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

Authors:

**GIUSEPPINA PENNISI**

**FRANCESCO ORSINI \***

**DANIELA GASPERI**

**SILVIA MANCARELLA**

**RABAB SANOUBAR**

**LIVIA VITTORI ANTISARI**

**GILMO VIANELLO**

**GIORGIO GIANQUINTO**

Research Centre for the Urban Environment on Agriculture and Biodiversity, Department of Agricultural  
Sciences, University of Bologna, Bologna, Italy

\*Corresponding author:

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## 26 Abstract

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

41

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

43

## 44 1 Introduction

45

46 Urban agriculture activities are commonly found in Europe and all over the world. These include the  
47 production of crop and livestock goods within cities and towns ([Zeza and Tasciotti 2010](#)), but also peri-urban  
48 agricultural areas which may provide product to the local population, such as vegetables, medicinal plants,  
49 fruit trees, ornamental plants, milk, meat and wool ([Lin et al. 2015](#)). Urban allotment gardens are one element  
50 of the urban green infrastructure that is becoming increasingly important in urban landscape planning. As  
51 reported in [Breuste and Artmann \(2014\)](#), they combine utility, social meaning, beauty, and several ecosystem  
52 services such as food supply ([Drescher 2004](#)), air filtering ([Davies et al. 2011](#)), urban temperature and climate  
53 regulation ([Phelan et al. 2015](#)), noise reduction ([Aylor 1972](#)), runoff mitigation ([Zhang et al. 2012](#)), and  
54 biodiversity development ([Lin et al. 2015](#)). However, plant cultivation within cities may present environmental  
55 risks associated to both air and soil pollution ([Alloway 2004](#)). In urban areas, air pollutants generally derive  
56 from artificial sources e.g. vehicular emissions and fossil fuel burning ([Agrawal et al. 2003](#)). Urban air  
57 pollution has increased rapidly in the past decades with fast industrialization, rapid growth of urban population,  
58 increase in vehicular traffic, badly maintained roads and human activities. Consequently, agricultural land  
59 adjacent to urban areas may be exposed to air pollutants of urban origin, which may result in contamination of  
60 horticultural products beyond precautionary values. When this happens, a dietary exposure to trace metals can  
61 result in significant human health risk ([Massaquoi et al. 2015](#)). An interesting study ([Säumel et al. 2012](#)),  
62 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<sup>2</sup>) is part of a large area of gardens (so-called Orti Salgari), built in the eighties, which occupies a surface of 27,000 m<sup>2</sup> 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<sup>3</sup>–10<sup>4</sup> vehicles d<sup>-1</sup>), the main motorway (A14, 1,200 m from the garden, 10<sup>5</sup> vehicles d<sup>-1</sup>), the railway (1,600 m from the garden, 700 trains d<sup>-1</sup>) 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<sup>-1</sup> 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.

137 *Acephala* or tuscan kale or black cabbage), Florence/Bulb fennel (*Foeniculum vulgare*, var. Azoricum cv.  
138 Carmo F1), and radish (*Raphanus sativus*, cv. Cherry Bell). Plants density was 5 plants m<sup>-2</sup> for kale and tomato,  
139 10 plants m<sup>-2</sup> for fennel, lettuce and basil, 25 plants m<sup>-2</sup> for radish, 35 plants m<sup>-2</sup> for onion.  
140 Treatments included growing media (soil vs peat) and fertilization management (mineral vs organic).  
141 Experimental design was a strip plot with 2 replicates with growing media on the main plot and fertilization  
142 management in the elemental plot. Each elemental plot hosted at least 6 plants.

## 144 2.2 Growing conditions

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

156 Plants were grown either on the allotment soil or onto elevated beds filled with commercial soil (Geotec, RO,  
157 Italy). Growing containers were built according to [Orsini et al. \(2014\)](#), using recycled pallets and black plastic  
158 films. Declared physical and biochemical features of commercial soil were Organic Carbon 25%, Organic  
159 Nitrogen 1%, peat 60%, salinity 0.6 dS m<sup>-1</sup>.

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

## 168 2.3 Chemical analyses

170 *Soil and peat analysis.* Prior to experimentation, after the applications of the different fertilizers, soil and peat  
171 samples were collected and analysed as reported below. Samples were air-dried and sieved (<2 mm). Measures  
172 of pH (pHmeter, Crison, Barcelona, Spain) were performed with distilled water on 1:2.5 w/v. Total organic  
173 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<sub>3</sub>) 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<sub>3</sub> 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<sub>3</sub> (suprapure, Merck, Kenilworth, New Jersey, USA) plus 2 mL of H<sub>2</sub>O<sub>2</sub> (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 Netherlands) 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):

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

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

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

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



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

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

$$219 \quad \text{DMI} = C_{\text{shoot}} * 0.085 * \text{DPC} / \text{BW} \quad (\text{eq. 3})$$

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

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

$$225 \quad \text{HRI} = \text{DMI} / \text{RfD} \quad (\text{eq.4})$$

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

## 230 **3 Results and discussion**

### 232 **3.1 Growing media characteristics and metal concentrations**

234 Soil pH measured in the top 20 cm layer was significantly ( $p < 0.001$ ) higher than that of peat (Table 1).  
235 Fertilization management did not influence pH of soil samples, whereas pH of peat samples were statistically  
236 different in the two diverse fertilization methods with higher values on samples treated with mineral fertilizer  
237 as compared to the organic ones (Table 1). The importance of this parameter is related to the influence that it  
238 has on all the reactions that occur in nature. The pH values of the soil encountered in the present study were in  
239 line with the regional average values, that, in Emilia Romagna soils, present pH values between 7 and 8.5,  
240 classified as sub-alkaline soils (ARPA-RER 2004). Average  $\text{CaCO}_3$  total content in soil samples was  
241 significantly ( $p < 0.01$ ) higher as compared to peat samples (+53%), which was possibly correlated with the  
242 differences in pH (Rowell 2014). On the other hand, organic matter was ten times higher in peat samples as  
243 compared to soil samples (Table 1). Both the values of total carbonates and organic carbon (Table 1) measured  
244 for soil samples were in line with the average values of the regional soils. No statistical differences in macro-  
245 element content was observed between organic and mineral samples (Table 1). Significantly ( $p < 0.001$ ) higher  
246 concentrations of Al, B, Ba, Fe, K, Mn were observed in soil samples; on the contrary, concentrations of Ca,  
247 Na, S, and Si were significantly higher in peat samples (Table 1). No significant differences in Mg, P, Sr and



248 Ti content were observed between soil and peat samples. Trace metal contents, however, widely varied  
249 between soil and peat samples. For every sample, the concentration of Cd was below the detection limit, and,  
250 only for soil samples Mo concentration was below the detection limit. Significant ( $p<0.001$ ) differences  
251 between soil and peat samples were revealed for every of the other microelements (As, Be, Co, Cr, Cu, Li,  
252 Mo, Ni, Pb, Sb, Sn, V, Zn), with greater values in soil samples (**Table 1**). Comparing average soil  
253 microelements concentrations with results of other researches in both urban and rural environments, the first  
254 consideration is that trace metal concentration in soils may vary substantially according to both geogenic and  
255 anthropic elements. As compared with other studies (Luo et al. 2011; Szolnoki et al. 2013; Izquierdo et al.  
256 2015), the soil Cr content hereby measured ( $91 \text{ mg kg}^{-1}$ , **Table 1**) turned out to be higher than previous  
257 evidences in urban gardens where it ranged 12 to  $31 \text{ mg kg}^{-1}$ . Alternatively, Cu, Pb and Ni (respectively  
258 accounting for 56, 44 and  $46 \text{ mg kg}^{-1}$ , **Table 1**) were within previously observed concentrations (Kabala et al.  
259 2009; Luo et al. 2011; Szolnoki et al. 2013; Jean-Soro et al. 2014; Izquierdo et al. 2015) in both urban and  
260 rural environments.

261

### 262 3.2 Trace metals content in vegetables' edible portions

263

264 Although potential health risks are generally associated with the assimilation of trace metals, there is a lack in  
265 legislation concerning threshold limits for most trace metals in marketed crops. The only available EU  
266 legislation (EU 2009) regulates upper limits of Pb and Cd in edible products. Consistently, Pb shall always be  
267 under 0.1, 0.3, and  $0.2 \text{ mg kg}^{-1}$  of fresh weight (respectively in legumes, brassica, and all other vegetables),  
268 which are about 1.2, 2.4 and  $3.6 \text{ mg kg}^{-1}$  on a dry weight basis. Furthermore, Cd threshold limits are set at  
269 0.05, 0.1, and  $0.2 \text{ mg kg}^{-1}$  of fresh weight, respectively in vegetables whose edible part is the fruit, the  
270 stem/root, or the leaf (EU 2009). Most dangerous elements beyond Pb and Cd are Cr, Cu, As, and Ni (Vittori  
271 Antisari et al. 2015). Within the experiments hereby described, some of these elements (namely Cd and As)  
272 were not detectable in vegetable samples. On the other hand, significant accumulation levels of Cr, Cu, Pb,  
273 and Ni were found, as shown in **Fig. 2** and **3**. The recovery intervals for these elements, based on the CRM  
274 482, were respectively  $3.99\pm0.51$  (Cr),  $7.03\pm0.19$  (Cu),  $2.19\pm0.49$  (Ni) and  $37.8\pm1.47$  (Pb). Given that no  
275 significant interaction between growing system and fertilization management on the edible product content of  
276 those trace metal was observed, the effects of these two factors will be separately described for each element.  
277 *Chromium*. Cr content in edible organs of radish, fennel, lettuce and basil was significantly higher in plants  
278 cultivated on soil as compared to those grown on peat, with greatest values in radish and lettuce (respectively  
279  $85$  and  $29 \text{ mg kg}^{-1}$ ) (**Fig. 2a**). Contrariwise, Cr accumulation in onion bulbs was higher in plants cultivated on  
280 peat. Finally, kale leaves and tomato fruit did not present significant differences between the two growing  
281 systems (**Fig. 2a**). Based on the fertilization managements (**Fig. 3a**), Cr accumulation was higher in samples  
282 of radish, onion, lettuce and tomato supplied with organic fertilizer. Highest concentrations were observed in  
283 radish and lettuce, respectively reaching 76 and  $26 \text{ mg kg}^{-1}$ . No significant differences could be associated  
284 with fertilization management in fennel, basil or kale (**Fig. 3a**).

285 *Copper*. Cu content in edible organs of radish, onion, fennel, lettuce, basil and tomato was significantly higher  
 286 in plants cultivated on soil as compared to those grown on peat, with greatest values in radish and basil  
 287 (respectively 19 and 17 mg kg<sup>-1</sup>) (**Fig. 2b**). No statistically significant differences could be observed in leaves  
 288 of soil- and peat- grown plants of kale, which also showed the lowest concentration (5 mg kg<sup>-1</sup>) among studied  
 289 species grown on soil (**Fig. 2b**). Considering fertilization management, significant differences in Cu  
 290 accumulation were detectable only in onion bulbs and tomato fruits, with a slightly higher (respectively +4  
 291 and +1 %) concentration in plants grown with mineral fertilizer as compared to same products supplied with  
 292 organic fertilization (**Fig. 3b**). Highest concentrations were observed in radish and lettuce (14 mg kg<sup>-1</sup>) (**Fig.**  
 293 **3b**).

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

302 *Nickel*. Ni content in edible organs of radish, fennel, lettuce, basil and kale was significantly higher in plants  
 303 cultivated on soil as compared to those grown on peat, with greatest values in radish and lettuce (respectively  
 304 7 and 3 mg kg<sup>-1</sup>) (**Fig. 2d**). Independently from the growing system, lowest accumulations were observed in  
 305 onion (0.10 mg kg<sup>-1</sup>), and tomato (0 mg kg<sup>-1</sup>) (**Fig. 2d**). Fertilization management did not affect Ni content in  
 306 edible tissues of the studied species, with exclusion of radish, where a 2.5-fold increase in Ni content was  
 307 associated with mineral fertilization regime (**Fig. 3d**).

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

318 When mean values of all studied vegetable species were compared, the adoption of simplified soilless systems  
 319 filled with non-contaminated growing substrate enabled to reduce trace metal contamination risk associated  
 320 with Cr (-70%, 6 vs 20 mg kg<sup>-1</sup>, in peat vs soil, respectively), Cu (-61%, 7 vs 13 mg kg<sup>-1</sup>), Pb (-45%, 0.8 vs 2.0  
 321 mg kg<sup>-1</sup>) and Ni (-81%, 0.3 vs 2 mg kg<sup>-1</sup>). 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.

335

### 3.3 Trace metal contamination indexes

337

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  $\mu\text{g kg}^{-1}$  Body Weight  $\text{d}^{-1}$ , 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  $\text{kg d}^{-1}$  Daily Plant Consumption, [Leclercq et al. 2009](#)) and 0.032, 0.270 and 0.951 in children (26 kg Body Weight and 0.13  $\text{kg d}^{-1}$  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  $\text{CaCO}_3$ ). 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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534 **Tables**

535 **Table 1.** Main soil features (pH, CaCO<sub>3</sub>, Organic matter and Organic C) and concentrations of macro-  
 536 elements (expressed as g kg<sup>-1</sup> dry weight) and micro-elements (expressed as mg kg<sup>-1</sup> dry weight) in soil and  
 537 peat samples added with organic fertilizer or mineral fertilizer. Different letters indicate significant  
 538 differences within row at P≤0.05; ns indicates non significant differences at P≤0.05 (n=6).

539

		Peat				Soil			
		Organic		Mineral		Organic		Mineral	
<b>pH (H<sub>2</sub>O)</b>	-	6.72	c	6.88	b	8.11	a	7.97	a
<b>CaCO<sub>3</sub></b>	g kg <sup>-1</sup>	35.15	b	39.95	b	59.9	a	55.05	a
<b>Organic Matter</b>	%	55.72	a	56.35	a	4.89	b	4.77	b
<b>Organic C</b>	%	22.27	a	22.67	a	1.96	b	1.90	b
<b>Al</b>	g kg <sup>-1</sup>	8.27	b	6.89	b	32.78	a	31.09	a
<b>B</b>	g kg <sup>-1</sup>	0.02	b	0.02	b	0.03	a	0.03	a
<b>Ba</b>	g kg <sup>-1</sup>	0.07	b	0.07	b	0.20	a	0.19	a
<b>Ca</b>	g kg <sup>-1</sup>	29.34	a	27.42	a	22.76	b	23.29	b
<b>Fe</b>	g kg <sup>-1</sup>	8.28	b	7.27	b	24.47	a	24.32	a
<b>K</b>	g kg <sup>-1</sup>	6.04	b	5.02	b	7.55	a	7.01	a
<b>Mg</b>	g kg <sup>-1</sup>	7.52	ns	6.70	ns	7.01	ns	7.04	ns
<b>Mn</b>	g kg <sup>-1</sup>	0.25	b	0.21	b	0.77	a	0.76	a
<b>Na</b>	g kg <sup>-1</sup>	1.87	a	1.80	a	0.81	b	0.72	b
<b>P</b>	g kg <sup>-1</sup>	1.63	ns	1.59	ns	1.77	ns	1.73	ns
<b>S</b>	g kg <sup>-1</sup>	1.14	a	1.21	a	0.17	b	0.19	b
<b>Si</b>	g kg <sup>-1</sup>	0.28	a	0.26	a	0.08	b	0.14	b
<b>Sr</b>	g kg <sup>-1</sup>	0.09	ns	0.10	ns	0.11	ns	0.11	ns
<b>Ti</b>	g kg <sup>-1</sup>	0.57	ns	0.46	ns	0.63	ns	0.56	ns
<b>As</b>	mg kg <sup>-1</sup>	3.63	b	3.56	b	6.81	a	7.23	a
<b>Be</b>	mg kg <sup>-1</sup>	0.36	b	0.30	b	1.43	a	1.39	a
<b>Cd</b>	mg kg <sup>-1</sup>	<DL		< DL		<DL		< DL	
<b>Co</b>	mg kg <sup>-1</sup>	4.71	b	4.15	b	13.13	a	12.78	a
<b>Cr</b>	mg kg <sup>-1</sup>	38.70	b	41.44	b	93.50	a	87.98	a
<b>Cu</b>	mg kg <sup>-1</sup>	37.98	b	34.56	b	56.46	a	56.13	a
<b>Li</b>	mg kg <sup>-1</sup>	12.08	b	10.52	b	45.08	a	44.15	a
<b>Mo</b>	mg kg <sup>-1</sup>	4.27	a	4.01	a	< DL		< DL	
<b>Ni</b>	mg kg <sup>-1</sup>	17.82	b	15.31	b	46.03	a	45.54	a
<b>Pb</b>	mg kg <sup>-1</sup>	18.96	b	15.15	b	48.66	a	40.12	a
<b>Sb</b>	mg kg <sup>-1</sup>	1.30	b	1.30	b	1.53	a	1.43	a
<b>Sn</b>	mg kg <sup>-1</sup>	1.44	b	1.41	b	4.30	a	3.75	a
<b>V</b>	mg kg <sup>-1</sup>	19.44	b	16.32	b	65.61	a	63.46	a
<b>Zn</b>	mg kg <sup>-1</sup>	74.13	b	71.01	b	106.3	a	108.8	a

540

541

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

Element	Species	BioAccumulation Factor		Translocation Factor		Health Risk Index		Health Risk Index	
						adult		children	
<b>Cr</b>	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
<b>Cu</b>	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
<b>Pb</b>	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
<b>Ni</b>	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

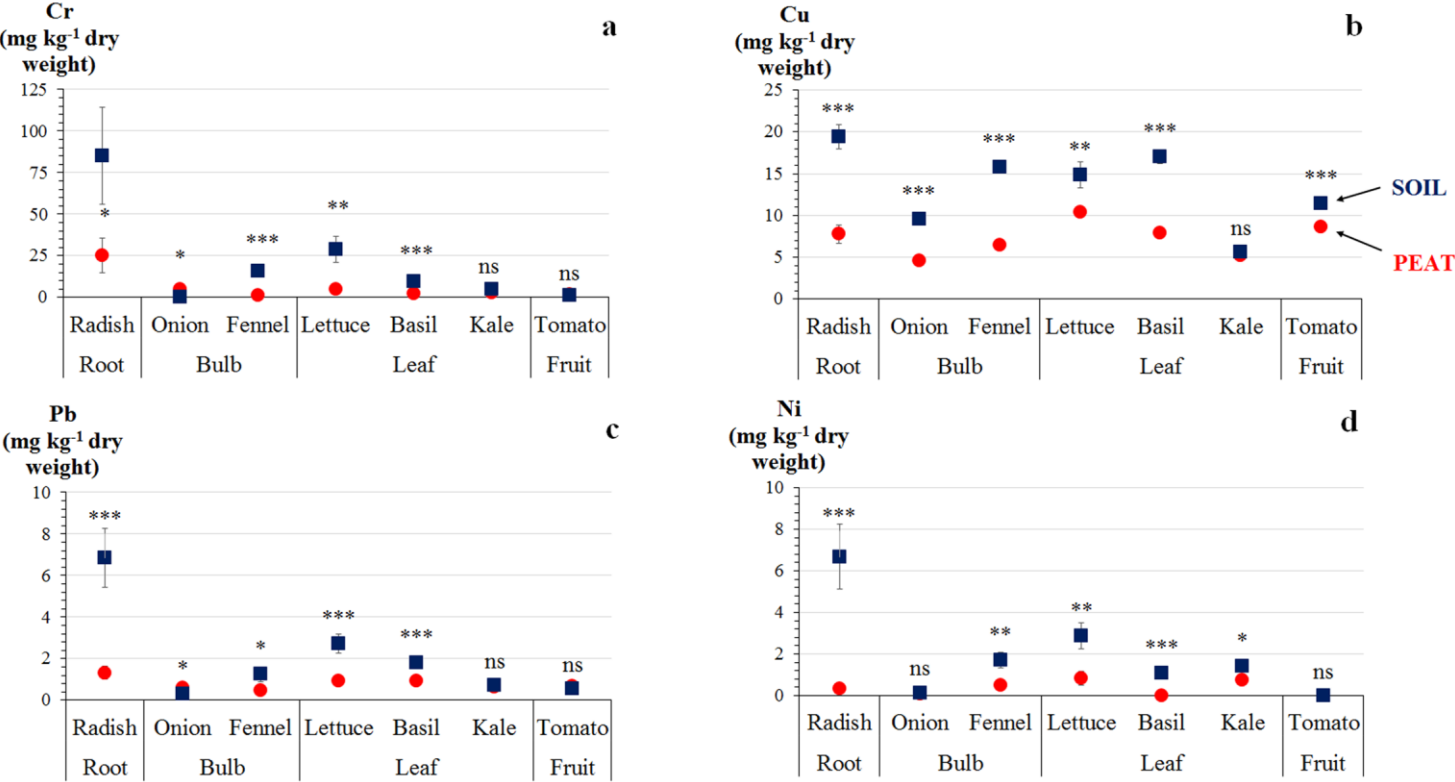
547 **Figures**

548 **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)  
549 and main railway (T).



550

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



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

