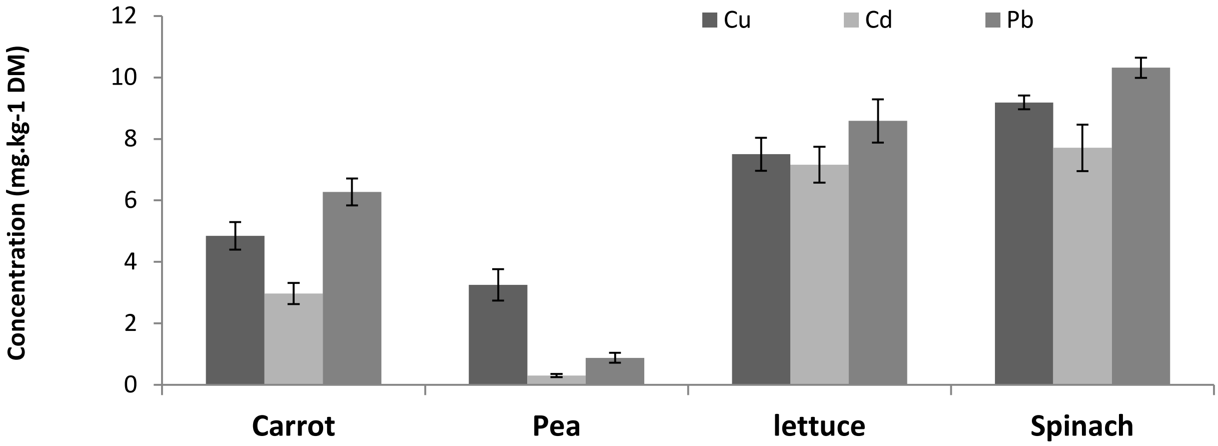

Figure 2: Mean total heavy metal concentration in the four vegetables species (mg.Kg^{-1} D.M.) (Means ± SD, n=4)

Comparing different experimental sites (Table 1), highly significant differences ($P<0.001$) were recorded for the majority of heavy metals; the highest concentrations were noted on site n°4.

Comparing the four sites, it appears that site 4 (farmer n°4) is characterized by a high level of all heavy metal, an average of 6.12 mg.kg^{-1} DM was recorded, while for the other sites, heavy metal level varies from 5.52 to 5.72 without significant difference (data not shown). Table 1 shows an important variation in the same site for all species and heavy metal. In general the highest concentration of Pb was recorded on the four sites. Site 1 and site 2, where carrot is grown, were characterized by a high concentration of this heavy metal (6,75 - 7,21 $mg.kg^{-1}$ D.M) while site the highest concentration of Pb was recorded on site 3 and site 4 either for lettuce (9,71 - 9,37 $mg.kg^{-1}$ D.M respectively) and spinach (10,99 - 10,70 $mg.kg^{-1}$ D.M, respectively). These vegetable couldn't be recommended on site 3 and 4. Comparing the four vegetable species, marked differences were exhibited between vegetables with regard to the mean concentrations of metals accumulated; the leafy vegetables appear to be high accumulators and would present a high risk for human health. For Cu, Cd and Pb the order of accumulation was: spinach > lettuce > carrot > pea.

Table 1: Mean concentrations ($mg.kg^{-1}$ D.M.) of Cd, Cu and Pb in the four vegetable species collected from the four sites.

	Carrot			Pea		
site	Cu	Cd	Pb	Cu	Cd	Pb
1	$4,38^{b*}$	$2,29^{b}$	$6,75^{a}$	$3,02^{b}$	$0,27^{b}$	$1,36^{a}$
2	$5,71^{a}$	$3,21^{a}$	$7,21^{a}$	$2,72^{bc}$	$0,36^{a}$	$0,78^{b}$
3	$3,81^{b}$	$2,56^{b}$	$5,91^{b}$	$2,51^{bc}$	$0,18^{bc}$	$0,72^{b}$
4	$5,49^{a}$	$3,83^{a}$	$5,23^{b}$	$4,75^{a}$	$0,41^{a}$	$0,67^{b}$
	lettuce			Spinach		
	Cu	Cd	Pb	Cu	Cd	Pb
1	$6,37^{b}$	$7,03^{ab}$	$8,67^{b}$	$9,86^{a}$	$6,67^{b}$	$9,56^{ab}$
2	$8,08^{a}$	$8,55^{a}$	$6,59^{c}$	$8,96^{a}$	$6,48^{b}$	$10,01^{a}$
3	$6,87^{ab}$	$7,34^{ab}$	$9,71^{a}$	$9,06^{a}$	$7,94^{ab}$	$10,99^{a}$
4	$8,69^{a}$	$5,72^{c}$	$9,37^{a}$	$8,87^{a}$	$9,75^{a}$	$10,70^{a}$

- Values in a same column followed by the same letter are not significantly different $P < 0.05$, DMRT. $n=4$.

3. Discussion

It was reported that soils may become contaminated by the accumulation of heavy metals and metalloids through many sources such as mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation [1]; [2]. In our study, the last factor is a major cause in El Ourdanine zone.

Neilson and his colleagues [14], reported that the uptake and bioaccumulation of heavy metals in vegetables are influenced by a number of factors such as climate, atmospheric depositions, the concentrations of heavy metals in soil, the nature of soil on which the vegetables are grown and the degree of maturity of the plants at the time of harvest.

In the other hand, Liu [28] and Kumar [29] found that increasing concentrations of heavy metals in soil increased the crop uptake. Field studies have found positive relationships between atmospheric metal deposition and elevated concentrations of heavy metals in plants and top soil [30;31]. Labanowski and his colleagues [32] reported that organic acids of low molecular weight naturally exuded by plant roots or produced by microbial activity have been hypothesized to influence the mobilization of nutrients or the translocation of metals in soil profiles.

In all the four experimental sites, Cu, Cd and Pb concentrations in the four species of vegetables based on their dry weight (Figure 2) show a difference in metal concentrations among these vegetables implied the different abilities and capacities to take up and accumulate these metals.

Cu element is essential for human and no specific data showed healthy implications of this metal, although excessive level of Pb was identified as contributing to anemia and neurological disorders [13]. Consumption of contaminated vegetables may pose risk to human health. Heavy metals determined in carrot, lettuce and spinach vegetables showed that the concentrations of Cu, Cd and Pb have often exceeded the safe limits of FAO/WHO. Pb concentration, however, exceed the safe limit especially in leafy vegetable, lettuce and spinach. This variation in the concentration may be due to the capacity of heavy metal accumulation in different species as well as to the high soil concentration in these heavy metals, since soil is usually irrigated by wastewater.

It was indicated that the magnitude of heavy metal deposition on vegetable surfaces varied with morpho-physiological nature of the vegetables [33]. Our results are in accordance with those reported by some authors who found that spinach and lettuce have a particularly high capacity to uptake heavy metals than in fruits or roots due to high levels of transpiration, translocation, and aerial deposition [34;23]. In contradiction with our results, Alexander and his colleagues [35] showed that five of the vegetables crops showed significantly higher concentrations (carrots, spinach, pea and lettuce all at $p < 0.001$ and French bean at $p < 0.05$) of Cd, Cu and Zn when grown in the metal-spiked soils compared with those in the untreated control soils. These differences may be attributed to the level of heavy metal concentration of soil before the experiment.

Levels of uptake and translocation of heavy metals can vary drastically among plant species as well as among crop cultivars [34;23]. Accumulation of Cu by two lettuce cultivars varied from the highest Cu concentration (12.55 mg/kg) for cv. Corsair, while Paris island cv. accumulated the lowest concentration (3.52mg/kg).

Comparing the ability to accumulate heavy metals six crop species were classified by Kuboi and his colleagues [27] as follow: French bean and pea, which had the lowest Cd contents, are members of the Leguminosae, which were classed as “low accumulators”. Carrot has intermediate concentrations of Cd and is a member of the Umbelliferae family, which are classed as “moderate accumulators”. The highest Cd concentrations found in this study were in lettuce (Compositae) and spinach (Chenopodiaceae), which were classed as “high accumulators”.

For Cd there are three limits for different types of vegetables: “leafy” vegetables 0.2 mg Cd kg^{-1} wet weight, “root” vegetables 0.1 mg Cd kg^{-1} wet weight and “all other” vegetables 0.05 mg Cd kg^{-1} wet weight [26].

In this context, Alexander and his colleagues [35] showed significant differences ($p = 0.034$) in Cu concentrations between carrot cultivars on the metal-spiked soil: cv. Nairobi showed the lowest (1.215 mg/kg) and cv. Amsterdam the highest mean concentrations (2.521mg/kg). On the other hand, they showed also that there is no significant differences ($p = 0.457$) in Pb concentrations between carrot cultivars when grown on the spiked soil. However, certain trends were evident with the cultivars Nantes, Nairobi and Mokum showing the lowest Pb concentrations (5.01, 5.04 and 5.18 respectively) and Ingot and Amsterdam the highest (7.23 and 6.31 respectively).

About spinach, Alexander and his colleagues [35] in contradiction with our results, show that there is no significant differences ($p = 0.095$) in Cd, Cu, Pb or Zn concentrations between spinach cultivars growing on the metal-spiked soil. Nevertheless, trends were evident, with cv. Bloomsdale showing the lowest Cd concentration (4.24 mg/kg) and Grodane, Mediana and Spartacus all having similar, higher Cd contents (6.94 mg/kg). In the case of Cu, the cultivars Mediana and Bloomsdale had the lowest concentrations and cv. Spartacus the highest mean concentration.

Plant adaptation results in an anatomical character, a physiological process or behavioral trait that evolved under the influence of natural selection because it improves the survival and reproductive success in the long term [36].

4. Conclusion

It must be concluded that, from these first data with regard to the suitability of vegetable crops for growing on contaminated soils, the accumulation ability of the four vegetables species investigated here showed significant differences. The lower accumulators (pea and carrot) would be most suitable for cultivation on contaminated soils. In general, both spinach and lettuce appear to be high accumulators of Cd and Pb that are considered a higher risk to human health than Cu. Although, leafy vegetable, lettuce and spinach, must be excluded in the four sites due to their higher concentration of all heavy metals.

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