

1 **Heavy metal accumulation in vegetables grown in urban gardens**

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23 **Abstract**

24 **Urban agriculture is increasingly popular for social and economical benefits. However, edible**  
25 **crops grown in cities can be contaminated by airborne pollutants, those leading to serious**  
26 **health risks. Therefore we need a better understanding of contamination risks of urban**  
27 **cultivation to define safe practices. Here we study heavy metal risk in horticultural crops**  
28 **grown in urban gardens of Bologna, Italy. We investigated the effect of proximity to different**  
29 **pollution sources such as roads and railways, and the effect of the growing system used, that is**  
30 **soil versus soilless cultivation. We compared heavy metals concentration in urban and rural**  
31 **crops. We focussed on surface deposition and tissue accumulation of pollutants during three**  
32 **years. Results show that in the city crops near the road were polluted by heavy metals, with**  
33 **up to 160 mg per Kg dry weight for lettuce and 210 mg/Kg for basil. The highest Cd**  
34 **accumulation of up to 1.2 mg/Kg was found in rural tomato. Soilless planting systems enabled**  
35 **a reduction of heavy metal accumulation in plant tissue, of up to -71% for rosemary leaves.**

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37 **Keywords: horticultural crops, urban and rural gardens, heavy metals, leaf leaching test,**  
38 **soilless**

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## 41 **1 Introduction**

42

43 Urban horticulture is spreading and becoming an essential feature of city planning in most cities of  
44 the world. Born as a complementary food-providing initiative, urban horticulture is now gaining  
45 value for many other essential roles it plays in the urban context (Ghosh et al. 2008; van  
46 Veenhuizen 2006). It provides ecosystem services, contributing to increase urban life quality  
47 through mitigation of the city climate, preservation and enhancement of biodiversity, reuse of urban  
48 wastes and contribution to the aesthetic satisfaction given by a greener urban environment (La  
49 Greca et al. 2011). Besides, urban horticulture has a wide field of social implications, for instance in  
50 the rehabilitation of people with addictions of various nature (alcohol, drugs), or for supporting and  
51 helping the elderly or the physically and mentally disabled (Muganu et al. 2010). Overall, its  
52 multifunctional role is recognized (Orsini et al. 2013), ranging from its contribution to food  
53 security, economic and environmental sustainability, preservation and implementation of the green  
54 space (Zasada 2011). However, growing food in the urban environment relies on different  
55 conditions as compared to traditional farming. In cities, horticultural gardens are distributed  
56 according to the available space (generally on marginal areas, e.g. close to railways, main roads, or  
57 nearby industrial areas) (Alloway 2004), rather than following rational and agronomical  
58 considerations (e.g. potential pollution, access to light, proximity to residential neighbourhoods).

59 It is recognized that the risk of contaminants accumulating in air, soil and water can influence the  
60 product quality and healthiness (Al Jassir et al. 2005; Leake et al. 2009). Given the health risk  
61 associated with their consumption, the European Union has defined maximum levels of lead and  
62 cadmium to be found in vegetables. Consistently, lead concentration should always be under 0.10,  
63 0.30 and 0.20 mg kg<sup>-1</sup> of fresh weight respectively in legumes, brassica and all other vegetables.  
64 Cadmium threshold limits expressed by European Union regulation are set at 0.05, 0.1 and 0.2 mg  
65 kg<sup>-1</sup> of fresh weight respectively in vegetables whose edible part is the fruit, the stem/root or the  
66 leaf (EU 2009).

67 Common pollutants in the urban environment are mainly anthropogenic, especially caused by  
68 emissions from road traffic, previous industrial use of the sites, atmospheric deposition from  
69 industrial activities and incinerators (Chen et al. 2005; Vittori Antisari et al. 2013). The main risk  
70 associated with urban soils is the presence of heavy metals deriving from intense human activities  
71 (Khan et al. 2008) and, more specifically, road traffic (Salvagio Manta et al. 2002). These elements  
72 may be absorbed by plants (Tei et al. 2010), although their accumulation across plant organs and  
73 between plant species may dramatically vary (Säumel et al. 2012). Within the city, areas with  
74 different load of pollutants can be distinguished: a garden settled nearby a road or a railway  
75 presents different conditions from one located in a courtyard or on a rooftop. Consistently, studies  
76 on heavy metals concentration have demonstrated that distance from the road and contamination are  
77 generally inversely correlated (Gherardi et al. 2009). Yet in the seventies, Lagerweff and Specht  
78 (1970) found that concentration of Cd, Ni, Pb and Zn in roadside soil and grass samples from  
79 several locations decreased with distance from traffic and with depth in the soil profile. Similar  
80 results were more recently obtained by Naszradi et al. (2004) and Bakirdere and Yaman (2008).  
81 Furthermore, the presence of buildings and trees as barriers for the pollutants was found to  
82 remarkably reduce road-induced pollution in the nearby gardens (Säumel et al. 2012). A weak point  
83 of previous researches is the absence of appropriate control when comparing urban to rural  
84 horticultural production. Most of the available cases addressed contaminations in urban- (Bakirdere  
85 and Yaman 2008; Bretzel and Calderisi 2006; Khan et al. 2008; Vittori Antisari et al. 2009), or  
86 rural- (Peris et al. 2007) cases only. On the other hand, when a comparison of urban *vs* rural  
87 horticultural good is claimed (Säumel et al. 2012), no reference to the growing conditions and  
88 provenance of the rural product is given.

89 In the present work, three years of experiments are presented, including results on rural *vs* urban  
90 products, distribution within the city and within gardens (proximity to different pollution sources,  
91 and distance from road), and adoption of different cultivation systems (soil *vs.* soilless).

92

## 93 2 Materials and Methods

94

95 A range of experiments was conducted between 2011 and 2013 in several sites within and nearby  
96 the city of Bologna (Fig. 1). Prior experimentation soil samples were collected from all study sites  
97 and analysed as follows. Soil samples were air-dried and sieved (<2 mm). pH (pHmeter, Crison,  
98 Barcelona, Spain) measures were performed with distilled water on 1:2.5 w:v. Total Organic  
99 Carbon was measured by Dumas combustion with a EA 1110 CHN elemental analyser (Thermo  
100 Fisher Scientific, Waltham, MA USA) after dissolution of carbonates with 2M HCl and the organic  
101 matter was obtained using 1.72 factors. Soil particle size distribution was determined by the pipette  
102 method (Gee and Bauder 1986) and total carbonates (CaCO<sub>3</sub>) were quantified by a volumetric  
103 method, according to Dietrich-Fruehing. The sites used for experimentation were:

- 104 - A rural control (coordinates 44°28'33'' N, 11°40'45'' E) from now on called  
105 CONTROL/RURAL, located nearby the small town of Medicina (about 16,000 inhabitants,  
106 35 km from Bologna, known as a vegetable crop cultivation area). The soil is UDIC  
107 CALCIUSTEPT Fine Silty, Superactive, Mesic (SSS, 2014), Cambic CALCISOL, Siltic,  
108 Hypocalcic (IUSS, 2014), with the following physic-chemical properties: sand (2-0.05 mm  
109 Ø) = 120 g kg<sup>-1</sup>; silt (0.05-0,002 mm Ø) = 580 g kg<sup>-1</sup>; Clay (>0,002 mm Ø) = 300 g kg<sup>-1</sup>; pH  
110 = 7.8; organic matter = 23.2 g kg<sup>-1</sup>; total CaCO<sub>3</sub> = 90.7 g kg<sup>-1</sup>.
- 111 - A traditional garden within the old city centre (coordinates 44°29'16'' N, 11°20'51'' E,  
112 from now on called CENTRE), in the old district were few ancient traditional gardens still  
113 exists. The soil is UDIFLUVENTIC HAPLUSTEPT Fine Silty, Superactive, Mesic (SSS,  
114 2014), Terric, Calcaric, CAMBISOL, Siltic, (IUSS, 2014), with the following physic-  
115 chemical properties: sand = 190 g kg<sup>-1</sup>; silt = 560 g kg<sup>-1</sup>; Clay = 250 g kg<sup>-1</sup>; pH = 7.9;  
116 organic matter = 18.3 g kg<sup>-1</sup>; total CaCO<sub>3</sub> = 120.5 g kg<sup>-1</sup>.
- 117 - Two gardens nearby the main railway (about 800 trains per day): a traditional one  
118 (coordinates 44°30'17'' N, 11°21'28'' E, from now on called RAILWAY/SOIL), and a

119 nearby rooftop soilless garden (coordinates 44°30'17'' N, 11°21'20'' E, from now on called  
120 RAILWAY/SOILLESS). Aerial distance between these two gardens is about 200 m. In  
121 traditional garden, the soil is UDIFLUVENTIC HAPLUSTEPT Fine Silty, Superactive,  
122 Mesic (SSS, 2014), Fluvic, Eutric, CAMBISOL, Siltic (IUSS, 2014), with the following  
123 physic-chemical properties: sand = 250 g kg<sup>-1</sup>; silt = 550 g kg<sup>-1</sup>; Clay = 200 g kg<sup>-1</sup>; pH = 7.5;  
124 organic matter = 16.8 g kg<sup>-1</sup>; total CaCO<sub>3</sub> = 10.9 g kg<sup>-1</sup>. The soilless garden uses coir as  
125 substrate (features provided by the supplier: bulk density = 0.06 g cm<sup>-3</sup>; pH = 7.4; EC = 1.7  
126 dS m<sup>-1</sup>).

127 - Two gardens nearby a main road of the city (via San Donato, 10<sup>3</sup>-10<sup>4</sup> vehicles day<sup>-1</sup>): two  
128 traditional gardens, placed 10 m from the street (coordinates 44°30'54'' N, 11°23'29'' E,  
129 from now on called ROAD/10) and 60 m from the street (coordinates 44°30'55'' N,  
130 11°23'33'' E, from now on called ROAD/60). Aerial distance between these two gardens is  
131 about 80 m. The soil in both gardens is UDIFLUVENTIC HAPLUSTEPT Loamy,  
132 Superactive, Mesic (SSS, 2014), Irragric, Fluvic, Calcaric, CAMBISOL, Loamic, (IUSS,  
133 2014), with the following physic-chemical properties: sand = 150 g kg<sup>-1</sup>; silt = 620 g kg<sup>-1</sup>;  
134 Clay = 230 g kg<sup>-1</sup>; pH = 8.0; organic matter = 21.6 g kg<sup>-1</sup>; total CaCO<sub>3</sub> = 181.7 g kg<sup>-1</sup>.

135

136

## 137 2.1 Plant material

138

139 During years 2011 and 2012, plant samples were collected from crops already grown in the gardens,  
140 according to their availability in each site. In these years, plant species considered included  
141 vegetable and aromatic plants, namely tomato (*Lycopersicon esculentum*), zucchini (*Cucurbita*  
142 *pepo* L.), chicory (*Cichorium intybus* L.), strawberry (*Fragaria x Ananassa*), eggplant (*Solanum*  
143 *melongena* L.), sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.), rosemary (*Rosmarinus*  
144 *officinalis* L.), and chilli pepper (*Capsicum annuum* L.), as well as some tree species, such as cherry

145 (*Prunus avium* L.), peach (*Prunus persica* L. Batsch), poplar (*Populus alba* L.), lime (*Tilia* L.), and  
146 maple (*Acer campestre* L.).

147 In 2013, plantlets of three species, namely tomato (cv Caramba 281, Seminis Inc., Oxnard, CA,  
148 USA), lettuce (cv Brasiliana, Eurosementi, Avellino, Italy) and basil (cv. Aromatico della Riviera  
149 Ligure, Arcoiris, Modena, Italia), were purchased from a local nursery (LACME, Medicina,  
150 Bologna, Italy). Transplanting was conducted in CONTROL/RURAL, ROAD/10, ROAD/60,  
151 RAILWAY/SOIL and CENTRE on April 15<sup>th</sup> (tomato and lettuce) and May 15<sup>th</sup> (basil). Each  
152 garden was provided with 9 plants per species.

153

## 154 2.2 Experimental protocols

155

156 - *Test#1: determination of pollutants as function of the distance of the road.* Plant samples  
157 were collected in 2011 and 2013 in ROAD/10 and ROAD/60 sites. Species considered in  
158 both gardens were tomato and zucchini in 2011 and tomato, lettuce and basil in 2013.

159 - *Test#2: comparison between different sources of pollution.* Two sites were selected for  
160 different sources of pollution: ROAD/10 and RAILWAY/SOIL. In 2011, leaves of lime,  
161 poplar and maple were sampled in ROAD/10, while in the RAILWAY/SOIL leaves of  
162 cherry, peach and poplar were collected. In 2013, tomato, lettuce and basil were collected  
163 from both gardens.

164 - *Test#3: comparison between the pollutants of horticultural crops grown in soilless and in  
165 soil.* In 2012 samples of sage, tomato, strawberry, basil, eggplant, rosemary, and chilli  
166 pepper grown in RAILWAY/SOIL and RAILWAY/SOILLESS were collected.

167 - *Test#4: urban vs. rural horticulture.* In 2013, according to the promising results obtained in  
168 the first two years and in order to overcome possible errors linked to differential mineral  
169 uptake due to species/cultivar, the analysis was extended to a great number of gardens  
170 within the city (CENTRE, RAILWAY/SOIL, ROAD/10 and ROAD/60). Furthermore, with

171 the aim of having a reference value in a rural environment, also the CONTROL/RURAL site  
172 was included. In all sites, same cultivars of tomato, lettuce and basil obtained from the same  
173 nursery were simultaneously grown.

174

### 175 2.3 Lab determinations

176

177 *Leaf leaching test.* The leaves of different species were sampled in glass jars of known tare weight.  
178 In the laboratory, leaves were weighed and then washed with water acidulated with HCl (pH ~5)  
179 (Vittori Antisari et al. 2012). Samples were shaken for 15 minutes and then water samples were  
180 collected in polyethylene beakers, evaporated in ventilated oven, and brought to 100 mL. Samples  
181 were then filtered, acidified with HNO<sub>3</sub> (65 % Suprapur, E. Merck, Germany; 1:100 v/v) and stored  
182 at 4°C until analysis. The major and trace elements were determined by Inductive Coupled Plasma  
183 Optical Emission Spectrometry (ICP-OES, Spectro Ametek, Arcos). The ICP-OES setting followed  
184 multi-standard solutions (CPI-International-Amsterdam) that reproduce the matrix effect present in  
185 samples and allow the lowering of Detection Limits (DL). Instrument response was assessed by  
186 measuring a standard sample (CRM 609 - Community Bureau of Reference – BCR).

187 In order to evaluate the deposition rate of pollutants the leaf area was determined in function of the  
188 leaf weight for three leaf samples. The calculated area/weight ratio ranges from 5.5 to 3.9 m<sup>2</sup> kg<sup>-1</sup>  
189 (Rutter et al. 2011).

190 *Analysis of leaf samples.* Clean leaves were dried in ventilated oven (T<40°C) and ground in a  
191 blender with blades made of pure titanium, carefully avoiding to introduce any further metal  
192 contamination to the samples (Vittori Antisari et al. 2012). Briefly, approximately 0.4 g of leaves  
193 sub-sample, weighted in Teflon bombs, were dissolved in 8 ml of H<sub>3</sub>NO<sub>3</sub> (suprapure, Merck, Roma,  
194 Italy) + 2 ml of H<sub>2</sub>O<sub>2</sub> (Carlo Erba, Milano, Italia) using a microwave oven (Milestone 2100,  
195 Sorisone, Bergamo, Italy). After cooling, solutions were made up to 20 ml with milli-Q water and  
196 then filtered with Whatmann 42 filter paper. The accuracy of the instrumental method and



197 analytical procedures used was checked by triplication of the samples, as well as by using reference  
198 material, which was run after every 10 samples to check for drift in the sensitivity. The analytical  
199 quality of the results was checked against the following reference materials, which certify values of  
200 the studied elements close to the measured ones: CRM 060 (aquatic plants), CRM 062 (Olive  
201 leaves) provided by the European Commission Institute for Reference Materials and Measurements.  
202 *Statistical analysis.* The experimental data were treated statistically using software packages (i.e.  
203 Excel, Statgraphic plus 5.0, Systat 12.0). The used one-way analysis of variance (ANOVA) test  
204 (Tukey's test,  $p \leq 0.05$ ) is a general technique that can be used to test the hypothesis that the means  
205 among two or more groups are equal. This is a non-parametric test used to determine if one of  
206 several groups of data tends to have more widely dispersed values than the other.

207

### 208 **3 Results and discussion**

209 Horticultural crops in urban or peri-urban areas are generally exposed to pollution risks, which  
210 include trace elements and organic contaminants (Säumel et al. 2012). The recent increase of areas  
211 for urban gardens in cities as well as the adoption of innovative (e.g. soilless) growing systems for  
212 urban cultivation arises the public concern on the produce safety. Overall, the range of trace  
213 elements concentration in the epigeous parts of the vegetables analyzed in the present study was  
214 similar to concentrations reported in previous studies (Alexander et al. 2006; Finster et al. 2004;  
215 Kachenko and Singh 2006; Murray et al. 2009), and always below limits expressed by European  
216 Union regulation (EU 2009) (Table 1). Field surveys in urban areas are to date scarce but crucial to  
217 determine health risks of urban horticulture (Säumel et al. 2012; Wong et al. 2006) and few studies  
218 have evaluated the role of exposition at different pollutant sources (Kelly et al. 1996; Li et al.  
219 2001). Differences in heavy metal pollution were however observed among study sites as reported  
220 in the following sections.

221

222 3.1 Heavy metal risk as affected by the garden distance from the road

223

224 A noticeable evidence related to traffic exposure has recently confirmed how lead concentration in  
225 plant tissue has been successfully reduced by the adoption of unleaded gasoline (Mielke et al.  
226 2011). However, environmental pollution associated to other metals (e.g. Ag, Cd, Ce, Ba) that are  
227 generally added to fuel as preservatives, result to be highly correlated with traffic exposure.  
228 Consistently, in order to assess the influence of the road distance on pollutant enrichment of  
229 horticultural crops, the trace elements concentration in leaf tissues from plants grown nearby the  
230 road (ROAD/10) was compared to the concentration found in vegetables grown on a more remote  
231 area of the urban garden (ROAD/60), as shown in Table 1. As and Hg concentrations were below  
232 detection limits (0.01 and 0.02  $\mu\text{g kg}^{-1}$  of dry weight, respectively), while Cd amount was detected  
233 only in tomato leaves in ROAD/10, in which a significant increase in the amount of Cr, Ni, Sn and  
234 Zn was also recorded. In zucchini, greater accumulation of Ni, Sn and Zn was associated to  
235 ROAD/10, whereas Ba concentration was higher in ROAD/60 samples. The stock of pollutant  
236 deposition ( $\text{g m}^{-2}$  of leaf area) on the leaves of tomato and zucchini (Table 1), highlighted a  
237 significant higher amount of most pollutants (As, Ba, Cu, Pb, Sb, Sn, V, Zn) deposited on both  
238 crops grown nearby the road (ROAD/10) compared with the ones located far from it (ROAD/60),  
239 therein confirming the deposition of these elements from road traffic as well as their high  
240 bioavailability and leachability from soils (Imperato et al. 2003; Madrid et al. 2002; Wong et al.  
241 2006). As a matter of fact, anthropogenic metals have been reported to be both easily bio-available  
242 in soils and easily diffused into the vegetable cuticle, through the stomata (Bianchini et al. 2013).

243

### 244 3.2 Effect of different pollution sources on heavy metal load

245

246 By analyzing the soluble pool of pollutants deposited on leaves of ornamental trees surrounding  
247 urban gardens exposed to road (ROAD/10) and railways (RAILWAY/SOIL) a different patterns of  
248 metals was distinguished (Table 2). Significantly higher amount of pollutants was found in the

249 deposition stock obtained from ROAD/10 as compared to RAILWAY/SOIL (Fig. 3, Vittori Antisari  
250 et al. 2012), except for As that resulted significantly higher on the surface of leaves collected from  
251 RAILWAY/SOIL (Table 2). Cd and Hg were not significantly different among samples. High  
252 deposition of particulate pollutants are intercepted by woodlands (Fowler et al. 1989) and urban  
253 trees are claimed to remove polluting particles from the air (Freer-Smith et al. 1997) absorbing  
254 atmospheric turbulence (Beckett et al. 2000; McPherson et al. 1994). Such phenomenon may be  
255 confirmed when comparing the behavior of deposition load on horticultural crops from that of  
256 ornamental tree leaves. As a consequence, the protection of the urban garden with ornamental trees  
257 resulted to be a sustainable solution to decrease the impact of both point and spread sources on the  
258 horticultural crops, therein leading to increased food safety.

259

### 260 3.3 Heavy metals risk in soil and soilless grown products

261

262 The investigation was performed to evaluate the differential metal accumulation between urban  
263 horticultural crops grown either on soil or in a soilless system. As shown in Fig. 2, samples from  
264 plants grown in either soilless (RAILWAY/SOILLESS) or soil systems (RAILWAY/SOIL) did not  
265 present differences in the total (expressed as sum of metals) concentrations. As, Cd and Hg  
266 concentrations were below the detection limits, while Cd and V were mainly found in soil-grown  
267 plants (data not shown). Significant differences in total accumulation were observed only in  
268 rosemary and eggplant samples (Fig. 2) as a consequence to greater Zn accumulation in soil-grown  
269 plants (data not shown). Consistently, depending on the species considered in the survey,  
270 differential heavy metal loads were confirmed, suggesting that accumulators (e.g. rosemary,  
271 Divrikli et al. 2006) should be avoided when cultivating contaminated soils (Fig. 2), in which  
272 soilless growing systems should also be preferred.

273

### 274 3.4 Heavy metal deposition and accumulation in rural and urban grown vegetables

275

276 The experiment simultaneously addressed the quantification of heavy metal deposition (Fig. 3A)  
277 and accumulation (Fig. 3B, C and D) in vegetable and aromatic species grown in rural and urban  
278 environments and as a consequence of the distance to pollution sources (e.g. main roads, railways).  
279 The pollutants deposited on the leaves were suddenly higher on ROAD/10, as highlighted by Fig.  
280 3A. The highest concentration of metals observed in tomato leaf tissues (Fig. 3D) as compared to  
281 basil and lettuce (Fig. 3B and C) was related to the dramatically higher Cu concentration (300 to  
282 1100 mg kg<sup>-1</sup> DW, data not shown). The elevated amount of Cu observed in tomato leaves could be  
283 explained as the consequence of foliar copper sulphate application for crop protection from  
284 diseases. Copper sulphate is generally overused in urban gardens, being the unique allowed product  
285 according to community garden rules (Tei et al. 2010). A peak in Cd concentration in leaves from  
286 all species was found in CONTROL/RURAL (0.4-1.2 mg kg<sup>-1</sup> of dry weight, data not shown). This  
287 could be the result of long-term soil fertilization (Tella et al. 2013) and Cd build-up in soils. Lettuce  
288 and basil (Fig. 3B and C) showed similar magnitudes of pollutants stored in their leaves and the  
289 maximum accumulation was detected in the urban garden nearby the road (ROAD/10, mean value  
290 184 mg kg<sup>-1</sup> of dry weight). Total metal concentration in tomato fruits was not significantly affected  
291 by washing, nor by the growing (rural or urban) environment and the distance from pollution  
292 sources (mean value 55.0±2.6 mg kg<sup>-1</sup> of dry weight, data not shown). Overall, the greater content  
293 of metals in tomato leaf tissue can be due to longer persistence of the plant in the field as compared  
294 to lettuce and basil which have a shorter vegetative cycle. Similarly, great Ba concentration was  
295 detected in the rosemary plants grown in RAILWAYS/SOIL as compared to other seasonal crops  
296 (Fig. 2).

297

#### 298 **4 Conclusion**

299 The concentration of heavy metals in urban grown vegetables is strictly related to the site in the city  
300 where plants are grown. When plants are cultivated nearby pollution sources (e.g. main roads), risks

301 of heavy metals accumulation is increased (about 1.5 folds when vegetables are grown 10 m from  
302 the road as compared to 60 m away). Prior consumption, vegetables grown nearby roads need to be  
303 carefully washed (deposition is increased 1.5 to 4 folds respectively in zucchini and tomato grown  
304 nearby the road as compared to those cultivated 60 m away). Overall pollutant accumulation in  
305 plant tissue is comparable to values found in rural areas, where Cd, mainly as a consequence to  
306 long-term soil fertilization, is generally higher (up to 1.2 mg kg<sup>-1</sup> of dry weight) than values found  
307 in urban products. Improper pest management, commonly experienced in allotment garden, resulted  
308 in excessive Cu accumulation (up to 1100 mg kg<sup>-1</sup> of dry weight in tomato fruits). These results  
309 should find application in the future planning and design of urban allotment gardens by public  
310 administrations. Given their increased relevance in shaping today's cities, allotments should be  
311 placed at safety distance from main roads or other pollution sources, and possibly surrounded by  
312 tree barriers. The suitability of available soils should be confirmed by preliminary analyses and  
313 whenever soils are not adequate, the adoption of soilless systems encouraged, although deeper  
314 studies for confirming the benefits of soilless cultivation systems in reducing heavy metals risks are  
315 however required. Finally, the present study should also call the attention on the possible risks faced  
316 by current rural cultivation systems: long-term soil fertilization may result in heavy metal build-up  
317 in soils, therein leading to potential contamination risks.

318

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440 **Tables**

441

442 Table 1. Trace elements accumulation and deposition in leaves of tomato and zucchini as affected by distance to main road (ROAD/10 and

443 ROAD/60). Values expressed as mg kg<sup>-1</sup> of dry weight (accumulation) and g m<sup>-2</sup> of leaf area (deposition). BDL: Below Detection Limit; SD:

444 Standard Deviation. ANOVA (Tukey test p&lt;0.05) test was performed on tomato and zucchini separately. ns: not significant differences at P≤0.05;

445 \*: significant differences at P≤0.05; \*\*: significant differences at P≤0.01.

		As	Ba	Cd	Cr	Cu	Hg	Ni	Pb	Sb	Sn	V	Zn
<b>Accumulation</b>													
<b>Tomato</b>	<b>ROAD/10</b>	BDL	32.10	0.20	0.80	13.10	BDL	2.38	0.28	0.44	18.30	0.10	144.40
	<i>SD</i>		0.40	0.00	0.30	0.30		0.02	0.12	0.04	0.80	0.00	0.30
	<b>ROAD/60</b>	BDL	34.40	BDL	0.10	11.50	BDL	0.36	0.40	0.32	15.80	0.10	38.20
	<i>SD</i>		0.50		0.00	0.10		0.04	0.00	0.00	6.40	0.00	0.40
Significance			ns	*	*	ns		*	ns	ns	ns	ns	*
<b>Zucchini</b>	<b>ROAD/10</b>	BDL	20.80	BDL	0.10	14.90	BDL	0.60	0.16	0.44	17.80	0.10	140.00
	<i>SD</i>		0.50		0.00	0.10		0.05	0.00	0.03	0.50	0.00	1.20
	<b>ROAD/60</b>	BDL	34.00	BDL	0.10	12.30	BDL	0.16	0.16	0.53	7.80	0.10	13.50
	<i>SD</i>		0.00		0.00	0.30		0.00	0.00	0.04	4.60	0.00	0.50
Significance			*		ns	ns		*	ns	ns	*	ns	*
<b>Deposition</b>													
<b>Tomato</b>	<b>ROAD/10</b>	0.3	18.2	0.1	0.9	68.8	BDL	3.5	3.6	0.2	0.3	0.5	100.1
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0
	<b>ROAD/60</b>	0.1	4.6	0.1	0.3	16.3	BDL	4.7	1.3	BDL	0.2	0.2	21.7
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.1	0.0	0.0
Significance		*	*	ns	*	*		ns	*	*	*	*	*
<b>Zucchini</b>	<b>ROAD/10</b>	0.2	13.5	0.0	0.1	16.1	BDL	3.6	2.3	0.3	0.7	0.6	26.2
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0
	<b>ROAD/60</b>	0.0	2.6	0.0	0.1	3.7	BDL	3.0	0.6	0.2	0.1	0.1	31.5
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0
Significance		*	*	ns	ns	*		ns	*	*	*	*	*

446

447

448 Table 2. Trace elements deposition in leaves of ornamental trees as affected by distance to main road (ROAD/10 and ROAD/60). Values expressed  
 449 as g m<sup>-2</sup> of leaf area. SD: Standard Deviation. ANOVA (Tukey test p<0.05) test was performed comparing the average values of metals  
 450 concentration. ns: not significant differences at P≤0.05; \*: significant differences at P≤0.05; \*\*: significant differences at P≤0.01.

451

		<b>As</b>	<b>Ba</b>	<b>Cd</b>	<b>Cr</b>	<b>Cu</b>	<b>Hg</b>	<b>Ni</b>	<b>Pb</b>	<b>Sb</b>	<b>Sn</b>	<b>V</b>	<b>Zn</b>	
<b>RAILWAY/ SOIL</b>	<b>Poplar</b>	0.93	11.31	0.08	1.42	34.25	0.06	3.88	0.75	0.12	1.45	0.30	84.75	
	<i>SD</i>	<i>0.01</i>	<i>0.73</i>	<i>0.00</i>	<i>0.02</i>	<i>0.99</i>	<i>0.00</i>	<i>0.13</i>	<i>0.10</i>	<i>0.01</i>	<i>0.06</i>	<i>0.03</i>	<i>0.75</i>	
	<b>Cherry</b>	1.21	14.04	0.04	1.72	55.05	0.06	5.45	0.91	0.24	1.67	0.44	57.52	
	<i>SD</i>	<i>0.03</i>	<i>1.09</i>	<i>0.00</i>	<i>0.03</i>	<i>1.15</i>	<i>0.00</i>	<i>0.15</i>	<i>0.16</i>	<i>0.01</i>	<i>0.05</i>	<i>0.03</i>	<i>0.87</i>	
	<b>Peach</b>	0.93	18.34	0.06	1.75	128.93	0.11	7.52	5.89	0.25	1.51	0.65	175.51	
	<i>SD</i>	<i>0.04</i>	<i>1.24</i>	<i>0.00</i>	<i>0.04</i>	<i>1.18</i>	<i>0.00</i>	<i>0.19</i>	<i>0.11</i>	<i>0.02</i>	<i>0.01</i>	<i>0.01</i>	<i>1.25</i>	
	Average	1.02	14.56	0.06	1.63	72.74	0.08	5.62	2.52	0.20	1.54	0.46	105.93	
	SD	0.56	1.60	0.03	0.08	2.70	0.05	0.60	0.80	0.12	0.07	0.09	12.80	
	<b>ROAD/10</b>	<b>Poplar</b>	0.12	44.74	0.22	3.05	168.42	0.17	16.30	11.41	1.13	2.25	1.54	597.42
		<i>SD</i>	<i>0.00</i>	<i>1.26</i>	<i>0.01</i>	<i>0.03</i>	<i>2.15</i>	<i>0.00</i>	<i>0.26</i>	<i>0.13</i>	<i>0.03</i>	<i>0.02</i>	<i>0.03</i>	<i>0.72</i>
<b>Maple</b>		0.30	43.35	0.24	3.39	110.11	0.14	9.50	10.90	0.91	2.51	1.62	511.06	
<i>SD</i>		<i>0.01</i>	<i>0.99</i>	<i>0.01</i>	<i>0.05</i>	<i>1.58</i>	<i>0.00</i>	<i>0.20</i>	<i>0.15</i>	<i>0.05</i>	<i>0.01</i>	<i>0.04</i>	<i>0.85</i>	
<b>Lime</b>		0.37	80.84	0.23	3.61	184.55	0.30	15.21	15.63	1.12	2.80	2.45	399.87	
<i>SD</i>		<i>0.02</i>	<i>0.75</i>	<i>0.02</i>	<i>0.04</i>	<i>1.96</i>	<i>0.00</i>	<i>0.23</i>	<i>0.20</i>	<i>0.06</i>	<i>0.01</i>	<i>0.10</i>	<i>1.14</i>	
Average		0.26	56.31	0.23	3.35	154.36	0.20	13.67	12.65	1.05	2.52	1.87	502.78	
SD		0.16	4.30	0.05	0.08	3.80	0.12	0.30	0.70	0.04	0.60	0.10	15.60	
Significance	*	*	ns	*	*	ns	**	*	*	*	*	**		

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## Figures

Figure 1. Sites used for sampling. Top box, localisation of the city of Bologna and the town of Medicina. *CONTROL/RURAL* samples were obtained from site (C). Bottom box, city of Bologna.

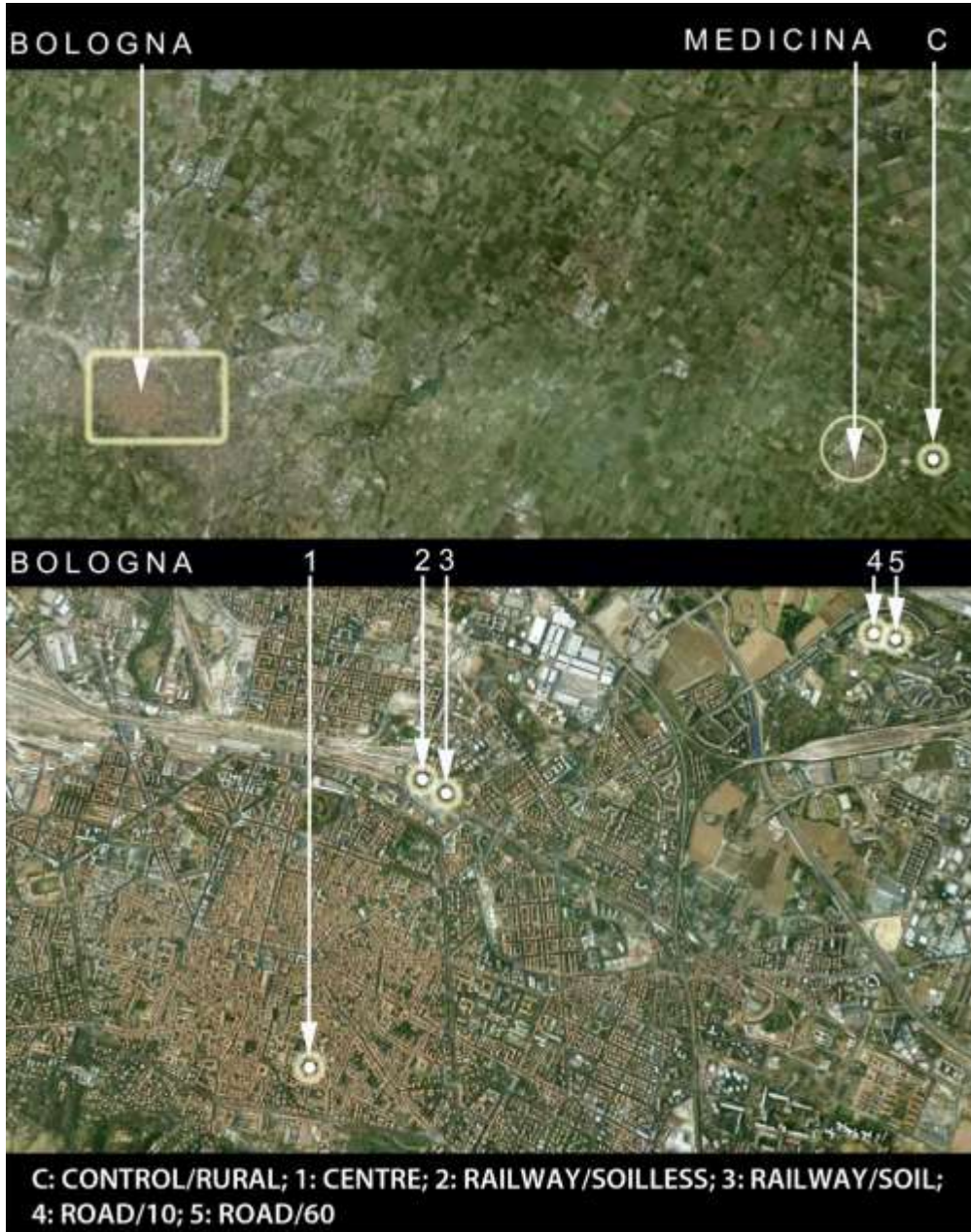


Figure 2. Cumulative concentration of selected heavy metals (As, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sb, Sn, V, Zn) on leaves of crops (tomato, sage, strawberry, pepper, rosemary, eggplant) grown on soil and soilless. Mean values, expressed as  $\text{mg kg}^{-1}$  of dry weight (DW), vertical bars represent standard deviation. ANOVA (Tukey test  $p \leq 0.05$ ) was performed in all study sites and different lowercase letters indicate a significant difference in metals content. ns = not significant differences at  $P \leq 0.05$ .

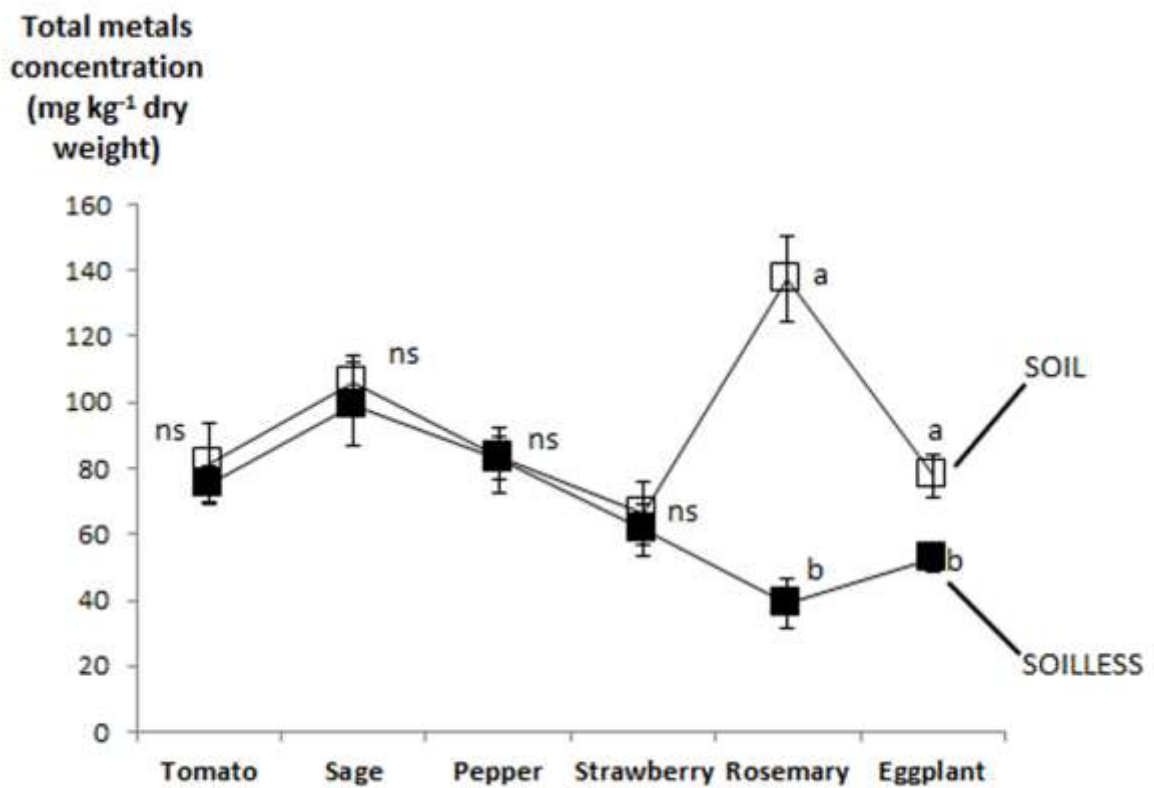


Figure 3. Cumulative pollutants (As, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sb, Sn, V, Zn) deposition and tissue concentration. Deposition (A) was determined in the water after washing, as affected to garden location and growing system adopted, and expressed as  $\text{g cm}^{-2}$  of leaf area (LA). Tissue cumulative concentration, expressed as  $\text{mg kg}^{-1}$  Dry Weight (DW), on leaves of crops (basil, A; lettuce, B and tomato, C). ANOVA (Tukey test  $p \leq 0.05$ ) was performed in all study sites and different lowercase letters indicate a significant difference in metals content.

