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Metal contamination of home gardens soils and cultivated vegetables in the province of Brescia, Italy: Implications for human exposure

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Abstract

Background—For the past century, ferroalloy industries in Brescia province, Italy produced particulate emissions enriched in manganese (Mn), lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), chromium (Cr), iron (Fe), aluminum (Al). This study assessed metal concentrations in soil and vegetables of regions with varying ferroalloy industrial activity levels.

Methods—Home gardens (n=63) were selected in three regions of varying ferroalloy plant activity duration in Brescia province. Total soil metal concentration and extractability were measured by X-ray fluorescence (XRF), aqua regia extraction, and modified Community Bureau of Reference (BCR) sequential extraction. Unwashed and washed spinach and turnips cultivated in the same gardens were analyzed for metal concentrations by flame atomic absorption spectrometry.

Results—Median soil Al, Cd, Fe, Mn, Pb, and Zn concentrations were significantly higher in home gardens near ferroalloy plants compared to reference home gardens. The BCR method yielded the most mobile soil fraction (the sum of extractable metals in Fractions 1 and 2) and all metal concentrations were higher in ferroalloy plant areas. Unwashed spinach showed higher metal concentrations compared to washed spinach. However, some metals in washed spinach were higher in the reference area likely due to history of agricultural product use. Over 60% of spinach samples exceeded the 2- to 4-fold Commission of European Communities and Codex

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Alimentarius Commission maximum Pb concentrations, and 10% of the same spinach samples exceeded 2- to 3-fold maximum Cd concentrations set by both organizations. Turnip metal concentrations were below maximum standard reference values.

Conclusions—Prolonged industrial emissions increase median metal concentrations and most soluble fractions (BCR F1+F2) in home garden soils near ferroalloy plants. Areas near ferroalloy plant sites had spinach Cd and Pb metal concentrations several-fold above maximum standard references. We recommend thoroughly washing vegetables to minimize metal exposure.

Keywords

metal; phytoavailability; ferroalloy industry emissions; vegetables; plant uptake; modified BCR sequential extraction procedure; Brescia; Italy

1. INTRODUCTION

Metal soil contamination resulting from anthropogenic activities has been demonstrated to be associated with health risks in nearby populations (Carrizales et al., 2006; Hinwood et al., 2004; Pruvot et al., 2006). Young children are highly susceptible to metal exposure via hand-to-mouth routes (Wang et al., 2010; Calabrese et al., 1997). Other exposure routes via inhalation/ingestion of re-suspended soil particulates (Harris and Davidson, 2005), and consumption of contaminated locally grown vegetables (Cambra et al., 1999, Hough et al., 2004) also occur in both children and adults. Since many communities rely on home gardens for dietary vegetable consumption, soil metal contaminations is a concern for residents in close proximity to industrial emissions (Yujing et al., 2005; Zheng N. et al., 2007).

Although Manganese (Mn) is a biologically essential mineral, it is also a harmful by-product of ferroalloy metal production. Chronic human Mn exposures may result in toxicity when concentrations exceed the nutritional homeostatic requirement. Higher lifetime Mn exposure was found to significantly increase the prevalence of Parkinsonian disturbances in Brescia province (Lucchini et al., 2007). Additionally, a significant positive association between soil Mn exposure and both impaired motor coordination and odor discrimination were observed among adolescents (Lucchini et al., 2012) and elderly (Zoni et al., 2012; Lucchini et al., 2014) who reside near ferroalloy emission sites located in Valcamonica, a valley in the Brescia province.

This study aimed to estimate the potential exposure among areas in Brescia, Italy with varying histories of ferroalloy activity. We examined concentrations and chemical mobility of typical ferroalloy industrial emission metals, including Mn, aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) in home garden soil and vegetables in the province of Brescia, Italy. Soil metal concentrations in ferroalloy-impacted areas were compared to non-ferroalloy areas within the same province.

2. MATERIALS AND METHODS

2.1 Study site

Three areas within the province of Brescia, Italy, were investigated for soil metal concentrations: i) Valcamonica, a pre-Alps valley where three ferroalloy industrial sites had

been active for about a century and ceased in 2001; ii) Bagnolo Mella, located in the southern plain of the province, where a ferroalloy plant has been active since the 1970s; and iii) Garda Lake, a region with no history of ferroalloy or other metal industry. Further details on these study areas have been reported (Ferri et al., 2012). In addition to soil sampling, home garden soils and cultivated vegetables were examined for metal analyses. Participating home gardens were 63 in total: 27 in Valcamonica, 9 in Bagnolo Mella and 27 in Garda Lake (Figure 1).

2.2 Soil samples

Approximately 500 g of soil from each of the 63 participating home gardens were collected from February to April 2010 before seasonal planting, in concordance with the official Italian method I.1 for soil chemical analysis (Ministerial Decree 13/09/1999). Each ~500 g soil sample consisted of 10–15 sub-samples of surface soil (~30–50 g per sub-sample, excluding the first 2–3 cm of surface soil) and were collected in dry, clean plastic ziplock bags using an ‘X-shape’ sampling grid across each garden. Information on the sampling area, date and depth of the soil sample, and use of fertilizers was recorded. In addition to home gardens, a series of undisturbed surface soil samples (n=14) were collected at incremental distances (80 m to 22 km) from the ferroalloy plant point sources for comparison with home gardens soils. An aliquot of each sample was screened for total metal concentration using a portable X-Ray Fluorescence (XRF) spectrometer (Niton, ThermoFinnigan). Collected samples were processed and analyzed in the laboratory using a single extraction method of aqua regia with analysis by flame atomic absorption spectroscopy, or using the BCR three step sequential extraction procedures with analysis by inductive-coupled plasma-optical emission spectrometry (ICP-OES).

2.2.1 XRF analyses—Total soil Cu, Cd, Cr, Fe, Mn, Pb, Zn concentrations were determined by portable X-ray spectrometer (model NITON XL3t) equipped with GPS locator. Composite soil sample measurements were taken before sieving and starting the sequential extractions. U.S. standard soil reference materials NIST-2709-a and NIST-2710 were used for XRF calibration before each measurement.

2.2.2 Modified BCR three-step sequential extraction procedure—Each composite soil sample was thoroughly mixed and an aliquot (~1 g ± 10%) was removed and oven-dried (65°C) to a constant weight for 24 h and sieved to <150 µm. A subset of soil samples was processed in triplicate. Sieved soil samples were analyzed with the modified BCR-three step sequential extraction procedure as previously described (Ferri et al., 2012; Pueyo et al., 2008; Rauret et al., 2000; Mossop and Davidson, 2003). First, soil metals were extracted in 40 mL of 0.11 M acetic acid (fraction 1). Second, 40 mL of 0.5 M hydroxylammonium chloride were added to the soil residue from fraction 1 and extracted at pH 1.5 (fraction 2); Next, the soil residue was extracted using 20 mL of 8.8 M Hydrogen Peroxide and 50 mL of 1.0 M Ammonium Acetate (fraction 3); Lastly, the residual soil fraction was then digested in 5 mL of 7.5 N HNO₃ at 80°C and extracted (fraction 4).

The modified BCR procedure yielded four fractions per soil sample. Fraction 1 (F1) reflected exchangeable and weak acid-soluble metals, and constituted the most mobile

fraction of soil metals potentially available to vegetables. This fraction of metals in the sorption complex is often bound to carbonates (Agnieszka M., 2004). Fraction 2 (F2) contains reducible metals, often associated with Mn and Fe oxides. Fraction 3 (F3) contains oxidizable metals associated with organic matter and sulfides. F3 metals are less mobile compared to F1 and F2, and are often incorporated into stable high molecular weight humic substances (Dilek B. 2011). The residual fraction (R) contains complex metals that are relatively immobile and unavailable for plants (Dilek B. 2011).

Metal extraction efficiency and reproducibility was evaluated using a soil standard reference material processed in triplicate with each soil extraction batch (BCR 483; sewage sludge amended soil certified by the Community Bureau of Reference). The reference material has certified values for Cd, Cr, Cu, Pb and Zn extracted using 0.05 mol L⁻¹ EDTA and 0.43 mol L⁻¹ acetic acid, which indicated readily extractable metal contents using the BCR sequential extraction procedure.

Concentrations of Al, Cd, Cr, Cu, Fe, Mn, Pb, Zn in soil extracts were determined by ICP-OES (Optima 4300 DV Series; PerkinElmer, Inc.) (Borgese et al., 2013). The relative amounts of metals extracted in F1 to R were determined and their sums normalized to 100% and results are described in terms of the percent distribution within each fraction. Total metal concentrations were calculated as the sum of values from all four fractions (F1, F2, F3 and R) obtained by the modified BCR sequential extraction procedure.

Average recovery of metals from the BCR 483 reference material was determined for each metal and fraction. This method resulted in a recovery range of 64–107% for each fraction extracted. The within-sample reproducibility of metal analysis was determined by analyzing a subset of composite soil sample extracts in triplicate. The relative standard error of samples processed and analyzed in triplicate was <5%, which was considered acceptable. Results are expressed as mg metal per kg of dry weight sample.

2.2.3 Aqua regia extraction procedure—The aqua regia procedure was also used to estimate speciation and mobility of metals in the soil. Soil samples were air dried at about 28–30°C in a shady area and ground to pass through a 0.2 mm sieve. Trace elements in soil samples were measured from aqua regia extractions, following the method XI.1 indicated by the specific Italian regulation (Ministerial Decree 13/09/1999).

Samples (~1g) were placed in 250 mL of Erlenmeyer flasks and 20 mL of H₂O₂ were added gradually to eliminate the organic matter. The suspension was heated and the volume was reduced to ~3 mL. Once cooled to room temperature, 9 mL HCL and 3mL HNO₃ were added to the samples and digested for 2 hours. The digested samples were filtered through an ash-free paper filter, diluted to 100 mL with deionized water and stored in polyethylene bottles until analysis. The near-total concentrations of Cd, Cr, Cu, Fe, Mn, Pb and Zn were determined in the digestive sample by F-AAS. Aluminum was not analyzed with the aqua regia procedure.

2.3 Vegetable samples

Two of the commonest vegetable plants in Italian home gardens were selected: spinach (a leafy vegetable) and turnip (a root vegetable). Seeds of spinach '*Spinacio lorelay*' and turnip '*Rapa of Milan with violet collar*' were planted in the home gardens of the three study areas by participating gardeners from April to May. Approximately 6 g of spinach seeds were spread over a 1 m² surface area. Three seeds of turnip were placed in each hollow with a sowing distance of ~20–30 cm between the rows and 15–20 cm on the row. After establishment, seedlings were thinned to one plant per hollow. The healthiest and strongest plants were chosen for analyses.

2.3.1 Samples harvesting and preparation—Plants were grown for 6–8 weeks and then harvested. Participating gardeners followed typical irrigation practices and were asked not to fertilize. Spinach and turnip samples were collected from a total of 47 and 54 home gardens, respectively. For spinach, 21 vegetable samples were collected from Valcamonica, 20 from Garda Lake and 6 from Bagnolo Mella. For turnip, 23 vegetable samples were collected from Valcamonica, 22 from Garda Lake, and 9 from Bagnolo Mella. After harvest and laboratory transportation, edible portions of vegetable sample were prepared for analysis. Edible spinach portions were randomly divided into two fractions. To estimate the amount of soil metals removed by washing, a portion of each vegetable sample was left unwashed and another portion was thoroughly washed with tap water. Washed and unwashed vegetable fractions were analyzed separately. Approximately 300 g of each vegetable fraction were homogenized using a blade homogenizer. Turnips were prepared for analyses by washing with tap water, peeling to remove the skin, and the edible portion of the turnip chopped for subsequent homogenization; ~300 g of chopped turnip was homogenized for digestion.

2.3.2 Digestion and analysis of the vegetable samples—Chemical analyses of vegetables were carried out by the Department of Food Chemistry's, Metal Laboratory in IZSLER in Brescia, using atomic absorption spectroscopy techniques, according to International Organization for Standardization guidelines. A total of 3 g of homogenized sample was weighed into Teflon vessels, and 2 mL (30% H₂O₂ and 8 mL 65% HNO₃) of trace metal grade were added to each vessel. The vessels were left for ~30 minutes at room temperature and then mineralized in MARSXpress microwave reaction system (CEM Corporation). The microwave and thermal programmes were as follows: Stage: 1; max power (W): 1600; ramp (min): 30.0; temperature: 200°C; hold (min): 20. After mineralization, each sample solution was poured into a 25 mL Class A precision flask and made up to the final volume with Milli-Q water (Millipore Corporation). In digested sample solutions, Pb and Cd were detected and quantified using a graphite furnace atomic absorption spectrometry (GF-AAS) system (model AAS240; Varian, Inc) with Zeeman background correction. Iron, Zn, and Mn were detected and quantified using a F-AAS system (Model 3110; PerkinElmer, Inc) using an air-acetylene flame; Cu was detected and quantified through both systems depending on its concentration in the mineralized sample. The analysis accuracy was evaluated for each batch using a spike recover approach, in which aliquots of vegetable digestates were fortified with known concentrations of metals, blank solutions, and the standard addition method. Concentrations of metals in vegetables

are expressed in mg metal kg⁻¹ fresh weight (fw) of vegetable sample. Spike recovery percentages on average were 60% for Cd, 76% for Zn, 82% for Cu and Pb, 83% for Mn and 89% for Fe. The lower value (60%) for Cd was due to the lower fortification than other metals.

2.3.3 Cd and Pb dietary contribution from locally grown vegetables—To assess the contribution of Cd- and-Pb-contaminated vegetables to dietary intake, two consumer groups were considered: children (3–10 years old), and those > 10 years old. To measure mean individual exposure, the FAO/WHO (1997) formula was applied:

$$\frac{\text{Mean vegetable contamination level } (\mu\text{g}/\text{kg}) \times \text{Mean individual daily vegetable consumption } (\text{kg}/\text{day})}{\text{Mean body weight } (\text{kg})}$$

Mean Pb and Cd exposures were based on locally grown spinach and turnip measurements in the three research sites. The mean individual spinach and turnip consumption for Italian subjects were based on the data reported by Leclercq (2009).

Mean individual exposure results were expressed in $\mu\text{g kg}^{-1}$ body weight (bw) day⁻¹ and compared to Cd Tolerable Daily Intake (TDI) of 0.36 $\mu\text{g kg}^{-1}$ bw day⁻¹ threshold set by the European Food Safety Authority (EFSA, 2006 and 2011). Since there is no agreement of a Pb exposure threshold for developmental neurotoxicity in children (< 7 years) and cardiovascular effects and nephrotoxicity in adults, no TDI has been indicated for Pb (EFSA, 2010; WHO, 2010). However, the EFSA established a mean lifetime dietary Pb exposure of 1.03 $\mu\text{g kg}^{-1}$ bw day⁻¹ for 3–10 yrs old children and of 0.68 $\mu\text{g kg}^{-1}$ bw day⁻¹ for overall European population (EFSA 2012). Since Pb TDI has not yet been established, the following EFSA estimated benchmark dose levels were used to compare to our calculated individual contamination concentration results: of 0.5 $\mu\text{g kg}^{-1}$ bw day⁻¹ for developmental neurotoxicity, 1.50 $\mu\text{g kg}^{-1}$ bw day⁻¹ for effects on systolic blood pressure, 0.63 $\mu\text{g kg}^{-1}$ bw day⁻¹ for chronic kidney disease.

3. STATISTICAL ANALYSIS

Data were summarized using empirical quartiles. Non-parametric tests (Kruskal-Wallis Test) were applied to compare the distribution of soil and vegetable measures between the three study areas, followed by the post-hoc non-parametric 'Nemenyi-Damico-Wolfe-Dunn' test (Hollander, Wolfe 1999). Bland-Altman plots were used to compare metal concentration measurement procedures. A log transformation was applied to stabilize the variance of the mean difference between the measurements. After log transformation was applied, the inverse logarithm of the difference represented the ratio for mean soil concentration measurements obtained. Correlations between fractions of soil metal concentrations and vegetable metal concentrations were determined using Spearman's rank correlation coefficient. The percentage of metal removal after washing was calculated by: [(unwashed-washed)/unwashed]*100 for each pair of vegetable samples. All statistical analysis were done using R (R Core Team, 2012). For all statistical tests of significance, alpha was set to 0.05.

4. RESULTS

4.1 Elements in soils

Descriptive statistics on soil metal concentration measurements using XRF, BCR modified sequential extraction with ICP-OES, and aqua regia extraction with F-AAS are reported in Table 1. The BCR modified procedure conferred significantly lower median concentration values than XRF, which was expected since the BCR modified procedure does not produce complete soil sample dissolution. Aqua regia extraction produced the highest median soil metal concentrations compared to both BCR modified and XRF procedures for all the analyzed metals except Mn (Supplementary Figure A).

For Mn, the inverse logarithm ratio between ICP-OES and F-AAS soil concentration measurements was 0.96 [i.e. ICP-OES underestimates F-AAS measurement by about the 4% (95% CI: 0.75, 1.21)]. The ratio between XRF and F-AAS was 1.32 [i.e. XRF overestimates F-AAS by 32% (95% CI: 0.99, 1.75)], and the ratio between ICP-OES and XRF was 0.73 (i.e. ICP-OES underestimates XRF by 27% (95% CI: 0.52, 1.02)).

According to BCR measurements, median soil concentrations were significantly higher in Valcamonica and Bagnolo Mella compared to the Garda Lake area for Al ($P < 0.001$ for both), Cd ($P < 0.001$; $P = 0.002$), Fe ($P < 0.001$ for both), Mn ($P < 0.001$ for both), and Zn ($P = 0.01$). Valcamonica median soil concentrations were significantly higher than Bagnolo Mella for Mn ($P = 0.003$) and Fe ($P < 0.001$). However, Cu median soil concentrations were significantly higher in Garda Lake compared to Bagnolo Mella ($P = 0.02$).

Median soil concentrations were compared to the Italian regulation maximum allowable concentration (n° 152, Annex 4-V5) for residential sites: 100 mg kg⁻¹ for Pb, 2 mg kg⁻¹ for Cd, 120 mg kg⁻¹ for Cu, 150 mg kg⁻¹ for Zn and Cr. The percentages of soil samples exceeding the maximum allowable concentrations for Pb were: 7% in Valcamonica, 0% in Bagnolo Mella, 4% in Garda Lake. For Cd, the soil sample percentages were: 85% in Valcamonica; 100% in Bagnolo Mella, 59% in Garda Lake. For Cu, the soil sample percentages were: 15% in Valcamonica, 0% in Bagnolo Mella, 11% in Garda Lake. For Cr, the soil sample percentages were: 0% in Valcamonica, 0% in Bagnolo Mella, 4% in Garda Lake. For Zn, the soil sample percentages were: 59% in Valcamonica, 33% in Bagnolo Mella, 22% in Garda Lake.

4.2 Metals in soil fractions

Results of Mn sequential extraction and the other metals are summarized in Table 2 and chemical partitioning percentages are shown in Figure 2. The only metal with the same chemical partitioning trend across the three study areas was Cd. Partitioning percentages of Cd for F2 and R for each site were: 30% and 55%, respectively, for Valcamonica, 27% and 52%, respectively, for Bagnolo Mella, and 30% and 44%, respectively, for Garda Lake. Remaining Cd was partitioned in F1 and F3 for Valcamonica samples (11% and 4%, respectively), Bagnolo Mella (17% and 4%, respectively) and Garda Lake (18% and 8%, respectively).

The highest Cr chemical partitioning percentages of soil samples occurred in F3 and R (30% and 49%, respectively) for Garda Lake, whereas for Valcamonica, the highest percentage occurred in F2 and R (22% and 56%, respectively) and for Bagnolo Mella (20% and 54%, respectively). The F1 was 3% for Valcamonica, 8% for Bagnolo Mella and 4% for Garda Lake samples.

The chemical Cu partitioning percentage for F1 was 2% for soil samples collected in Valcamonica, and 0% for both Bagnolo Mella and Garda Lake samples. Higher Cu partitioning percentages were demonstrated in F2 for Valcamonica and Bagnolo Mella samples (38% and 31%, respectively) compared to Garda Lake area (18%). The highest Cu percentage in F3 in Garda Lake samples was 41% compared to Valcamonica and Bagnolo Mella (23% for both).

The percentage of Pb was highest in F2 of both Valcamonica and Bagnolo Mella samples (78% and 56% respectively). Similarly, the highest Zn concentrations occurred in F2 in Valcamonica and Bagnolo Mella samples (41% and 39%, respectively). The highest percentages of Al and Fe occurred in the R fraction in Valcamonica and Bagnolo Mella as well. The bivariate association between Al and Fe showed the highest positive correlation ($\rho=0.78$, $P=0.03$) among the metals considered in this study.

Although Mn was found to be highly extractable by weak acids, it was the most extracted analyte in F2 versus F1 in soil samples collected in Valcamonica and Bagnolo Mella. The order of Mn extracted soil fractions across the three study areas was the following: F2 (66%) > F1 (18%) > R (14%) > F3 (2%) in Valcamonica; F2 (67%) > F1 (19%) > R (11%) > F3 (3%) in Bagnolo Mella and F2 (52%) > F1 (32%) > R (12%) > F3 (4%) in Garda Lake soils. Low contents of Mn in the R fraction were obtained in Garda Lake. The Mn partitioning percentage contained in F2 was similar in samples near historic or currently active ferroalloy plant operations (~68%) that include Valcamonica and Bagnolo Mella home gardens. The Mn percentage was also higher in these areas than in Garda Lake home gardens (~52%).

4.3 Metal concentrations in vegetables

Trace metal concentrations in unwashed and washed spinach, as well as turnip samples grown in the home gardens of Valcamonica, Bagnolo Mella and Garda Lake are reported in Table 3. The highest metal concentrations were found to occur in leafy vegetables for all three study areas whilst the lowest concentrations of trace metals were found in root vegetables.

In the home gardens, the concentrations of Fe ($P=0.003$) and Cu ($P=0.05$) measured in unwashed spinach were significantly higher in Bagnolo Mella compared to Valcamonica and Garda Lake. In washed spinach, Pb concentrations ($P=0.02$) and Zn concentrations ($P=0.004$) in the washed spinach were significantly higher in Garda Lake compared to Bagnolo Mella. Turnip Cu concentrations were significantly higher in Garda Lake compared to Valcamonica and Bagnolo Mella ($P<0.001$ and $P<0.001$, respectively) areas. Washed spinach had the least median metal concentration across all study sites.

A comparison of Cu, Fe, Mn, and Zn concentrations in vegetables with findings from previous studies are listed in Table 4. The overall mean metal concentrations in the vegetables were lower or similar to reference values. Turnip Cu concentration level collected from Garda Lake were approximately double the concentrations deemed safe for human consumption by the Food Composition and Nutrition Tables (Souci et al., 2008), but below the unsafe concentration according to the US National Nutrient Database for Standard Reference (USDA, 2011).

A high percentage of spinach produced in the kitchen gardens on the Garda Lake, Valcamonica and Bagnolo Mella (65%, 62% and 17%, respectively) exceeded the Pb safety limit guidelines set by the Commission of the European Communities and the Codex Alimentarius Commission. Ten percent of spinach collected in the Garda Lake area exceeded both limits for Cd concentration. The turnip samples did not exceed these standards.

4.4 Spinach and Turnip consumption and dietary intake assessment for Cd and Pb

Estimates of daily Pb and Cd intake due to vegetable consumption are reported in Table 5. The estimated daily intake of Pb and Cd due to spinach consumption was higher in Valcamonica and Garda Lake. Based on spinach consumption alone, daily intake of Pb and Cd reached almost 60% of the mean lifetime dietary level for Pb and the TDI level for Cd., The mean and 95th percentile daily intake for children on the Garda Lake area were 52% and 116%, respectively, of the EFSA's benchmark dose level for Pb-induced developmental neurotoxicity. In other words, spinach consumption would provide 52% of the maximum Pb daily intake reference value. Estimated daily intake of Pb and Cd due to turnip consumption was similar across study sites.

4.5 Correlation between soil extractable metal concentration and plant metal concentrations

Turnip metal concentrations were correlated with soil concentrations for F3 Cu ($\rho=0.35$; $P=0.01$) and total Cu ($\rho=0.47$; $P<0.001$). Turnip metal concentrations were also correlated with soil F1 Pb ($\rho=0.31$; $P=0.03$); with soil F3 Pb ($\rho=0.29$; $P=0.04$), with R Pb ($\rho=0.28$; $P=0.04$) and with soil F1 Cd ($\rho=0.31$; $P=0.02$). No other correlation resulted between the turnip metal concentrations and the soil fractions extracted. No significant correlation was noted between metal concentrations in washed spinach leaves and extractable metal concentrations in different fractions of soils.

5. DISCUSSION

Total metal concentrations in surface soil showed higher levels on Mn, Cd, Fe, Zn in the areas of current (Bagnolo Mella) and historical (Valcamonica) metal emission from ferroalloy industry compared to the reference area (Garda Lake). As an area with the longest ferroalloy activity history, Valcamonica soil contained the highest total metal concentrations. Intermediate total metal concentrations were found in Bagnolo Mella and reflect current, shorter relative history (~40 yrs) of ferroalloy plant activities.

Each of the analytical methods used served different purposes in the investigation of local ferroalloy plant activity impact on metal contamination in home garden soils and cultivated vegetables. Portable XRF for was used for field-based screening of surface soil metal levels. The aqua regia digestion of soil was useful in determining total extractable metals and modified BCR sequential extraction method to estimate metal fractionation in the soil in addition to the chemical mobility and the potential bioavailability of metals to cultivated plants.

Cadmium, Mn and Zn were extracted using modified BCR primarily in F1 in all study sites, which is the most chemically labile fraction of the soil samples. F1 extractions of Cd and Mn ($\rho=0.66$), Cd and Zn ($\rho=0.65$), Mn and Zn ($\rho=0.54$) were strongly correlated and highly significant ($P<0.001$), suggesting similar chemical mobility of these elements across the three sites. In particular, Cd partitioning percentage trend for each fraction were similar across all three areas of Brescia and this was in agreement with previous studies since Cd is an easily removed and chemically labile metal (Sahuquillo et al, 2003). High percentages of Mn and Zn in F1 were probably due to the close association of these metals with carbonates (Marin, Giresse, 2001).

Extracted F2 concentrations, which include reducible metals bound to Fe and Mn oxides, were significantly higher in Al, Fe, Mn and Zn for Valcamonica and Bagnolo Mella soil samples compared to Garda Lake reference samples. High concentrations of these metals in F2 extracted soils reflected the synthetic oxide reactions typical of ferroalloy production (i.e. MnO_2 or Mn_3O_4). The relationship of these metals to the historic presence of ferroalloy plant emissions was further demonstrated by comparing both extracted soil and home garden samples and proximity to current or historic ferroalloy plant emissions. Total Mn concentrations were higher in the most ferroalloy-impacted soils compared to Mn in Garda Lake home gardens with no history of metallurgic activities. Surface metal soil concentrations declined with greater distance from the closest ferroalloy plant, which supports the notion that plant emissions are a substantial source of higher soil metal concentrations (Ferri et al, 2012). Our data on Mn fractionation were in agreement with previous results examining metal loadings and mobility amongst the same three sites in Brescia, Italy (Borgese, 2013). Large differences were found in F2 between the reference area and the areas of ferroalloy industrial activity. Another study also found that the Mn partitioned percentage of R from an area of anthropogenic heavy metal emissions in Turkey was lower than Mn percentage of R from agricultural area soils (Tokalioglu et al, 2010). This supports an anthropogenic origin of Mn because the R extraction, which consists of immobile and less phytoavailable metals bound to organic matter, was proportionally higher in areas with no history of ferroalloy industrial activity.

The highest Al and Fe partitioning percentages in this study also occurred in the R fraction, indicating that these metals are strongly bound to minerals and biologically unavailable to plants (Borgese, 2013). However, across the three study sites, F2 and total Al and Fe concentration differences between the Garda Lake reference area and Valcamonica and Bagnolo Mella were highly significant.

Conversely, Cr, Cu, and Pb concentrations of F3 soil extractions were higher in Garda Lake compared to Valcamonica and Bagnolo Mella, reflecting the strong complexation of these metals with soil rich in organic matter and/or sulfides. This has been observed in a similar metal speciation study for Cu and Pb in sediments of Algeciras Bay, Spain (Diaz et al., 2011). An increase in Pb and Cu in F2 was expected since these two metals are adsorbed by clay minerals, organic matter, Fe, and are co-precipitated with Fe and Mn oxides (Favas, 2011). After F3 metal-organic complexations, the availability of these metals to plants is reduced (Gupta and Sinha, 2006). According to Udom et al. (2004), metal-organic complexation also decreases metal mobility in soils. Therefore, even though the Cu soil concentrations in Garda Lake contained greater levels of these metals in total, the higher Cu concentrations in F3 fraction means that Cu was less mobile and less bioavailable to plants compared to Cu contaminating Valcamonica and Bagnolo Mella soils.

The poor correlation of soil extractable metal concentration and plant metal concentrations found in this study was consistent with similar prior studies (Oyeyiola et al., 2011; Olayinka et al., 2011; Anawar et al., 2008; Menzies et al., 2007) and may be due to a number of factors. Phytoavailability of soil metals is influenced by soil composition and chemistry (pH, oxygen content, etc.), organic matter content and composition, metal content and speciation, microbial activity and the biochemical/molecular processes of plants that regulate metal uptake and utilization (Agbenin J, 2009). A study in Turkey has also found correlations between soil and plant bioavailability to vary by plant type (Tokalioglu S., 2006). Furthermore, bioavailability of these metals to humans is further complicated by additional factors such as differences in origin, sorption behavior, clay content and pH dependence (Ljung et al., 2007).

Direct soil contamination due to re-suspension of soil particulates and direct airborne particulate matter deposition onto plant surfaces likely distorted the relationship between soil metal content and plant accumulation. For spinach leaves, which are more exposed to airborne deposition than turnips, no significant correlation was found for any extraction fraction of the studied metals. Several other bio-monitoring studies had demonstrated that plants accumulated trace elements from the atmosphere (Bargagli 1998; Rossini Oliva and Valdes, 2004; De Temmerman and Hoenig, 2004). A principal component analysis examining the relationship between vegetable metal content, agricultural soil and airborne particulate matter in northern Greece found that the dominant pathway for Pb, Cr and Cd trace elements in vegetable leaves originated primarily from the atmosphere (Voutsas et al., 1996) while in Spain found a direct relationship between soil Cu and Cu concentrations in particulate matter (Rossini Oliva and Fernandez Espinosa., 2007). Other studies on vegetables harvested at planting sites in inner-city Berlin, Germany and Nanjing, China have demonstrated significantly higher Pb concentrations in edible crops than areas of less urban traffic (Säumel et al., 2012; Fang et al., 2011). The pollutants emitted from the more traffic-heavy Garda Lake area than Valcamonica and Bagnolo Mella, also contributed to the disproportionately greater Pb median spinach concentrations in this reference area. For edible turnip portions growing beneath the soil with less susceptibility to airborne matter contamination, lower concentrations of all metals were found compared to both washed and unwashed spinach. Furthermore, across study sites, the variation of Pb turnip concentrations among Garda Lake, Valcamonica and Bagnolo Mella was not statistically different.

Despite low correlations between soil and plant metal concentrations, the daily flux of outdoor particle deposition was very low compared to loadings of metal-enriched particulates in the soil. It is unlikely that the soil metal concentrations varied after planting. It is more likely that the lack of correlation between soil metal extractions and plant metal accumulations was due to the specific plant species as well as contributing factors from the environment. There had been no noted disturbance of soils or reason to expect that metal soil concentrations would change during the plant growth process. Farmers were instructed not to use fertilizers or fungicides since doing so may contaminate soils with trace metals (Murray et al, 2011). Additionally, samples of soil and vegetables were retrieved around the same time of harvesting in order to optimally relate vegetable surface metal content with soil. Furthermore, the consistent finding of greater metal concentrations on unwashed versus washed spinach and even lower metal concentrations on root vegetables across all three study sites were consistent with our expectations regarding plants grown on undisturbed soil.

Although the extraction procedures were able to classify soil metal fractions, they may not be a true reflection of plant metal accumulation. Rather, sequential metal extraction can be used to disentangle effects of the chemical mobility and bioavailability of metals in soils and their uptake by plants (Kennedy et al, 1997; Dilek et al., 2011).

Spinach consumption constituted a substantially higher proportion of Cd and Pb metals compared to turnip consumption (Table 5). For spinach consumption, estimated contribution to Pb TDI was high for children and persons greater than 10 years old. Similarly, the contribution of Cd to percentage of TDI for both children and persons greater than 10 years old was substantial, even though the contamination concentrations were lower than the the EU Regulation limit 1881/2006. This is consistent with the EFSA report of a mean Cd oral intake in Europe is close to the tolerable weekly intake (TWI) of 2.5 $\mu\text{g kg}^{-1} \text{bw}$ (EFSA 2007). Some subgroups such as vegetarians and smokers, can exceed the TWI estimations by about 2-fold (EFSA 2007). Since vegetables are a staple food, a mean contribution close to 15–20% of the TDI is highly relevant (Chen et al., 2011). In addition to vegetable ingestion, children may obtain metal exposure in areas of past ferroalloy industrial activity from soil ingestion (Wang et al., 2010). Children are particularly vulnerable to consequences of high Pb exposure since increased Pb absorption during development leads to adverse health outcomes.

The concentrations of Pb and Cd in plants grown for commercial distribution are regulated by legislative standards that we used to compare concentrations measured in home-garden vegetables (Douay, 2007). Over 60% of spinach samples, including washed spinach, exceeded the 2- to 4-fold Commission of European Communities and Codex Alimentarius Commission maximum international Pb concentrations standards. Ten percent of the same spinach samples exceeded 2- to 3-fold maximum Cd concentrations set by both organizations. Turnip metal concentrations were below maximum standard reference values.

The Pb content of spinach was reduced by more than four-fold by washing with tap water. In standard preparation of vegetables for consumption, thoroughly washing vegetables with tap water was effective in reducing plant metal concentrations. Iron was the most efficiently removed from the spinach leaves by washing. As a common component of dust, Fe adheres

to the leaf surface until washing away part of the deposition fraction. Thus, both mobility and water solubility of contaminants are factors strictly related to the washing effect (Zwolinski et al., 1998). The results obtained in this study suggest that washing efficacy depends on the metal contaminant in addition to metal plant concentrations.

6. CONCLUSION

High concentrations of soil metal-oxides, inferred by the relatively high percentage of metals in the F2 fraction, reflect typical ferroalloy emissions. The low correlation between the soil metal concentrations and vegetable metal concentrations suggests that metal concentrations in different fractions of soils extracted using the modified BCR procedure were poor predictors of phytoavailability.

Spinach grown in areas near historic or current ferroalloy industrial activity are contaminated with higher metal concentrations than recommended by European standards, and thus poses a threat to human health. The trend of higher metal concentrations in unwashed versus washed samples demonstrates that washing vegetables with tap water was able to remove metallic elements deposited on spinach leaf surface. However, washing with fresh tap water may not be sufficient in reducing the Pb TDI for children, since even washed spinach in some home gardens did not conform to Pb concentrations by both of the international food standard guidelines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Total soil metal concentration and extractability were measured by X-ray fluorescence (XRF), aqua regia extraction, and modified Community Bureau of Reference (BCR) sequential extraction.
- Unwashed and washed spinach and turnips cultivated in the same gardens were analyzed for metal concentrations by flame atomic absorption spectrometry.
- Median soil Al, Cd, Fe, Mn, Pb, and Zn concentrations were significantly higher in home gardens near ferroalloy plants compared to reference home gardens.
- Over 60% of spinach samples exceeded the 2- to 4-fold Commission of European Communities and Codex Alimentarius Commission maximum Pb concentrations, and 10% of the same spinach samples exceeded 2- to 3-fold maximum Cd concentrations set by both organizations.

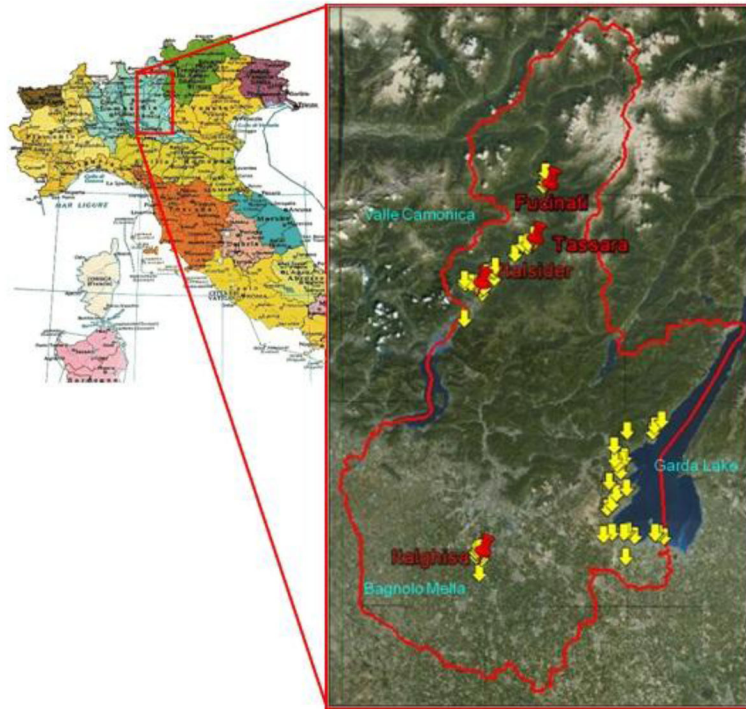


Figure 1. Collection areas (arrows) and ferroalloy plant locations (pins) in the areas of Valcamonica, Bagnolo Mella, and Garda Lake.

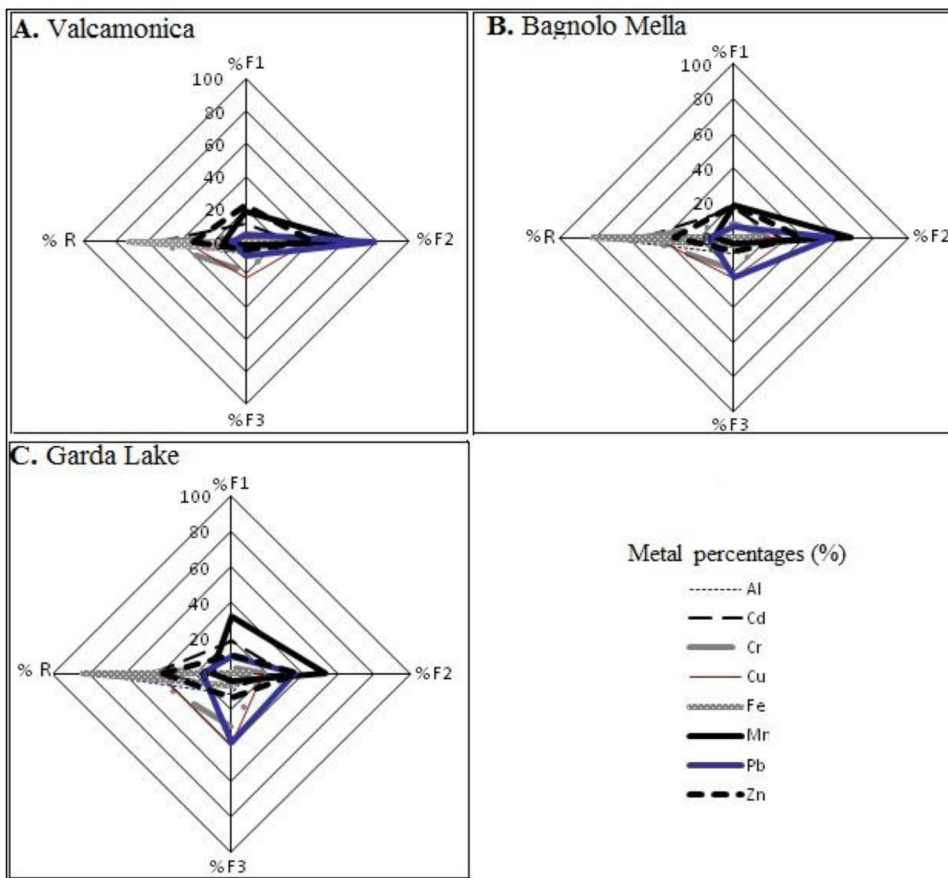


Figure 2. Total percentages of each metal concentration for each of the modified Community Bureau of Reference (BCR) sequential extraction fractions: Fraction 1 (F1); Fraction 2 (F2); Fraction 3 (F3) and Residual (R) for soils collected in the three study areas.

Table 1
Total soil metal concentrations (expressed in mg kg⁻¹ of dry mater) at three different study areas determined by modified Community Bureau of Reference (BCR) sequential extraction, aqua regia procedure and X-Ray Fluorescence (XRF)

Heavy metals	Method	Study sites			p values §
		Valcamonica Median (IQR)	Bagnolo Mella Median (IQR)	Garda Lake Median (IQR)	
Al	BCR modified	10514 (4493–19169)	10280 (8030–11803)	6948 (3334–11201)	<0.001
	BCR modified aqua regia procedure	2.54 (1.29–4.16)	2.38 (2.05–2.70)	2.04 (1.00–2.60)	<0.001
Cr	BCR modified aqua regia procedure	1 (0.00–3.00)	2 (1.00–3.00)	3 (1.00–4.00)	2*
	BCR modified aqua regia procedure	15.63 (6.96–34.4)	18.11 (13.3–30.5)	17.85 (7.21–277)	0.28
Cu	BCR modified aqua regia procedure	93 (47.0–120)	50 (43.00–64.00)	77 49.00–690.00	150*
	XRF	69.93 (25.1–116)	29.02 (24.2–65.5)	84.21 (36.1–237)	<0.001
	BCR modified aqua regia procedure	64.65 (24.8–373)	31.24 (23.8–58.7)	66.69 (23.4–162)	0.005
	XRF	74 (28.0–410)	43 (30.0–84.0)	87 (36.0–237)	120*
Fe	BCR modified aqua regia procedure	29037 (16414–37502)	21854 (17547–24508)	15813 (7952–23660)	<0.001
	XRF	14173 (7648–19629)	11768 (8568–13654)	9268 (3431–12570)	<0.001
	BCR modified aqua regia procedure	17160 (15920–18040)	17400 (16880–17680)	15680 (13040–17320)	<0.001
	XRF	1337 (690–2570)	806 (552–1440)	577 (442–893)	<0.001
Mn	BCR modified aqua regia procedure	982 (473–2113)	623 (466–1357)	393 (288–747)	<0.001
	XRF	1025 (493–1612)	712 (555–1254)	437 (315–658)	<0.001
	BCR modified aqua regia procedure	62.1 (34.0–127)	45.6 (21.4–59.3)	39.9 19.0–212	0.005
Pb	BCR modified aqua regia procedure	47.1 (24.2–109)	43.08 (27.0–50.0)	37.3 (16.9–128)	0.07
	XRF	65 (36.0–125)	90 (47.0–147)	64 (50.0–207)	100*
Zn	BCR modified aqua regia procedure	225.1 (90.7–643)	178 (89.7–216)	145 (65.8–458)	0.01
	XRF	172 (70.4–426)	128 (70.3–192)	113 (32.4–305)	0.01
	BCR modified aqua regia procedure	258 (92.0–845)	220 (114–275)	153 (58.0–474)	150*

§ comparison between Valcamonica, Bagnolo Mella and Garda Lake

* Maximum allowable concentration.

Median metal concentrations (mg kg^{-1}) in different modified Community Bureau of Reference (BCR) extracted fractions of soils from the three study areas.

Table 2

Element	Fraction	Study site			p value §
		Valcamonica Median (IQR)	Bagnolo Mella Median (IQR)	Garda Lake Median (IQR)	
Al	F1	32.2 (0.11–65.8)	10.1 (3.59–17.4)	0.11 (0.11–7.49)	<0.001
	F2	2120 (871–8890)	1280 (33.0–3110)	24.1 (0.90–3430)	<0.0001
	F3	881 (335–1360)	820 (486–1270)	768 (342–1250)	0.15
	R	7780 (3270–13900)	8620 (5980–9980)	5220 (2980–9030)	0.002
	Total (BCR + R)	10500 (4490–19200)	10300 (8030–11800)	6950 (3330–11200)	<0.001
Cd	F1	0.26 (0.09–0.62)	0.44 (0.27–0.55)	0.37 (0.15–0.50)	0.01
	F2	0.63 (0.40–1.69)	0.62 (0.41–0.98)	0.56 (0.30–1.08)	0.2
	F3	0.09 (0.03–0.39)	0.1 (0.04–0.15)	0.15 (0.03–0.61)	0.1
	R	1.43 (0.44–2.33)	1.3 (0.94–1.50)	0.86 (0.03–1.41)	<0.001
	Total (BCR + R)	2.54 (1.30–4.16)	2.38 (2.05–2.71)	2.04 (1.00–2.60)	<0.001
Cr	F1	0.52 (0.20–0.93)	1.58 (1.05–1.99)	0.74 (0.46–5.71)	<0.001
	F2	3.3 (1.20–13.4)	2.24 (1.45–8.04)	1.61 (0.22–253)	0.1
	F3	2.82 (0.02–17.3)	2.16 (1.42–12.6)	3.74 (0.67–49.6)	0.05
	R	9.04 (3.66–16.9)	9.25 (7.48–14.8)	10.3 (4.81–15.8)	0.6
	Total (BCR + R)	15.63 (6.96–34.5)	18.1 (13.3–30.5)	17.8 (7.21–277)	0.3
Cu	F1	1.09 (0.08–8.19)	0.079 (0.07–0.08)	0.08 (0.07–7.18)	<0.001
	F2	18.1 (2.95–323)	7.07 (0.09–25.0)	0.1 (0.10–93.0)	0.0004
	F3	12.6 (1.24–87.6)	6.85 (0.70–32.9)	21.9 (0.58–130)	0.02
	R	24.6 (3.42–45.6)	19 (5.16–31.7)	26.3 (4.07–49.0)	0.1
	Total (BCR + R)	64.6 (24.8–373)	31.2 (23.8–58.7)	66.7 (23.4–163)	0.005
Fe	F1	4.37 (0.04–7.85)	0.44 (0.04–1.06)	0.71 (0.04–4.76)	<0.001
	F2	2710 (1670–7850)	1290 (350–3840)	372 (6.63–5420)	<0.001
	F3	571 (373–1350)	512 (240–814)	468 (147–1140)	0.07
	R	10300 (3600–16400)	9710 (7700–10800)	6660 (3230–11400)	<0.001
	Total (BCR + R)	14200 (7650–19600)	11800 (8570–13700)	9270.00	<0.001
Mn	F1	165 (108–280)	124 (97.2–179)	134 (94.2–198)	0.005

Element	Fraction	Study site			p value §
		Valcamonica Median (IQR)	Bagnolo Mella Median (IQR)	Garda Lake Median (IQR)	
Pb	F2	681 (264–1650)	419 (280–996)	205 (116–477)	<0.001
	F3	19.1 (10.7–98.6)	17 (11.0–84.5)	13.2 (5.73–80.3)	0.04
	R	127 (35.2–308)	75.1 (50.7–120)	49.4 (21.7–68.0)	<0.001
	Total (BCR + R)	983 (473–2110)	623 (466–1360)	393 (288–747)	<0.001
	F1	2.19 (0.74–4.46)	2.31 (0.78–4.06)	3.49 (2.24–4.84)	<0.001
Zn	F2	35.9 (19.8–84.7)	22.1 (6.30–36.1)	7.53 (1.12–53.10)	<0.001
	F3	3.53 (0.56–25.1)	3.09 (0.56–30.9)	11.7 (0.57–88.9)	0.01
	R	4.33 (0.60–16.5)	3.67 (2.23–12.7)	5.3 (0.62–31.2)	0.4
	Total (BCR + R)	47.1 (24.2–109)	43.1 (27.0–50.0)	37.3 (16.9–128)	0.07
	F1	34.5 (10.1–117)	28.7 (5.76–37.7)	11.5 (1.29–40.6)	<0.001
	F2	58.2 (22.6–247)	47.8 (33.1–70.1)	35.4 (4.63–108)	0.03
	F3	7.52 (2.16–24.7)	6.86 (4.50–23.8)	8.62 (1.49–156)	0.5
	R	50.8 (16.3–149)	45.3 (26.7–70.6)	37.4 (13.8–102)	0.1
	Total (BCR + R)	172 (70.4–426)	128 (70.3–192)	113 (32.4–305)	0.01

§ comparison between Valcamonica, Bagnolo Mella and Garda Lake using Kruskal-Wallis test

Table 3

Median metal concentrations (mg Kg⁻¹ fresh weight) in unwashed and washed spinach “S” (*Spinacea Oleracea*) and turnip “B” (*Brassica Rapa*) from home gardens from three study sites. The number of vegetable samples per study site and vegetable type is given in parentheses.

Element	Plant	Study site			P value §
		Valcamonica Median (IQR) (S=21; B=9)	Bagnolo Melia Median (IQR) (S=6; B=9)	Gardia Lake Median (IQR) (S=20; B=22)	
Cd	Unwashed spinach	0.05 (0.02–0.14)	0.06 (0.02–0.13)	0.06 (0.01–0.36)	0.63
	Washed spinach	0.04 (0.01–0.10)	0.02 (0.01–0.06)	0.05 (0.01–0.31)	0.07
	Turnip	0.01 (0.01–0.02)	0.02 (0.01–0.04)	0.01 (0.01–0.04)	0.002
Cu	Unwashed spinach	0.9 (0.67–3.10)	2.11 (0.82–3.06)	1.62 (0.52–40.50)	0.05
	Washed spinach	0.72 (0.37–1.00)	0.56 (0.42–1.04)	0.85 (0.40–14.50)	0.15
	Turnip	0.4 (0.28–1.08)	0.37 (0.28–0.59)	0.76 (0.36–5.21)	<0.0001
Fe	Unwashed spinach	93.2 (20.0–510)	537 (312–820)	166 (18.8–639)	0.003
	Washed spinach	37.5 (9.20–96.8)	28.9 (8.92–43.6)	30.5 (7.28–126)	0.47
	Turnip	5.48 (2.95–13.7)	4.14 (3.70–6.90)	4.31 (1.62–18.7)	0.32
Mn	Unwashed spinach	5.43 (0.58–16.4)	7.77 (1.31–32.0)	4.87 (1.60–18.0)	0.86
	Washed spinach	1.82 (0.15–4.73)	0.59 (0.06–2.34)	2.12 (0.95–8.50)	0.05
	Turnip	0.92 (0.52–1.52)	1.08 (0.86–1.74)	1.01 (0.71–1.50)	0.05
Pb	Unwashed spinach	0.69 (0.07–2.20)	0.95 (0.21–3.69)	0.89 (0.08–3.30)	0.21
	Washed spinach	0.15 (0.04–0.43)	0.07 (0.04–0.19)	0.21 (0.07–1.00)	0.03
	Turnip	0.01 (0.00–0.06)	0.02 (0.01–0.03)	0.02 (0.01–0.13)	0.06
Zn	Unwashed spinach	9.03 (5.60–28.0)	9.54 (3.95–13.8)	10.15 (7.44–20.0)	0.18
	Washed spinach	6.1 (2.80–18.0)	4.37 (2.97–5.33)	6.99 (4.10–13.1)	0.006
	Turnip	2.18 (1.22–4.68)	2.81 (2.21–4.91)	2.59 (1.04–7.50)	0.03

§ Comparison between Valcamonica, Bagnolo Melia and Gardia Lake using Kruskal-Wallis Test

Table 4

Mean metal concentrations in spinach and turnips (mg kg^{-1}) reported in literature.

Water and trace elements	Reference		
	Italian Food Composition database (BDA, 2008)	Food Composition and Nutrition Tables (Souci et al., 2008)	USDA National Nutrient Database for Standard Reference (USDA, 2011)
		Spinach	
Water	90.1 g	91.2 g	91.4 g
Cu	1.6 mg kg^{-1}	0.9 mg kg^{-1}	1.3 mg kg^{-1}
Fe	29.000 mg kg^{-1}	34.000 mg kg^{-1}	27.000 mg kg^{-1}
Mn	/	5.99 mg kg^{-1}	8.97 mg kg^{-1}
Zn	14.3 mg kg^{-1}	6.7 mg kg^{-1}	5.3 mg kg^{-1}
		Turnip	
Water	93.3 g	89.9 g	91.87 g
Cu	/	0.56 mg kg^{-1}	0.85 mg kg^{-1}
Fe	6 mg kg^{-1}	3.83 mg kg^{-1}	3 mg kg^{-1}
Mn	/	0.68 mg kg^{-1}	1.34 mg kg^{-1}
Zn	0.80 mg kg^{-1}	2.30 mg kg^{-1}	2.7 mg kg^{-1}

