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Assessment of heavy metals transfer from a moderately polluted soil into the edible parts of vegetables

M. Di Salvatore, G. Carratù and A. M. Carafa *

Dipartimento AR.BO.PA.VE, Università degli Studi di Napoli "Federico II" Facoltà di Agraria, 80055 Portici, Italy.
**e-mail: carafa@unina.it, mdisalva@unina.it*

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Abstract

In the present study we have grown plants of *Brassica oleracea* L., *Lactuca sativa* L. and *Lycopersicon esculentum* L., widely cultivated in the Mediterranean area, on garden soil contaminated with different amounts of cadmium, copper, nickel and zinc. The aim was to assess the translocation and accumulation of metals into the edible parts of the vegetables in question. Soil contamination caused no symptoms of suffering in the species tested; the quantities of metal detected in the edible parts varied significantly both in relation to the metal quantity in the growth substrate and in relation to the plant genotype. The quantities of metals absorbed by the plants were below the critical level of toxicity for the species in question; however, for Cd and, to a lesser extent, Zn, the tolerability levels for foodstuffs were exceeded. This indicates that the use of plants grown on contaminated soils may constitute a hazard for human health.

Key words: Heavy metals translocation, soil contamination, vegetables, cadmium, zinc, copper.

Introduction

Heavy metals are natural components of the earths crust, however, today soil contamination with heavy metals is an environmental problem on a global scale and it is becoming increasingly important as industrialisation increases. In most cases pollution by heavy metals stems from factory emissions, though we should not underestimate pollution resulting from the application of organic, urban and industrial waste to soils and that arising from normal farming practices (use of pesticides, fertilisers, irrigation with contaminated water) [1-5]. Due to their strong affinity for organic matter, heavy metals tend to accumulate in soils and limited but prolonged treatments can lead to their increase, especially in the humic fraction [6]. Plants constitute the reservoir through which trace elements move in the food chain towards animals and humans starting from soil, water and the air. Very often plant chemical composition is modified without damage being easily visible, and plants grown in contaminated soils contain higher quantities of heavy metals than plants grown in non-contaminated soils [7-9]. Heavy metals intake by human populations through food has been reported in many countries [10-13]; so heavy metal pollution of agricultural soils is a major environmental problem that can affect plant productivity and, above all, food quality and human health. In recent years, owing to uncontrolled and illegal disposal of various urban and industrial waste materials, numerous agricultural lands in Campania region have suffered increasing heavy metal pollution, in fact a mixture of metallic trace elements arise from waste disposal, and this represents the principal concern for food production and consumer health. In programmes to safeguard consumer health, it is of primary importance to know the degree of translocation of heavy metals from soils to plants used as food crops, and studies on the absorption of metals by food plants grown on soils with such contamination levels as not to cause phytotoxicity symptoms are of great practical interest. Indeed, eating plants produced in such soils could expose unknowing consumers to the risk of ingesting doses of metals that exceed the law and that, in the long term, could cause cases of subacute or chronic intoxication. The main aim of the research we were conducting in this field was to establish whether there is a direct relation between pollution and the quality of some food plants. We therefore set up an ecotoxicological trial, growing three widely used vegetable species in soils simultaneously contaminated with non-phytotoxic quantities of Zn, Cu, Cd and Ni, in order to evaluate: a) their ability to translocate heavy metals found in agricultural soils to edible plant parts, b) whether the quantities absorbed may in some way become a hazard for human health. For this study broccoli, lettuce and tomato were selected because their cultivation is widespread in Mediterranean environments, especially in Campania, while Zn, Cu, Cd and Ni were selected because they are considered to be among the most environmentally toxic pollutants in intensely cultivated areas [13].

Materials and Methods

Soil sampling: Pot experiments were conducted on a garden soil (soil A or control) collected in Torre del Greco (NA) and two artificially polluted soil samples (soil B and C). Before polluting the soil, soil A was air-died, passed through an 8-mm sieve to remove the largest stones and poured into a large pot to facilitate soil manipulation. Soil C was obtained by adding to soil A solutions containing different concentrations of copper, zinc, nickel and cadmium nitrate to give concentrations of metals that it is possible to find in a contaminated agricultural soil. To stabilize the soil sample [14], soil C was placed for four weeks, in a greenhouse, at ambient temperature (10-28°C); during the "stabilization period" the soil was carefully mixed several times and watered lightly. Soil B was obtained by mixing soil A and soil C in equal parts. It was decided to pollute the garden soil with metals rather than use contaminated soil for the potential problems that would arise in procuring, transporting and handling contaminated soil. Furthermore, the addition of the four metals to the same soil would mimic the situation which could occur in real agricultural soil contaminated by various elements, and all plants would be exposed to the same effects.

Plant material and growth conditions: Seedlings of *Brassica oleracea* L. subsp. *Botrytis cimosa* (var. Calabrese Tardivo), *Lactuca sativa* L. (var. Biondo Liscio da Taglio) and *Lycopersicon esculentum* L. (var. S. Marzano Nano), vigorous and of uniform size (approx.10 cm tall) were grown in 5 l plastic pots containing soil A, B or C in a greenhouse illuminated with natural light at ambient temperature (10-28°C). Each pot contained 3 kg of soil and one seedling; every soil/plant combination was replicated five times in a completely randomized design. Plants were watered with tap water if necessary, and care was taken that the water never leached out of the pots. Fertilizer (Nitrophoska Gold - BASF Agritalia) was applied once (lettuce), twice (sprouting broccoli) and three times (tomato), during the course of experiments. Plants were grown simultaneously from April to June; cultivation was extended for variable times for each species until marketable maturity was reached; in each species the edible parts were present and consisted in leaves for lettuce, inflorescences for broccoli and berries for tomato.

Soil analysis: Soil analyses were conducted on the fine part (<2 mm) according to Italian standard procedure [16]: soil pH was measured in bi-distilled water using a 1:2.5 w:v soil/solution ratio, cation exchange capacity (CEC) was determined using $BaCl_2$-triethanolamine. Organic matter (OM) was determined by loss on ignition [16]. Basic characteristics of soil A are as follow: sand 77%, silt 14%, clay 9%, $pH_{(H2O)}$ 6.71, organic matter 3.85, CEC 19.20 meq/100 g. Total heavy metal contents in soil were determined digesting the soil samples with aqua regia [17]: in brief, 1 g of soil samples was treated for 20 minutes with 3 ml of hydrogen peroxide, then 9 ml of superpure HCl and 3 ml of superpure HNO_3 were added and the samples were mineralised in a Mega FKV microwave oven. The final volume was diluted to 50 ml with bi-distilled water; concentrations of Cd, Ni, Cu and Zn were determined by flame atomic absorption spectroscopy (AAS Perkin Elmer Lambda 3).

Plant analysis: When the normal stage of maturity required for the commercial vegetable market was reached, each plant was harvested, roots were cut off and discarded, the aerial parts were thoroughly washed first with tap water and then twice with deionized water and oven dried at 70°C until constant weight was obtained and weighed. From these values the biomass production was estimated. Because present work was focused on the soil-plant-human pathway of heavy metals, only the dry edible portions of the crops were ground in a mill, 0.5 g of plant powder was weighed in a Teflon container, a superpure nitric acid/hydrogen peroxide (6:1) mixture was then added and the samples were mineralised in a Mega FKV microwave oven. The digest was diluted in 50 ml bi-distilled water and determination of metal contents was carried out with a Perkin Elmer Lambda 3 model atomic absorption spectrophotometer with AAS- flame and AAS-graphite furnace HGA 700 depending on the heavy metals. To determine Cu, Cd and Ni contents deuterium background correction was used; the standard-addition procedure was used in all determinations. AAS-flame and AAS-graphite furnace conditions employed in these determinations are summarised in Table 1.

Statistical analysis: Data were subjected to two-way ANOVA with soil treatments and plant species as independent factors. The Newman-Keuls test ($P \leq 0.05$) was used to evaluate differences between treatments and interactions means, using XL Stat software package.

Table 1. Wavelength, slit width and lamp current used in the AAS and GF-AAS determination of different elements with their detection limits.

Element	Wavelength (nm)	AAS			GF-AAS		
		Slit width (nm)	Lamp current (mA)	Detection limit (ppm)	Slit width (nm)	Lamp current (mA)	Detection limit (ppb)
Ni	232	0.2	3.5	3.0	0.7	30	25
Cd	228.8	0.7	3.5	0.5	0.7	30	1
Cu	327.4	0.7	3.5	2.0	0.7	30	5
Zn	213.9	0.7	3.5	0.4	0.7	30	1

Table 2. Means total concentrations (mg/kg) of the metals in polluted and control soils (n = 5).

Soil	Ni	Cd	Cu	Zn
A	23	0.29	53	132
B	65	3.6	95	247
C	108	7	137	469
Limit values[a]	120	2.1	120	150

[a] Limit values of contaminants acceptable in agricultural soils according to M.D. 471/99

Results and Discussion

Heavy metal contents of the soils: The quantities of heavy metals found in soils used for the experiments are reported in Table 2. According to current regulations on the limit values of contaminants acceptable in soils [18], whether agricultural or other, soil A is a non-contaminated soil; soil B and C may be considered as being polluted by Cd and Zn and by Cd, Zn and Cu, respectively. The quantities of Ni may be considered normal. It is well known that the uptake of heavy metals from soil is not a simple function of total soil heavy metal content because soil characteristics, such as soil pH, ion exchange properties and organic matter, affect the mobility of metals in soil [5, 19, 20]; moreover, the plant itself plays an active role towards mobilizing and uptake of metals bound in soil with considerable differences among plant species [20, 21]. Nevertheless, in our work soil contamination was determined by measuring the total quantity of metals found in the soil rather than the elements available, since according to several authors [4, 13] extraction methods are of limited interest for predicting the availability of metals to plants in heavy metal-contaminated soils; on the contrary this type of analysis is a valid tool to ascertain soil contamination [23] and supplies data that can be compared with the findings of many other studies of surface soil contamination [24]. In agricultural environments and in most natural environments in conditions of slight pollution, plants may well be grown in substrates in which the concentrations of heavy metals, as in our experiment, exceed those established [18]; moreover, in many intensive agricultural soils (vineyards, orchards) both in Italy and abroad [4, 25, 26], quantities of metals have been found which are higher than soils limit values.

Effect of soil contamination on plant growth: In the species tested, soil contamination does not cause any visible symptom of plant suffering, nor does it affect plant growth. Indeed, there are no statistically significant differences in the biomass produced by the three species (Fig. 1). The failure to inhibit growth is due to the fact that, though the metals absorbed and transferred to the edible parts increase along with the increase in their soil concentrations, the values are below the critical toxicity level for plants [27].

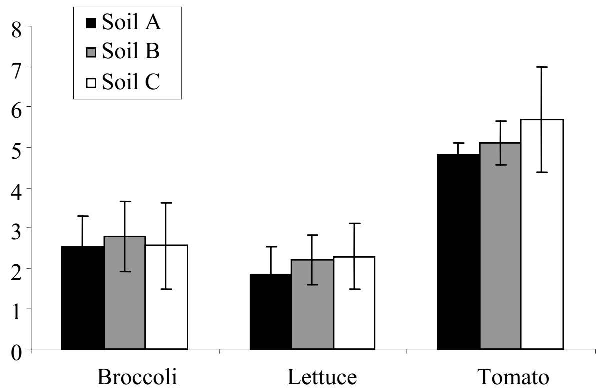

Figure 1. Effect of soil contamination on biomass production (g dry wt. plant $^{-1}$).

Absorption and transfer of metals within the edible parts: The species tested absorb and transfer in variable quantities the metals in the soil; the quantities of metals found in the edible parts depend both on the species in question and on the level of soil contamination (Table 3). Sprouting broccoli showed a more marked tendency to transfer Ni to the aerial parts, followed by tomato and lettuce in that order. The quantity of metal transferred to the tomato fruits and the broccoli leaves increased in proportion with the increase in the quantity of Ni in the soil, while in lettuce the increase was more than proportional. Nickel is one of the more mobile and

Table 3. Heavy metal concentrations[a] (µg/g dry weight) in edible portions of plants.

Plant	Soil	Ni	Cd	Cu	Zn
Lettuce		0.305f	0.012f	6.8b	55.9c
Broccoli	A	1.906cd	0.016f	4.4c	45.6d
Tomato		0.918ef	0.080f	2.15f	35.4e
Lettuce		1.450de	4.3cd	9.1a	101b
Broccoli	B	3.8b	3.3e	4.6c	91.3b
Tomato		2.273cd	4d	2.77e	36.1e
Lettuce		2.484c	6.6b	9.2a	121a
Broccoli	C	5.4a	4.8c	6.4b	120a
Tomato		4.1b	8.5a	3.4d	44.5d

[a] Data are the means of five repetition. Different letters within the same column for each metal indicate a significant difference at 5% level.

bioavailable heavy metal ions [28, 29] that may be present in both industrially contaminated and unpolluted soil. Although there is currently no clear evidence of Ni importance in plant metabolism, actually this metal at low concentration is considered an essential micronutrient for higher plants [30, 31]. Plants absorb it easily from the soil and, until the critical toxicity level is reached in plant tissues, absorption is correlated positively to the Ni concentration in the soil [24]. The lettuce leaves, given the same soil, contained a larger quantity of Cu than the broccoli and tomato fruits. Analyzing the behaviour of each species as the substrate varied, it was noted that the increase in metal in the edible parts is not proportional to the increase in metal in the soils: lettuce grown in soils B and C contained the same quantities of Cu, and broccoli grown in soils A and B has the same quantity of Cu; in the tomatoes the Cu content, though lower than in the other two species, increased along with the Cu concentration in the soil. Broccoli and lettuce transferred more Zn to the edible parts than tomato. The Zn concentration in the two vegetables, given the same soil, is similar; by contrast, the Zn concentration in the tomato fruits was decidedly lower, recording values more or less similar to the control. It should be noted that the degree of Zn and Cu transfer to the edible parts falls in the more polluted soils and the increase in concentration in the leaves, albeit statistically significant, is not proportional to the increase in total quantities found in the soil.

Zinc and copper are essential micronutrients to higher plants and are involved in several metabolic processes [32, 33]. Above certain treshold these elements are toxic but, due their role of micronutrients, plant cannot exclude their uptake. In order to resist the damaging effect of the excess of heavy metals in the soil, plants have adopted different strategies which are active not only in hyper-accumulators, where they are definitely accentuated but also in normal plants, and they vary according to the plant genotype [1]. Some of the most effective regulation mechanisms are exclusion, reduced transfer to the shoot of metals adsorbed at the cell walls of the root, chelation and compartmentation of the metals in the vacuole through the production of organic acids and formation of metal-binding polypeptides called phytochelatins [34]. When there is an increase in soil concentrations without reaching phytotoxicity levels, the plant is able to effectively control regulation mechanisms to maintain a fairly constant tissue concentration. As regards Cu and Zn one of the most effective regulatory mechanisms is the reduced transfer to the shoot of metals found in the root apoplast [33, 35, 36]. In our experiments we did not analyse roots for two reasons: first, they do not constitute the edible part in the plants considered in our research and, secondly, analysis of roots leads to results which are definitely misleading as it is in practice impossible to free them of the soil particles that adhere to them [4]. We believe, in accordance with several authors [26, 35, 36], that most of the Cu and Zn remains immobilized at the root level and that only part of the metals absorbed is translocated to the aerial part of the shoot. Furthermore in our experiments plants are exposed to metals simultaneously and antagonistic interactions between elements can occur and one or more trace elements might affect uptake of other trace elements in the plant [32, 37, 38].

Cadmium warrants treatment on its own: cadmium content in agricultural soils should usually be on the threshold of detectability [39]. However, it worryingly increases in the event of the following being applied to the soil: compost, domestic and industrial waste, slurry from livestock farms and phosphate fertilisation [3, 40, 41] ; in Great Britain, in heavily polluted sewage sludge soils, concentrations of up to 150 mg/kg have been found [42]. In our tests the cadmium content in plants grown in contaminated soils increased considerably: while in control trials it remains at about 0.1 µg/g DM, the situation changes dramatically when plants were grown in contaminated soil where the quantity of Cd in tomato fruits exceeded 8.5 µg/g. In our experiments cadmium was absorbed and transferred to the edible parts in this order: tomato> lettuce> broccoli. Though it is not an essential element and is highly toxic for plants where it produces oxidative stresses [43], in the soil-plant system it is easily absorbed and transported by transpiration stream [44] to stem, leaves and fruits [45], especially when the content in total Cd is not very high [3]. The non-accumulator plants, like those that we tested, in the presence of low to moderate Cd produce phytochelatins that bind the metal and accumulate it in the vacuole, making it harmless [46].

In Fig. 2 we compare the quantities of metals transferred to the edible parts with limits of tolerability for foodstuffs reported in the literature [24]. As may be seen, the Cu and Ni quantities found in plant edible parts are always lower than the tolerance limits; for Zn the limits are exceeded by lettuce grown in soils B and C and by broccoli grown in soil C. As regards Cd, the tolerance limits were exceeded by the three plants grown in soils contaminated by the metal. Of the metals found in the soils examined, cadmium is undoubtedly the most hazardous for consumer health. It may accumulate in the human body and entail renal disfunctions, damage to the skeleton, deficits in the reproductive apparatus nor can one rule out possible cancerogenous action [47]. Plants are the main source of Cd in the diet and several scholars are convinced that the quantities absorbed through food, even at very low concentration, may constitute a hazard for human health [48, 49]. To safeguard the health of its citizens, the EU issued Regulation 466/2001 [50] which sets maximum levels for certain contaminants in foodstuffs. As regards cadmium, it has been established that in fruits it cannot exceed 0.05 mg/kg fresh weight and 0.2 mg/kg fresh weight in leaf vegetables. In the edible parts of the species tested, taking into account that dry weight in the leaves or fruits is on average 10% of fresh weight, we always found higher Cd quantities than those indicated by EU legislation.

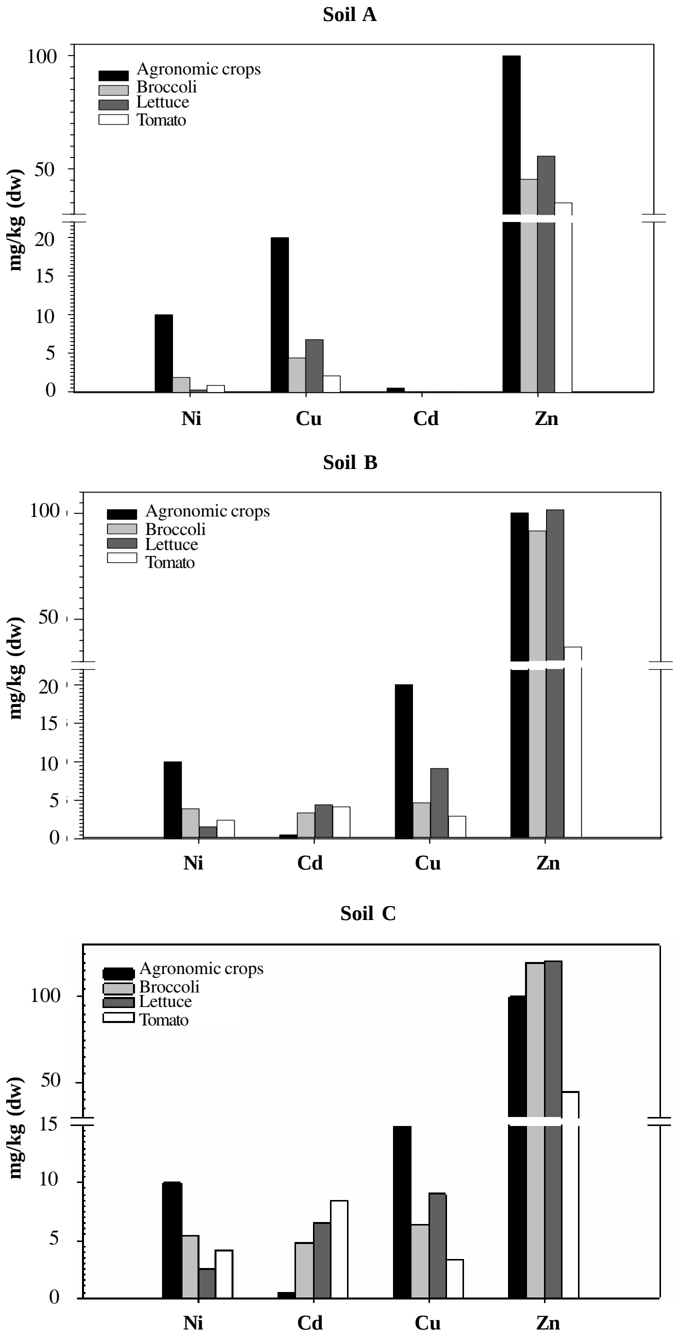

Figure 2. Comparison between heavy metals concentrations in edible parts of plants grown in soil A, B and C and permissible levels in agronomic crops.

Conclusions

The effect of heavy metals on plant growth and metabolism in many of the experiments conducted in recent decades was studied by using either hydroponic culture or soil experiments, but always in the presence of large quantities of heavy metals [35, 36, 51-53]. Such experiments, though very important in terms of scientific knowledge, are of limited practical value as such high levels of heavy metals are unlikely to be found in agricultural soils. In terms of food product safety, experiments in which plants are exposed simultaneously to relatively low quantities of metals appear more useful, as it is thus possible to ascertain the uptake of heavy metals for each species and appropriately appraise the hazardousness of metals found in food crops. In recent years research along such lines has been intensified[5, 8, 43, 54, 55]. Likewise, our experiments aimed to ascertain, in the soil-plant system, the transfer of metals in the substrate towards the edible parts and their hazardousness. Our results agree with the extensive research findings on the absorption of contaminants by plants grown in soils polluted by heavy metals: the quantities of metals absorbed varies not only in relation to the quantity of the same metals in the growth substrate, but also in relation to the plant genotype used[54, 56-58]. Given the results of our experiments, it seems necessary to point out that the pollution of soils with low or moderate quantities of heavy metals, especially for Cd and to a lesser extent for Zn, may constitute a hazard for consumer health. In fact, healthy-looking vegetables actually have high heavy metal content and agricultural produce from such soils may contribute substantially to the intake of heavy metals in the diet. In order to soundly evaluate the potential risk deriving from the use of plants grown in contaminated soils, it appears necessary to combine the monitoring of agricultural soils to detect the presence of heavy metals whit the study of plant absorption of such contaminants in order to ensure that plants are safe for human consumption.

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