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Soilless system on peat reduce trace metals in urban grown food: unexpected evidence for a soil origin of plant contamination.

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Abstract

Urban horticulture is increasingly popular for social and economic benefits. However, edible urban crops may be contaminated by airborne pollutants, thus leading to serious health risks. Therefore, a better understanding of contamination risks of urban cultivation is needed in order to define safe practices. In particular, whereas it is commonly accepted that the contamination of urban grown food comes from airborne pollutants, little is known on a possible contamination by soils. Here we studied trace metal risk in horticultural crops grown in an experimental urban allotment garden in Bologna, Italy. Seven experiments were conducted between June and November 2015 on tomato, sweet basil, onion, lettuce, kale, bulb fennel and radish. Treatments included two growing systems, soil and soilless, and two fertilization managements: mineral and organic. Trace metal concentrations were measured in soils, substrates and edible plant tissues. We identified preferentially translocated metals by partitioning analysis of tomato, sweet basil and kale. Results showed that crops grown on soilless system have a lower metal content, of -70% for Cr, -61% for Cu, -45% for Cd and -81% for Ni, compared with those grown on soil. This finding demonstrates that the major contamination risk is urban area is unexpectedly related to soil pollution.

Keywords: urban gardening; soilless cultivation; food safety; heavy metals; plant nutrition.

1 Introduction

Urban agriculture activities are commonly found in Europe and all over the world. These include the production of crop and livestock goods within cities and towns (Zezza and Tasciotti 2010), but also peri-urban agricultural areas which may provide product to the local population, such as vegetables, medicinal plants, fruit trees, ornamental plants, milk, meat and wool (Lin et al. 2015). Urban allotment gardens are one element of the urban green infrastructure that is becoming increasingly important in urban landscape planning. As reported in Breuste and Artmann (2014), they combine utility, social meaning, beauty, and several ecosystem services such as food supply (Drescher 2004), air filtering (Davies et al. 2011), urban temperature and climate regulation (Phelan et al. 2015), noise reduction (Aylor 1972), runoff mitigation (Zhang et al. 2012), and biodiversity development (Lin et al. 2015). However, plant cultivation within cities may present environmental risks associated to both air and soil pollution (Alloway 2004). In urban areas, air pollutants generally derive from artificial sources e.g. vehicular emissions and fossil fuel burning (Agrawal et al. 2003). Urban air pollution has increased rapidly in the past decades with fast industrialization, rapid growth of urban population, increase in vehicular traffic, badly maintained roads and human activities. Consequently, agricultural land adjacent to urban areas may be exposed to air pollutants of urban origin, which may result in contamination of horticultural products beyond precautionary values. When this happens, a dietary exposure to trace metals can result in significant human health risk (Massaquoi et al. 2015). An interesting study (Säumel et al. 2012), explored the relationship between local traffic burden and the trace metal concentration in the edible biomass of different horticultural crops cultivated by gardeners in the inner city of Berlin, Germany, analysing the influence of traffic burden and of the existence of barriers between cultivation sites and nearby streets. The study shows that a higher overall traffic burden increases trace metal content in the crop biomass while the presence of barriers between cultivation site and roads strongly reduces trace metal content. As reported in Vittori Antisari et al. (2015), the concentration of trace metals in urban grown vegetables is strictly related to the site in the city where plants are grown, with vegetables grown nearby main roads generally presenting greatest pollution levels. Urban soils can also be contaminated as they are often located on old urban sites, impacted by human activities, such as industrial activities, road traffic, waste dumps and demolition sites (Jean-Soro et al. 2015). They may contain hazardous substances that can be assimilated by plants grown in these spaces and become a danger for human health. Among major pollutants in urban soils, potential toxic elements (As, Hg, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sn, Zn and Se), can be accumulated in foodstuffs and enter into the food chain, causing serious problems to human health. Most important sources of trace metals in urban soils are atmospheric depositions, agricultural input (e.g. fertilizers, pesticides and manure), use of low quality compost, industrial activities, refineries. Exposure to metals can occur through a variety of processes. For As and Cd, intake of plant-derived food represents a major fraction of potentially health-threatening human exposure (Clemens and Ma 2016).

In Europe, urban gardens are primarily managed by the elders using traditional management practices often related to the wide use of synthetic products, mainly fertilizers, herbicides and pesticides (Szolnoki et al., 2013). One of the main problems that are generally encountered is the contamination risk associated with weed and pest control, generally managed by application of chemicals. In 2001, a study showed that about 90% of the German allotment holders use pesticides (UM 2014). Pesticides kill pests, but they may have a negative effect on the useful garden fauna (e.g. pollinators, pest predators), may cause water, air and soil pollution, as well as contamination of the edible products. Consistently, in recent years, the use of pesticides is being reduced or even completely banned in allotment gardens, and their application has been dramatically reduced as gardener awareness has grown (Barthel et al. 2010). Soil fertilization, on the other hand, is generally applied by mineral inorganic fertilizers, which are easy to apply and allow the supply of the right dose of nutrients. However, cases of over-application and soil and water contamination by nitrates and phosphates have been repeatedly reported in urban gardens, due to the low agricultural skills of hobby farmers (Tixier and de Bon 2006). However, the potentially high environmental impact related to the considerable amount of inputs needed to support the production may be avoided when sustainable practices are adopted. Weed management may be addressed by using organic mulching, therein also improving moisture retention and preservation of soil fertility. Pest control may be addressed in different ways (Voigt et al. 2016), including selection of disease and pest resistant plants, adoption of companion plantings, using trap crop plants that attract pest insects saving the nearby main crop, application of natural insecticides (e.g. Neem oil and Pyrethrum), application of pests' diseases (e.g. *Bacillus thuringensis*), and integration in the garden of flowering plants that attract beneficial insects (both pollinators and natural pest enemies). Organic fertilization (e.g. by using manure from livestock or poultry, or compost from vegetable waste) has the function of preserving soil characteristics allowing the

proper crop development (Tixier and de Bon 2006). Organic fertilizers are characterized by slow and long-lasting action, and have beneficial effects on soil microflora, soil texture and water holding capacity.
With the increase of world urban population, which in 2007 has surpassed the rural population, the surface of the city grows and evolves often uncontrollably. This leads to a continuous increase in impervious surfaces and the consequent consumption of arable soils. In such conditions, urban horticulture development follows a growing trend, representing not only a source of food, but also becoming a possible source of income (Caldeyro-Stajano 2004). The two main constraints to the spread of urban horticulture are the shortage of soil and water. Soil in the cities may be contaminated by air and soil pollutants, therefore cultivation of edible products may not be feasible and, in general, in the cities often availability of land is scarce (Orsini et al. 2013). In addition, water availability constitutes a further problem in the urban environment, since in some areas there can be difficult access to drinking water. The adoption of simplified soilless cultivation systems allows overcoming these constraints (Tixier and de Bon 2006). Among the different soilless systems, most commonly adopted in urban gardens are those that make use of solid substrates in containers generally built on wood and waterproofed with a plastic film (Orsini et al. 2014). The aim of the hereby illustrated research is to propose alternative/innovative agricultural techniques instead of those typically used by local gardeners (on soil cultivation with mineral or organic fertilizers purchased in local shops). Consistently, the risk of trace metals contamination in vegetables cultivated in an experimental urban allotment garden was investigated by comparing different growing systems (soil and soilless) and fertilization managements (mineral and organic).

2 Materials and Methods

The experimental allotment garden was previously identified (Vittori Antisari et al. 2015) as the most contaminated by trace metals in terms of both tissue accumulation and leaf deposition in comparison with other allotment gardens situated in different areas of the city and nearby different sources of contamination. The experimental allotment garden (**Fig. 1**, with a surface of 40 m^2) is part of a large area of gardens (so-called Orti Salgari), built in the eighties, which occupies a surface of 27,000 m^2 and is composed by 398 allotment gardens. It is situated nearby a main road of the city of Bologna (via San Donato, 300 m from the garden, 10^3–10^4 vehicles d^{-1}), the main motorway (A14, 1,200 m from the garden, 10^5 vehicles d^{-1}), the railway (1,600 m from the garden, 700 trains d^{-1}) and it is placed 10 m from the local street (coordinates 44° 30′ 54″ N, 11° 23′ 29″ E). Moreover, the city incinerator (Frullo, 600 t d^{-1} of urban waste processed) is located 3,000 m away, north-east of the garden, with prevailing winds in direction East.

2.1 Plant material and experimental design

Seven experiments were conducted between June and November 2015. Plant species used were tomato (*Lycopersicon esculentum*, cv San Marzano), sweet basil (*Ocimum basilicum*, cv. Napoletano), onion (*Allium cepa*, cv. Gialla di Stoccarda), lettuce (*Lactuca sativa*, cv. Four Seasons), kale (*Brassica oleracea* var.

Acephala or tuscan kale or black cabbage), Florence/Bulb fennel (*Foeniculum vulgare*, var. Azoricum cv. Carmo F1), and radish (*Raphanus sativus*, cv. Cherry Bell). Plants density was 5 plants m^{-2} for kale and tomato, 10 plants m^{-2} for fennel, lettuce and basil, 25 plants m^{-2} for radish, 35 plants m^{-2} for onion.

Treatments included growing media (soil vs peat) and fertilization management (mineral vs organic). Experimental design was a strip plot with 2 replicates with growing media on the main plot and fertilization management in the elemental plot. Each elemental plot hosted at least 6 plants.

2.2 Growing conditions

Some species (basil, tomato and radish) were sown in plastic containers filled with peat at the experimental greenhouse facilities of the Agricultural Sciences Department of the University of Bologna under controlled environmental conditions (25°C and 50% relative humidity). As they reached adequate growth (June 5, for basil and tomato and September 28 for radish, at respectively 50, 28 and 10 days after sowing), seedlings were transplanted at the experimental allotment garden. Other species (lettuce, onion, kale, fennel) were purchased from a local retailer of products for agriculture (normally used by local gardeners, Garden Cap, Cadriano, BO, Italy) and transplanted at the experimental allotment garden (respectively on June 5 for onion, and on September 18, for lettuce, kale and fennel). Irrigation was provided daily with a drip irrigation system, supplying 9 and 6 L m^{-2} d^{-1}, respectively in summer (June to September), and early fall (October). Later on in the season (November), irrigation was not supplied.

Plants were grown either on the allotment soil or onto elevated beds filled with commercial soil (Geotec, RO, Italy). Growing containers were built according to Orsini et al. (2014), using recycled pallets and black plastic films. Declared physical and biochemical features of commercial soil were Organic Carbon 25%, Organic Nitrogen 1%, peat 60%, salinity 0.6 dS m^{-1}.

No pest control treatments were applied during the crop cycle. Consistently, organic and conventional management protocols were diversified only according to the plant nutrient management. Plants grown under mineral fertilization management were supplied with an N-P-K (5-7-14) chemical fertilizer (Tris, produced by ALFE, MN, Italy), in measure of 50 g m^{-2}. Organic crop management was obtained by adding 125 g m^{-2} of mature horse manure (Fertistall pellet, Agrilinea, FE, Italy), with the following declared features Nitrogen 2%, Organic Carbon 24%. The different doses of fertilizer were calculated to give the same amount of Nitrogen in the two treatments.

2.3 Chemical analyses

Soil and peat analysis. Prior to experimentation, after the applications of the different fertilizers, soil and peat samples were collected and analysed as reported below. Samples were air-dried and sieved (<2 mm). Measures of pH (pHmeter, Crison, Barcelona, Spain) were performed with distilled water on 1:2.5 w/v. Total organic carbon was measured by Dumas combustion with a EA 1110 CHN elemental analyser (Thermo Fisher

Scientific, Waltham, MA, USA) after dissolution of carbonates with 2 M HCl, and the organic matter was obtained using 1.72 factors. Soil particle size distribution was determined by the pipette method (Gee and Bauder 1986) and total carbonates ($CaCO_3$) were quantified by a volumetric method, according to Dietrich-Fruehing.

The metal contents was determined according to Vittori Antisari et al. (2013). Briefly, the soil (0.25 g) was treated with aqua regia (2 ml HNO_3 65% plus 6 ml suprapur HCl 37%, suprapur grade Carlo Erba, MI, Italy) in a microwave oven and the metal concentrations were determined by ICP-OES. The analysis of each sample was replicated three times and compared with analyses of the International Reference Materials (BCR 141) and laboratory internal standards (MO and ML), which was run after every 10 samples to check changes in sensitivity. Controls with only reagents were also determined.

Vegetable samples collection and analysis. At full maturity (at respectively 38, 76, 76, 69, 69, 69 and 60 days after transplanting, for basil, tomato, onion, lettuce, kale, fennel and radish), samples of the different plant organs (roots, bulbs, stems, leaves, fruits) were collected for biochemical determinations. At harvest, fresh weights were recorded and dry weights were determined after washing in tap water and drying at 58°C per 72 hours. Samples were dried in ventilated oven (T<40 °C) and ground in a blender with blades made of pure titanium, carefully avoiding to introduce any further metal contamination to the samples (Vittori Antisari et al. 2012). Briefly, approximately 0.25 g of leaf sub-sample, weighted in Teflon bombs, was dissolved in 8 mL of HNO_3 (suprapure, Merck, Kenilworth, New Jersey, USA) plus 2 mL of H_2O_2 (Carlo Erba, MI, Italy) using a microwave oven (Milestone 2100, Sorisone, BG, Italy). After cooling, solutions were made up to 20 mL with Milli-Q water and then filtered with Whatmann 42 filter paper. The major and trace elements were determined by inductive coupled plasma optical emission spectrometry (ICP-OES, Spectro Ametek, MI, Italy). The ICP-OES setting followed multi-standard solutions (CPI International, Amsterdam, The Netherland) that reproduce the matrix effect present in samples and allow the lowering of detection limits. Instrument response was assessed by measuring a standard sample (CRM 482 - Community Bureau of Reference, BCR).

Statistical analysis. The experimental data were statistically analysed using two ways analysis of variance (ANOVA) and means of field replicates (n=6) were compared using Least Significant Difference (LSD) test.

2.4 Contamination indexes

BioAccumulation Factor. For the different trace metals, the BioAccumulation Factor (BAF) was calculated by (eq. 1):

$$BAF = C_{shoot}/C_{soil} \quad \text{(eq. 1)}$$

where C_{shoot} and C_{soil} are metals concentration in the plant edible portion of the shoot (mg kg^{-1} dry weight) and soil (mg kg^{-1} dry weight), respectively (Ma et al. 2001; Cluis 2004).

Translocation Factor. Trace metals translocation from root to shoot was measured by Traslocation Factor (TF) calculated as described below (eq. 2).

$$TF = C_{shoot}/C_{root} \quad \text{(eq. 2)}$$

where C_{shoot} and C_{root} are metals concentration in the edible part of the shoot (mg kg^{-1} dry weight) and the one of the root of plant (mg kg^{-1} dry weight), respectively. Whenever C_{shoot} is equal or greater than C_{root} (TF>1), metals are effectively translocated from root to the shoot, whereas root compartmentation occurs whenever C_{root} is greater than C_{shoot} (Baker and Brooks 1989; Zhang et al. 2002; Fayiga and Ma 2006).

Daily Metal Intake and Health Risk Index. The Daily Metal Intake and Health Risk Index are indexes relating to daily estimated consumption as well as health risks from the consumption of contaminated food. The US Environmental Protection Agency's reference doses (US-EPA IRIS 2006) were used as reference points. The Daily Metal Intake (DMI) was estimated using the eq. 3:

$$DMI = C_{shoot} * 0.085 * DPC / BW \qquad \text{(eq. 3)}$$

where C_{shoot} is the concentration of metals in the edible part of the shoot of the plant (as mg kg^{-1} dry weight), multiplied by a conversion factor of 0.085 to convert dry weight vegetable metal content to fresh weight, according to Rattan et al. (2005). Daily plant consumption (DPC) and body weight (BW) were estimated based on Leclercq et al. (2009).

Based on the Daily Metal Intake, it was possible to calculate the Health Risk Index, based on eq. 4:

$$HRI = DMI/RfD \qquad \text{(eq.4)}$$

where RfD is the Reference Dose, which, for the studied elements is respectively 1.5 (Cr), 0.04 (Cu), 0.004 (Pb) and 0.02 (Ni) (mg kg^{-1} BW d^{-1}) (US-EPA IRIS 2006; Jan et al. 2010). HRI values >1 are considered to pose health risks (Cui et al. 2004; Rattan et al. 2005).

3 Results and discussion

3.1 Growing media characteristics and metal concentrations

Soil pH measured in the top 20 cm layer was significantly ($p<0.001$) higher than that of peat (**Table 1**). Fertilization management did not influence pH of soil samples, whereas pH of peat samples were statistically different in the two diverse fertilization methods with higher values on samples treated with mineral fertilizer as compared to the organic ones (**Table 1**). The importance of this parameter is related to the influence that it has on all the reactions that occur in nature. The pH values of the soil encountered in the present study were in line with the regional average values, that, in Emilia Romagna soils, present pH values between 7 and 8.5, classified as sub-alkaline soils (ARPA-RER 2004). Average $CaCO_3$ total content in soil samples was significantly ($p<0.01$) higher as compared to peat samples (+53%), which was possibly correlated with the differences in pH (Rowell 2014). On the other hand, organic matter was ten times higher in peat samples as compared to soil samples (**Table 1**). Both the values of total carbonates and organic carbon (**Table 1**) measured for soil samples were in line with the average values of the regional soils. No statistical differences in macro-element content was observed between organic and mineral samples (**Table 1**). Significantly ($p<0.001$) higher concentrations of Al, B, Ba, Fe, K, Mn were observed in soil samples; on the contrary, concentrations of Ca, Na, S, and Si were significantly higher in peat samples (**Table 1**). No significant differences in Mg, P, Sr and

Ti content were observed between soil and peat samples. Trace metal contents, however, widely varied between soil and peat samples. For every sample, the concentration of Cd was below the detection limit, and, only for soil samples Mo concentration was below the detection limit. Significant ($p<0.001$) differences between soil and peat samples were revealed for every of the other microelements (As, Be, Co, Cr, Cu, Li, Mo, Ni, Pb, Sb, Sn, V, Zn), with greater values in soil samples (**Table 1**). Comparing average soil microelements concentrations with results of other researches in both urban and rural environments, the first consideration is that trace metal concentration in soils may vary substantially according to both geogenic and anthropic elements. As compared with other studies (Luo et al. 2011; Szolnoki et al. 2013; Izquierdo et al. 2015), the soil Cr content hereby measured (91 mg kg^{-1}, **Table 1**) turned out to be higher than previous evidences in urban gardens where it ranged 12 to 31 mg kg^{-1}. Alternatively, Cu, Pb and Ni (respectively accounting for 56, 44 and 46 mg kg^{-1}, **Table 1**) were within previously observed concentrations (Kabala et al. 2009; Luo et al. 2011; Szolnoki et al. 2013; Jean-Soro et al. 2014; Izquierdo et al. 2015) in both urban and rural environments.

3.2 Trace metals content in vegetables' edible portions

Although potential health risks are generally associated with the assimilation of trace metals, there is a lack in legislation concerning threshold limits for most trace metals in marketed crops. The only available EU legislation (EU 2009) regulates upper limits of Pb and Cd in edible products. Consistently, Pb shall always be under 0.1, 0.3, and 0.2 mg kg^{-1} of fresh weight (respectively in legumes, brassica, and all other vegetables), which are about 1.2, 2.4 and 3.6 mg kg^{-1} on a dry weight basis. Furthermore, Cd threshold limits are set at 0.05, 0.1, and 0.2 mg kg^{-1} of fresh weight, respectively in vegetables whose edible part is the fruit, the stem/root, or the leaf (EU 2009). Most dangerous elements beyond Pb and Cd are Cr, Cu, As, and Ni (Vittori Antisari et al. 2015). Within the experiments hereby described, some of these elements (namely Cd and As) were not detectable in vegetable samples. On the other hand, significant accumulation levels of Cr, Cu, Pb, and Ni were found, as shown in **Fig. 2** and **3**. The recovery intervals for these elements, based on the CRM 482, were respectively 3.99±0.51 (Cr), 7.03±0.19 (Cu), 2.19±0.49 (Ni) and 37.8±1.47 (Pb). Given that no significant interaction between growing system and fertilization management on the edible product content of those trace metal was observed, the effects of these two factors will be separately described for each element.

Chromium. Cr content in edible organs of radish, fennel, lettuce and basil was significantly higher in plants cultivated on soil as compared to those grown on peat, with greatest values in radish and lettuce (respectively 85 and 29 mg kg^{-1}) (**Fig. 2a**). Contrariwise, Cr accumulation in onion bulbs was higher in plants cultivated on peat. Finally, kale leaves and tomato fruit did not present significant differences between the two growing systems (**Fig. 2a**). Based on the fertilization managements (**Fig. 3a**), Cr accumulation was higher in samples of radish, onion, lettuce and tomato supplied with organic fertilizer. Highest concentrations were observed in radish and lettuce, respectively reaching 76 and 26 mg kg^{-1}. No significant differences could be associated with fertilization management in fennel, basil or kale (**Fig. 3a**).

Copper. Cu content in edible organs of radish, onion, fennel, lettuce, basil and tomato was significantly higher in plants cultivated on soil as compared to those grown on peat, with greatest values in radish and basil (respectively 19 and 17 mg kg^{-1}) (**Fig. 2b**). No statistically significant differences could be observed in leaves of soil- and peat- grown plants of kale, which also showed the lowest concentration (5 mg kg^{-1}) among studied species grown on soil (**Fig. 2b**). Considering fertilization management, significant differences in Cu accumulation were detectable only in onion bulbs and tomato fruits, with a slightly higher (respectively +4 and +1 %) concentration in plants grown with mineral fertilizer as compared to same products supplied with organic fertilization (**Fig. 3b**). Highest concentrations were observed in radish and lettuce (14 mg kg^{-1}) (**Fig. 3b**).

Lead. Pb content in edible parts of radish, fennel, lettuce and basil was significantly higher in plants cultivated on soil than those cultivated on peat (**Fig. 2c**), with values above EU safety thresholds only observed in radish. Contrariwise, onion showed opposite results, with Pb accumulation being greater in onion cultivated on peat as compared to same plants grown on soil (**Fig. 2c**). In general, radish and lettuce had the higher accumulation level (respectively 7 and 3 mg kg^{-1}). Kale and tomato fruit did not present significant differences between the two growing systems. Fertilization management did not affect Pb content in edible tissues of the studied species, with exclusion of lettuce, where a significant 2-fold increase in Pb content was associated with organic fertilization regime (**Fig. 3c**).

Nickel. Ni content in edible organs of radish, fennel, lettuce, basil and kale was significantly higher in plants cultivated on soil as compared to those grown on peat, with greatest values in radish and lettuce (respectively 7 and 3 mg kg^{-1}) (**Fig. 2d**). Independently from the growing system, lowest accumulations were observed in onion (0.10 mg kg^{-1}), and tomato (0 mg kg^{-1}) (**Fig. 2d**). Fertilization management did not affect Ni content in edible tissues of the studied species, with exclusion of radish, where a 2.5-fold increase in Ni content was associated with mineral fertilization regime (**Fig. 3d**).

In order to understand the potential health risk of the vegetables grown in the allotment garden, a comparative analysis against values obtained in previous similar studies (Douay et al. 2013; Säumel et al. 2012; Warming et al. 2015) was performed. Accordingly, and despite the apparently high content in soils, Cr values were relatively low (with exclusion of radish that most accumulated Cr, with values of 85 mg kg^{-1}, **Fig. 2**). On the other hand, Cu and Ni values were generally higher than previous experiences and Pb values varied dramatically across species (with greatest values again on radish, about 7 mg kg^{-1}, **Fig. 2**). A previous study (Säumel et al. 2012) addressed the quantification of trace metal contaminants in allotment gardens and food purchased at local markets in the city of Berlin, showing lower values of Cu and Ni as compared to the hereby presented results. This suggests that crops able to better exclude or compartmentalize those two elements should be preferred at the experimental site.

When mean values of all studied vegetable species were compared, the adoption of simplified soilless systems filled with non-contaminated growing substrate enabled to reduce trace metal contamination risk associated with Cr (-70%, 6 vs 20 mg kg^{-1}, in peat vs soil, respectively), Cu (-61%, 7 vs 13 mg kg^{-1}), Pb (-45%, 0.8 vs 2.0 mg kg^{-1}) and Ni (-81%, 0.3 vs 2 mg kg^{-1}). Apparently, the greater values of contaminants associated with on-soil cultivation should be related to the growing media content (**Table 1**). Furthermore, the observed differences found in the pH values of soil and peat (**Table 1**) may have influenced the toxic mineral uptake, given that substrate pH affect differently the solubility and availability of the elements, as commonly known (e.g. Kumpiene et al. 2008; Zeng et al. 2011).

Organic fertilization strategies did not affect substantially biochemical features and trace metal concentrations of the growing media, as illustrated in **Table 1**. In the present study, however, some crop-specific differences in trace metal accumulation in edible tissue were evidenced, with a general raise in Cr in radish and in Cr and Pb in lettuce grown under organic fertilization. On the other hand, mineral fertilization resulted in greater Ni in radish and Cu in onion and tomato. Comparative studies of organic versus mineral fertilization have rarely addressed the effect on trace metal accumulation (Chu and Wong 1987; Singh et al. 2010; Zaccone et al. 2010; Christensen and Elsgaard 2014), often with controversial results (Liñero et al. 2015). Consistently, a priority of the research is to address the understanding of how organic matter may affect (e.g. by chelating minerals) plant uptake of contaminants.

3.3 Trace metal contamination indexes

In order to identify the crop capacity to exclude dangerous trace metals, the BioAccumulation Factor was used to understand which species were most resilient to ion distribution in the growing media (Rezvani and Zaefarian 2011). Consistently, crops may be classified as hyperaccumulators, accumulators and excluders, based on the value of their BioAccumulation Factor being above, equal or below 1 unit. Accordingly, all studied species acted as excluders, although to a different extent (**Table 2**). While highest values were associated with radish roots, the lowest ones were observed for Cr and Ni in edible organs of tomato, confirming the plant capability to avoid toxic elements loading into fruits (Ali and Al-Qahtani 2012). Once a toxic element enters in plant by the root, it can either be translocated to shoots or compartmentalized within roots. An indicator of the plant ability to avoid translocation of such elements into shoot is provided by the Translocation Factor (Rezvani and Zaefarian 2011). The higher its value, the greater the rate of element that is transferred to shoots. Among studied crops, three species (kale, sweet basil and tomato) representing three different plant families (respectively Brassicaceae, Lamiaceae and Solanaceae) were studied for their trace metal compartmentation in different plant organs (**Table 2**). For the sake of this publication, Translocation Factor was calculated by relating the element concentration in the edible plant organ (leaf or fruit) to the concentration of the same element in roots. Accordingly, no differences were observed on Cr, whereas greatest values of Translocation Factor for Cu were observed in sweet basil and for Pb and Ni in kale, confirming the hypothesis that lowest translocation occurs to fruits as compared to leaves in plants (Tiwari et al. 2011). Consistently, a study over 15 plant native species grown on trace metal contaminated sites of Florida, showed that both Pb and Cu absorbed at root level were only partially translocated to shoots (Yoon et al. 2006).

In order to better address the comprehension of the potential health risks associated with the consumption of allotment garden vegetables, the application of Daily Metal Intake and Health Risk Index was performed on the vegetables grown on the garden soil. Accordingly, values of Daily Metal Intake were extremely low (ranging 0 to 20 μg kg^{-1} Body Weight d^{-1}, data not shown) and this resulted in limited Health Risk Index values. Health Risk Index was greatest in radish for Cr, Cu and Pb (**Table 2**), reaching respectively 0.015, 0.13 and 0.45 in adults (70 kg body weight and 0.22 kg d^{-1} Daily Plant Consumption, Leclercq et al. 2009) and 0.032, 0.270 and 0.951 in children (26 kg Body Weight and 0.13 kg d^{-1} Daily Plant Consumption, Leclercq et al. 2009) (**Table 2**). The same index was not affected by the crop species in neither adults (0.026) nor children (0.055) when Ni was considered (**Table 2**). Health Risk Index estimated on both adults and children resulted to be below the toxicity level of 1 (US-EPA IRIS 2006), although index value associated with Pb was greatest in radish, confirming its associated health risks when grown in contaminated environments.

4 Conclusions

The experimental garden presented moderately high levels of Cr, whereas Cu, Pb and Ni were within ranges observed in both urban and rural vegetable gardens assessed in previous studies. Indeed, while Cr content in edible organs were lower than values observed in previous studies, Ni and Cu were higher than previous evidences. Furthermore, Pb concentration in edible radish roots resulted to be above risk thresholds indicated by the EU. Nonetheless, for all studied trace metals, Daily Metal Intake and Health Risk Index of plants grown on soil were below the threshold value for health risks. Among studied plants, radish most accumulated toxic elements, which resulted in significantly greater risk from all other studied crops, reaching values close to toxicity for Pb when Health Risk Index was associated with children consumption. All studied crops presented BioAccumulation Factor values always below 1, although with highest values for Cr, Cu, Pb and Ni in radish. Translocation Factor of Cu from root to shoot was greater in leaves of sweet basil, while of Pb and Ni in kale, as compared to the translocation to fruits in tomato. Plants grown on peat in a simplified soilless systems showed reduced content of Cr, Cu, Pb and Ni as compared to those grown on soil, due to differences in the growing media in terms of concentration of the element and biochemical properties (pH and $CaCO_3$). Organic fertilization resulted in increased Cr and Pb and reduce Cu and Ni in the edible organs of some of the studied crops. According to the results of the present study, the adoption of soilless growing systems where plants are grown on peat may be a feasible strategy to cope with trace metal contamination risk in urban allotment garden. The absence of clear differences in trace metal accumulation as affected by either mineral or organic fertilizers, on the other hand, suggests that deeper studies should be conducted in order to define proper fertilization strategies in potentially polluted environments.

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Tables

Table 1. Main soil features (pH, $CaCO_3$, Organic matter and Organic C) and concentrations of macro-elements (expressed as g kg^{-1} dry weight) and micro-elements (expressed as mg kg^{-1} dry weight) in soil and peat samples added with organic fertilizer or mineral fertilizer. Different letters indicate significant differences within row at P≤0.05; ns indicates non significant differences at P≤0.05 (n=6).

		Peat				**Soil**			
		Organic		Mineral		Organic		Mineral	
pH (H_2O)	-	6.72	c	6.88	b	8.11	a	7.97	a
$CaCO_3$	g kg^{-1}	35.15	b	39.95	b	59.9	a	55.05	a
Organic Matter	%	55.72	a	56.35	a	4.89	b	4.77	b
Organic C	%	22.27	a	22.67	a	1.96	b	1.90	b
Al	g kg^{-1}	8.27	b	6.89	b	32.78	a	31.09	a
B	g kg^{-1}	0.02	b	0.02	b	0.03	a	0.03	a
Ba	g kg^{-1}	0.07	b	0.07	b	0.20	a	0.19	a
Ca	g kg^{-1}	29.34	a	27.42	a	22.76	b	23.29	b
Fe	g kg^{-1}	8.28	b	7.27	b	24.47	a	24.32	a
K	g kg^{-1}	6.04	b	5.02	b	7.55	a	7.01	a
Mg	g kg^{-1}	7.52	ns	6.70	ns	7.01	ns	7.04	ns
Mn	g kg^{-1}	0.25	b	0.21	b	0.77	a	0.76	a
Na	g kg^{-1}	1.87	a	1.80	a	0.81	b	0.72	b
P	g kg^{-1}	1.63	ns	1.59	ns	1.77	ns	1.73	ns
S	g kg^{-1}	1.14	a	1.21	a	0.17	b	0.19	b
Si	g kg^{-1}	0.28	a	0.26	a	0.08	b	0.14	b
Sr	g kg^{-1}	0.09	ns	0.10	ns	0.11	ns	0.11	ns
Ti	g kg^{-1}	0.57	ns	0.46	ns	0.63	ns	0.56	ns
As	mg kg^{-1}	3.63	b	3.56	b	6.81	a	7.23	a
Be	mg kg^{-1}	0.36	b	0.30	b	1.43	a	1.39	a
Cd	mg kg^{-1}	<DL		< DL		<DL		< DL	
Co	mg kg^{-1}	4.71	b	4.15	b	13.13	a	12.78	a
Cr	mg kg^{-1}	38.70	b	41.44	b	93.50	a	87.98	a
Cu	mg kg^{-1}	37.98	b	34.56	b	56.46	a	56.13	a
Li	mg kg^{-1}	12.08	b	10.52	b	45.08	a	44.15	a
Mo	mg kg^{-1}	4.27	a	4.01	a	< DL		< DL	
Ni	mg kg^{-1}	17.82	b	15.31	b	46.03	a	45.54	a
Pb	mg kg^{-1}	18.96	b	15.15	b	48.66	a	40.12	a
Sb	mg kg^{-1}	1.30	b	1.30	b	1.53	a	1.43	a
Sn	mg kg^{-1}	1.44	b	1.41	b	4.30	a	3.75	a
V	mg kg^{-1}	19.44	b	16.32	b	65.61	a	63.46	a
Zn	mg kg^{-1}	74.13	b	71.01	b	106.3	a	108.8	a

Table 2. Contamination indexes of soil-grown vegetables at the experimental site. BioAccumulation Factor, Translocation Factor, and Health Risk Index for adults (70 kg body weight and 0.22 kg d^{-1} daily plant consumption) and children (26 kg body weight and 0.13 kg d^{-1} daily plant consumption). Different letters indicate significant differences at P≥0.05 (n=6).

Element	**Species**	**BioAccumulation Factor**		**Translocation Factor**		**Health Risk Index** adult		**Health Risk Index** children	
Cr	Fennel	0.100	c	-		0.003	e	0.006	e
	Kale	0.060	e	0.260	a	0.001	c	0.002	c
	Lettuce	0.210	b	-		0.005	b	0.011	b
	Onion	0.060	f	-		0.0001	g	0.000	g
	Radish	0.780	a	-		0.015	a	0.032	a
	Basil	0.080	d	0.260	a	0.002	d	0.004	d
	Tomato	0.030	g	0.440	a	0.0002	f	0.001	f
Cu	Fennel	0.220	d	-		0.105	g	0.219	g
	Kale	0.120	g	0.330	c	0.037	c	0.078	c
	Lettuce	0.270	b	-		0.099	d	0.206	d
	Onion	0.140	f	-		0.064	f	0.133	f
	Radish	0.270	a	-		0.130	a	0.270	a
	Basil	0.260	c	1.170	a	0.114	b	0.236	b
	Tomato	0.220	e	0.520	b	0.076	e	0.159	e
Pb	Fennel	0.020	d	-		0.084	e	0.174	e
	Kale	0.020	e	0.780	a	0.049	d	0.102	d
	Lettuce	0.050	b	-		0.182	b	0.379	b
	Onion	0.020	g	-		0.019	g	0.041	g
	Radish	0.110	a	-		0.457	a	0.951	a
	Basil	0.040	c	0.750	a	0.121	f	0.252	f
	Tomato	0.020	f	0.390	b	0.038	e	0.079	e
Ni	Fennel	0.030	d	-		0.023	a	0.048	a
	Kale	0.030	c	0.660	a	0.019	a	0.040	a
	Lettuce	0.050	b	-		0.038	a	0.080	a
	Onion	0.003	f	-		0.002	a	0.004	a
	Radish	0.080	a	-		0.089	a	0.186	a
	Basil	0.010	e	0.220	b	0.014	a	0.030	a
	Tomato	0.000	g	0.000	c	0.000	a	0.000	a

Figures

Figure 1. Location of the experimental garden (X) in respect to main pollution sources, namely the city incinerator (I), Via San Donato (R), A14 motorway (M) and main railway (T).

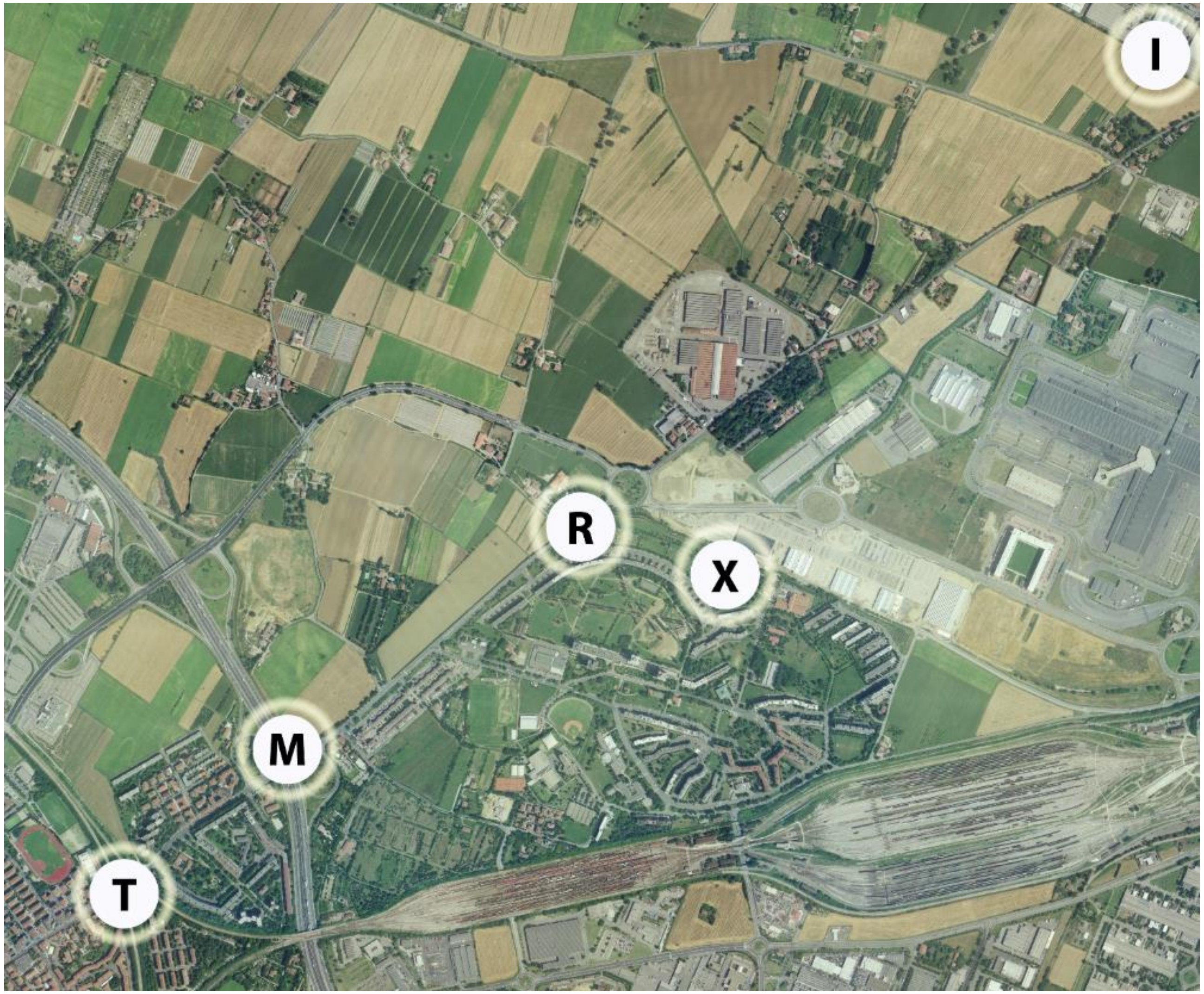

Figure 2. Accumulation of Cr (a), Cu (b), Pb (c) and Ni (d) (expressed in mg kg^{-1} dry weight) in edible portions of radish, onion, fennel, lettuce, basil, kale and tomato according to growing system (soilless on peat in red or traditional on soil in black). Vertical bars represent standard deviation. Symbols (*) indicate significant differences at P≤0.05 (*), 0.01 (**) or 0.001 (***), whereas 'ns' indicates non significant differences at P≤0.05 (n=6).

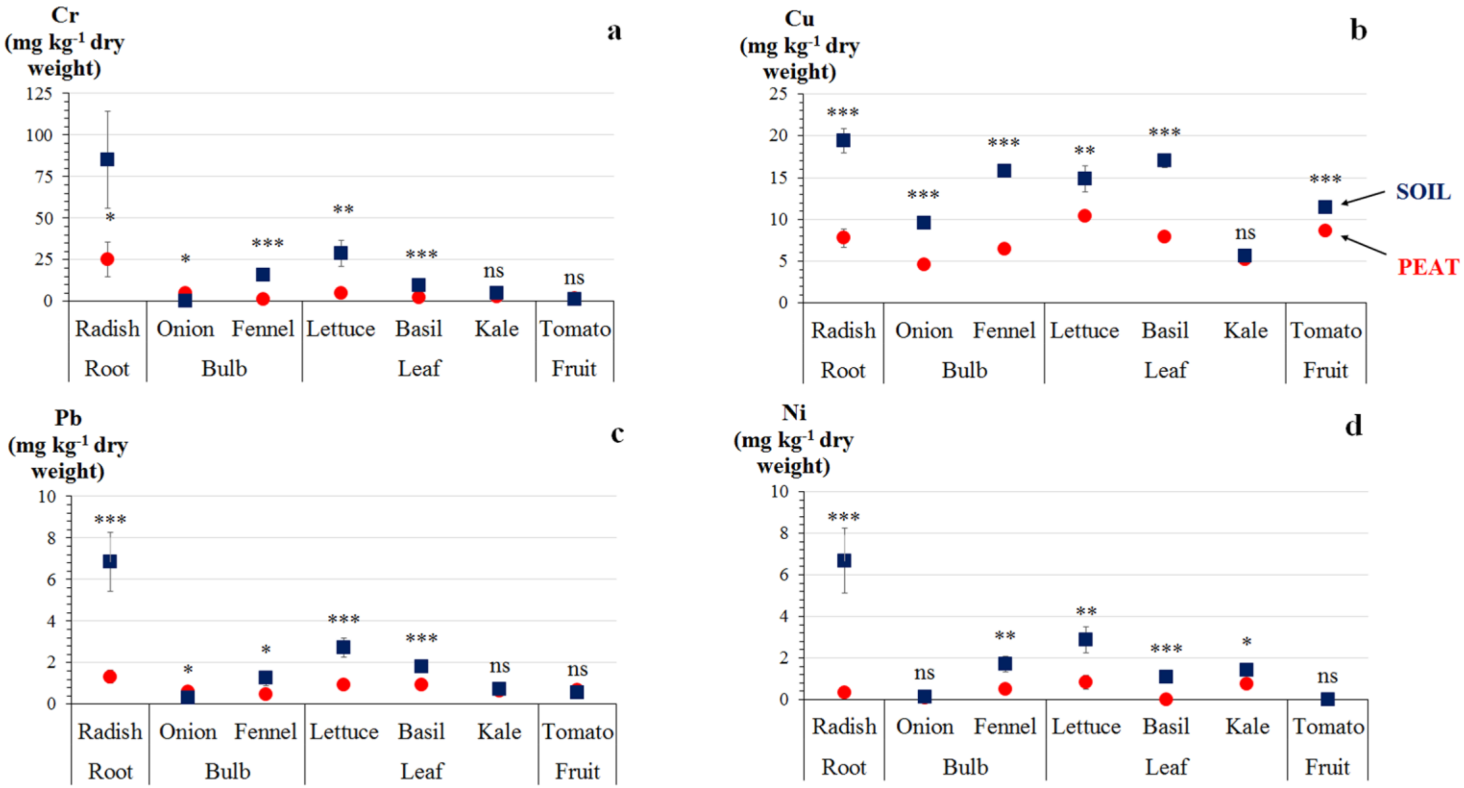

Figure 3. Accumulation of Cr (a), Cu (b), Pb (c) and Ni (d) (expressed in mg kg^{-1} dry weight) in edible portions of radish, onion, fennel, lettuce, basil, kale and tomato according to fertilization management (organic in red and mineral in black). Vertical bars represent standard deviation. Symbols (*) indicate significant differences at P≤0.05 (*), 0.01 (**) or 0.001 (***), whereas 'ns' indicates non significant differences at P≤0.05 (n=6).