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Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite

Mery Malandrino[a*], Ornella Abollino[a], Sandro Buoso[a], Agnese Giacomino[a], Carmela La Gioia[a], Edoardo Mentasti[a]

[a] University of Torino, Department of Analytical Chemistry, Via Pietro Giuria 5, 10125 Torino, Italy

E-mail addresses: mery.malandrino@unito.it; ornella.abollino@unito.it; sandro.buoso@unito.it; agnese.giacomino@unito.it; carmela.lagioia@unito.it; edoardo.mentasti@unito.it

Corresponding author. Mery Malandrino
Università degli Studi di Torino
Dipartimento di Chimica Analitica
Via Pietro Giuria 5
10125 Torino (Italy)

Abstract

We evaluated the distribution of 15 metal ions, namely Al, Cd, Cu, Cr, Fe, La, Mn, Ni, Pb, Sc, Ti, V, Y, Zn and Zr, in the soil of a contaminated site in Piedmont (Italy). This area was found to be heavily contaminated with Cu, Cr and Ni. The availability of these metal ions was studied using Tessier's sequential extraction procedure: the fraction of mobile species, which potentially is the most harmful for the environment, was much higher than that normally present in unpolluted soils. This soil was hence used to evaluate the effectiveness of treatment with vermiculite to reduce the availability of the pollutants to two plants, *Lactuca sativa* and *Spinacia oleracea,* by pot experiments. The results indicated that the addition of vermiculite significantly reduces the uptake of metal pollutants by plants, confirming the possibility of using this clay in amendment treatments of metal-contaminated soils. The effect of plant growth on metal fractionation in soils was investigated. Finally, the sum of the metal percentages extracted into the first two fractions of Tessier's protocol was found to be suitable in predicting the phytoavailability of most of the pollutants present in the investigated soil.

Keywords. contaminated soil; heavy metals; sequential extraction; *in situ* immobilization; vermiculite; edible plants.

1. Introduction

Peri-urban agriculture has been assuming greater significance in the last years due to an increase in population and urbanization. The input of metal pollutants to such marginal agricultural lands through sewage sludge and industrial effluents (Chhonkar et al., 2000a,b; Rattan et al., 2002, 2005) is a matter of concern because of the persistence of these metals in soils, uptake by crops and accumulative effects in animal and human beings (Gupta and Gupta, 1998). One remediation technology for metal-contaminated soil includes excavation of soil followed by washing and disposal of the treated material (U.S. Environmental Protection Agency, 1991). However, this remediation strategy is very expensive and gives rise to a considerable amount of wastes. Although phytoremediation, i.e. the use of plants for ameliorating metal-contaminated sites, has received considerable attention in recent years, one of the major problems associated with this approach is low metal removal rates (McGrath et al., 2002; Pierzynski et al., 2000; Rattan et al., 2002). Hence, a logical and rational remediation process appears to be chemical stabilization, i.e. metal immobilisation by using different amendments. Common methods for immobilisation of metals in soil are to apply lime, phosphates, organic matter residues and other natural or synthetic additives, like zeolites, beringite, hydrous oxides of Al, Fe and Mn (Bolan and Duraisamy, 2003; Gworek, 1992; Khattak and Page, 1992; Vangronsveld et al., 1990). Altogether the chemical stabilization method is surely a relatively simple and cost-effective remediation technique for contaminated sites and hence it merits systematic investigation.

For these reasons, in recent years, many researchers studied the behaviour of natural organic and inorganic materials having high adsorption capacity and which are particularly abundant and inexpensive, in order to use them as low-cost effective amendments for on-site remediation of metal-contaminated soils.

Clay minerals, such as montmorillonite and vermiculite, have a high cation exchange capacity and high specific surface area associated with their small particle sizes. Such properties have made these materials the target of several adsorption studies. Previous investigations in our laboratory showed that vermiculite, a widespread natural clay, has a high total capacity toward some heavy metals (Malandrino et al., 2006). In the present work we have determined the extent and distribution of contamination in a site polluted by heavy metals and the metal availability by Tessier's fractionation method. Then we have evaluated the effectiveness of the treatment with vermiculite on the uptake of pollutants present in the investigated soil by two plants, *Lactuca sativa* and *Spinacia oleracea*. Finally we have studied the changes in metal availability after application of vermiculite.

2. Experimental

2.1. Site description

The investigated site is located in northeast Piedmont, Italy, near the town of Borgomanero, in the province of Novara, as reported in Figure 1. The area, now uncultivated and characterised by a high environmental deterioration, was used as permanent meadow and woodland in the past. The contamination occurred because of the repeated floods of a small stream, which today has a new course, caused by the insufficient size of the stream bed with respect to the flow in rainy periods. The stream collected the wastewaters of local industries, some of which operating in the electroplating field, and its floods caused an accumulation of contaminants, mainly of inorganic nature, in the soil. The extension of the polluted area is estimated between 20,000 and 100,000 m^2. The core of the contaminated zone is about 3,000 m^2 wide: it is flat and covered by a layer of black sludge about 1.50 m deep carried by the floods, where a scant vegetation grows. The rest of the area is covered by trees and spontaneous plants. The land in the zone is made of alluvial deposits.

Figure 1. Geographical map of Piedmont and detail of the area investigated (grey square).

2.2. Apparatus and reagents

A Delta 320 Mettler Toledo pH-meter provided with a combined glass-calomel electrod and an incorporated thermal probe was used for pH measurements.

Sample dissolution for the determination of total concentrations was performed with a Milestone MLS-1200 Mega (Milestone, Sorisole, Italy) microwave laboratory unit.

Metal determinations were carried out with a Varian Liberty 100 model (Varian Australia, Mullgrave, Australia) inductively coupled plasma-atomic emission spectrometer (ICP-AES). The calibrations were always performed with standard solutions prepared in aliquots of sample blanks. High purity water (HPW) produced with a Millipore Milli-Q system was used throughout. All the reagents used were of analytical grade. Standard metal solutions were prepared from concentrated stock solutions (Merck Titrisol).

2.3. Soil sampling and characterization

The soil material was sampled in several points, both in the site core and in the surrounding area where self-sown vegetation is growing. Namely, samples B1 and B3 were collected at the surface in the site core and samples B2 and B4 immediately below, at a depth of 10 cm; finally, samples B6 and B7 were taken at the border of the site, at the surface and at a depth of 10 cm respectively. All samples were collected with plastic tools and transferred into polyethylene bags. Subsequently they were air-dried, sieved through a 2-mm sieve, ground in a centrifugal ball mill and stored in plastic bags prior to laboratory analysis. Untreated soil aliquots were used in pot experiments.

Grain size distribution, pH, organic carbon, organic matter and cation exchange capacity (CEC) were determined according to the official methods of soil analysis of the Italian legislation issued in 1999 (Ministerial Decree, 1999) using the following procedures: Esenwein's pipette method for particle size distribution; a 1:2.5 soil – 1 M KCl suspension for pH measurements; the Walkley-Black method for organic carbon and organic matter content; the barium chloride method for CEC.

2.3.1. Total metal content

For the determination of the total metal concentrations acid digestion in a microwave oven was chosen as the dissolution procedure.

Sample aliquots of 500 mg were treated with a mixture of 10 ml of aqua regia and 4 ml of hydrofluoric acid in PTFE bombs. Four heating steps of 5 min each (250, 400, 600, 250 W respectively), followed by a ventilation step of 25 min, were applied. Then 1.4 g of boric acid were added, and the bombs were further heated for 5 min at 250 W and again cooled by ventilation for 15 min. The resulting solutions were filtered with paper filters and diluted to 100 ml with HPW. The solutions were employed for the ICP-AES analysis.

2.3.2. Tessier sequential extraction procedure

This sequential extraction procedure (Tessier et al., 1979, Tessier et al., 1980) partitions the metals into five operationally defined chemical fractions: extractable and exchangeable (1 M $MgCl_2$, agitation for 1 h), bound to carbonates (1 M CH_3COONa, plus CH_3COOH (pH 5), agitation for 5 h), bound to Fe and Mn oxides (0.04 M $NH_2OH·HCl$ in 25% CH_3COOH, agitation for 6 h at the temperature of 96 ± 3 C°), bound to organic matter and sulphides (0.02 M HNO_3 and 5 ml of 30% H_2O_2, agitation for 5 h at the temperature of 85 ± 2 C°) and residual. The fifth fraction was not considered because it is mainly present as scatter within the crystal lattices of the rocks and minerals that constitute the soil and it may be released only in the long term (Abollino et al. 2006; Davidson et al. 1998).

After each extraction the suspension was centrifuged for 20 min at 4000 rpm. The solution was separated, while the precipitate was washed with 10 ml of HPW and centrifuged again for 5 min. The washing water then was added to the supernatant, while the precipitate was used for the subsequent extractions. The extracts were diluted to 25 (first fraction), 50 (second fraction) or 100 (next two ones) ml, stabilised by addition of 25, 50 or 100 µl of concentrated nitric acid respectively and analysed.

2.4. Vermiculite properties

Vermiculite was supplied by Aldrich. It is a naturally occurring mineral which contains a small amount of crystalline silica in the form of quartz.

The main properties of the clay are reported in a previous paper (Malandrino et al., 2006). Altogether it has a good cation exchange capacity (CEC = 40.08 meq/100 g) and a high pH at the point of zero charge (pH_{zpc} = 8.63). Potassium is the principal exchangeable ion present in the interlayer of this clay.

2.5. Pot experiments with lettuce and spinach

The soil sampled in the center of the site was put in polyethylene pots (5 kg in each pot) and treated by adding vermiculite (500 g). At the same time an aliquot of the soil and one of unpolluted soil were left unamended and used as reference. Each treatment was performed in triplicate. *Lactuca sativa* and *Spinacia oleracea* were planted in separated pots. The pots were laid out at room temperature (25 °C) and they were watered three times a week with 500 ml of tap water. Plants grown in the control pots were harvested after one month. The vegetables grown in the pots filled with polluted soil amended with vermiculite were harvested twice: after one month and after two months. All harvested plants were oven-dried at 60 °C for 16 h, then ground in an agate mortar. 0.2 g of ground plant material were digested with 10 ml of concentrated HNO_3 in a microwave oven, using the following heating program: 5 min at 250 W, 5 min at 400 W, 5 min at 600 W, 5 min at 250 W and 25 min of air ventilation. After cooling, the digestion solutions were filtered with paper filters and diluted to 50 ml. The concentrations of Cr, Cu, Mn, Ni, Pb and Zn in the digests were determined by ICP-AES.

The transfer capability of heavy metals from soil to the edible part of vegetables was described using the translocation factor, calculated according to the following formula:

TF = metal concentration in edible plant parts (mg kg^{-1} dry weight) / metal concentration in substrate (mg kg^{-1} dry weight) (Cui et al., 2004; Greger et al., 2007; Li et al., 2010).

At the end of the pot experiments, soil samples were taken out of each pot, air-dried, sieved through a 2 mm sieve and ground. Sub-samples were used to determine the pH changes and other sub-samples were used to investigate changes in the metal fractionation with Tessier’s sequential extraction procedure (section 2.3.2).

3. Results and discussion

3.1. Soil characterization

Table 1 reports particle size distribution and some general characteristics, namely percentages of organic carbon, organic matter and CEC, of the soils examined in this study.

The average percentage of organic matter is higher in the uncontaminated soil than in the contaminated soil; this result was not unexpected since the soil used as reference was chosen to obtain a good growth of vegetables and it hence is rich in humus and other organic substances. As to particle size analysis, the most abundant components are sand and fine sand in the uncontaminated and in the contaminated soil respectively. Therefore both soils can be considered as “loamy sand” according to the USDA textural triangle (USDA Forest Service, Soil Conservation Service, 1983). The clay percentage in the uncontaminated soil is higher than in the contaminated soil, but this is not sufficient to explain the high CEC evidenced in the former. The most likely explanation of this trend is that part of this capacity is due to the higher humus content of this soil.

3.1.1. Total metal content

The pH and total content of 15 metals together with the relative standard deviations are reported in Table 2. It must be borne in mind that elements such as Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn are present in unpolluted soils at what can be defined “background level”, both as a result of natural phenomena, such as the contribution of the parent material, and of common anthropogenic activities. We can suspect or confirm the presence of pollution when the concentrations are higher than the typical values for soils found in literature and exceed the levels present in the nearby areas. In this study, in order to define the presence and level of contamination, the concentrations of elements were compared with the normal ranges in soils (Alloway, 1990) and Earth’s crust (Turekian and Wedepohl, 1961) and with the maximum acceptable levels in soils according to the Italian Legislation (Ministerial Decree 2006) for the reclamation of contaminated sites (Table 3). Italian limits depend on land use, and are lower for public and private green areas and residential sites (“A” limits) and higher for industrial areas (“B” limits).

The soil samples considered have low pH values (mean: 5.10; range: 4.06 – 6.08), therefore they can be defined as acid (from very strongly acid to moderately acid). Such pH values are however within the range normally found in typical temperate environment (4 – 8), pH values in these samples result however in agreement with this range. The pH of the soils investigated can be likely low owing to the low buffering by Al; in fact this element is present at lower concentrations than the mean concentrations in soil and Earth’s crust.

High concentrations of Cd, Cr, Cu, Ni, Pb and Zn were found in Borgomanero site. In particular, the contents of Cr, Cu and Ni exceeded Italian “B” limits, and those of Cd, Pb and Zn were higher than “A” limits. The presence of Cu, Cr and Ni could be due to an input from the effluents of electroplating industries.

The elements of mainly geochemical origin, such as Al, La, Mn, Y and Zr, are instead present at concentrations lower than the typical values in soil and Earth’s crust. This may be due to a "dilution effect” of the black sludge carried by the floods in this area on the native lithogenic element content.

The contamination is high in the soil samples collected in the core of the site and it seems to decrease in the soil samples collected at the border of site, where the spontaneous vegetation is present.

As a whole, the investigated metals can be divided into two groups: (1) Cd, Cr, Cu, Ni, Pb and Zn, whose concentrations are heavily affected by anthropogenic inputs, and (2) Al, Fe, La, Mn, Sc, Ti, V, Y and Zr, which are mainly of geochemical origin, even if a contribution from human activities (especially for Fe) cannot be excluded.

3.1.2. Metal mobility

We studied the fractionation of metals with Tessier’s protocol. This procedure has been applied and accepted by a large group of specialists (e.g. Irvine et al., 2009; Rico et al., 2009; Lopez Sanchez et al., 1996) even if, like all others sequential extraction procedures, it suffers from several drawbacks, such as lack of selectivity and element redistribution during extraction, and it provides operationally defined results (Bermond and Yousfi, 1997; Gómez-Ariza et al., 1999). In any case the partitioning of the metals into the different fractions is a suitable tool to estimate mobility and plant availability of many elements in soils (Sager et al., 2007) and gives an indication of their potential harmful effects (Yukselen and Gokyay, 2006). Figures 2a and 2b show the sequential extraction results. The data are expressed as percent fractions of the total concentration.

Figure 2. Percentages extracted into the first four fractions according to Tessier’s procedure (untreated contaminated soil) for: a) Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn and b) Al, La, Sc, Ti, V, Y and Zr.

The results obtained clearly show that the samples collected in the centre of the site (B1 – B4) are characterised by higher percentages of metals extracted into the first two fractions, hence they seem to be polluted in a higher extent. This could be the explanation for the absence of spontaneous vegetation in the centre of the site. In fact many researchers (Sager et al., 2007; Lua et al., 2003; Li et al., 1995; Delmas et al., 2002) affirm that the exchangeable fraction, or the exchangeable plus bound to carbonates fractions, should be readily available to plant roots.

The amounts found in the first fraction in B1 – B4 samples are very high, in particular for Cd, Cu, Ni, Pb and Zn. Also a relatively high percentage of Y was released: generally this element has a geological origin and it is not extracted into the first fractions at detectable levels. Nevertheless it is not possible to ascertain whether this behaviour is due to an anthropogenic origin, since its fractionation with Tessier's protocol or with other sequential extraction schemes was rarely considered (Abollino et al., 2006). The relatively large $MgCl_2$-extractable fraction of the above-mentioned elements in this soil is also due, at least in part, to the low soil pH.

The percentages extracted into the second fraction are lower than in the first one for most of the metals considered, even if they are higher than those usually reported for contaminated soils (Abollino et al., 2002; Lu et al., 2003). Altogether the percentages extracted into the first two fractions of Tessier are much higher than the ones obtained for agricultural soils of Piedmont studied in a previous work (Abollino et al., 2002). In such unpolluted soils, most elements (with the exception of Al, Fe, Mn, Ti) were undetectable by ICP-AES in the first fraction and were typically present at levels below 1 % in the second one. In particular Cd, Cu, Ni, Pb and Zn, whose total concentrations in B1-B4 samples are higher than the Italian "A" limits, were extracted at percentages between 4.62 and 32.50 % and between 3.17 and 20.45 % respectively into the first and second fraction, while the extraction percentages of the same metals from the agricultural soils were lower than 0.8 and 4 % respectively. These results clearly indicate that these metals can be identified as pollutants in Borgomanero soil.

The percentages of metals extracted into the third fraction are usually higher than those present in the previous ones, reaching 77.83 % for Cd in B7 sample. The main soil pollutants are extracted into this fraction at high percentages with the exception of Cu that, instead, is principally released into the fourth fraction. This behaviour is in agreement with the general findings that this element forms stable complexes with organic matter (Wong et al., 2002). Instead, Mn is extracted at low percentages into the third fraction and it is mainly present in the residual fraction; this probably occurs because it derives from the dissolution of crystalline oxides containing this element.

The behaviour of Cr is in agreement with its features of inert metal and with the results of many other studies, which reported a low availability for this element both in clean and contaminated soils (e.g. Burt et al., 2003; Abollino et al., 2006). It is interesting however to note that this element is present at high percentages in the third and fourth fraction, while usually it is extracted nearly exclusively in the residual fraction. It is possible that this element was discharged in the soil investigated as Cr(VI). This form is more mobile than Cr(III) and is considered the most toxic form of Cr. Cr(VI) is a strong oxidising agent, having a high positive reduction potential, and in the presence of soil organic matter is reduced to Cr(III) (Bartlett and James, 1988; Alloway 1990). This reduction is more rapid in acid soils, like that of Borgomanero, than in alkaline ones. Some researchers found that, following reduction of Cr(VI), Cr in soil was present as hydrated oxides of Cr(III) mixed with or occluded in Fe oxides (Cary et al., 1977). This is a possible explanation for the behaviour of Cr in this soil.

The other elements (Al, Fe, La, Mn, Sc, Ti, V and Zr) were extracted at low percentages in the first four fractions, showing that they are strongly bound to the soil matrix and, hence, can be considered as constituents of the soil.

In general, a clear differentiation is evident between the two groups of elements evidenced above. In particular the metals identified as pollutants (Cd, Cr, Cu, Ni, Pb and Zn) are characterised by a high mobility and hence they could be released into the environment upon a change in ionic strength, soil pH or redox potential. Instead the elements identified as lithogenic (Al, Fe, La, Mn, Sc, Ti, V, Y and Zr) are mainly associated to the residual fraction and this is a further confirmation of their natural origin.

3.2. Pot experiments with lettuce and spinach

We considered two soil samples: a composite sample of Borgomanero soil and an uncontaminated soil used as control. Our previous studies on synthetic solutions showed that vermiculite is a very efficient sorbent for heavy metals (Malandrino et al., 2006; Abollino et al., 2008). In order to test the behaviour of this clay in a real scenario, we studied its effectiveness as soil amendment in reducing the phytoavailability of the metal pollutants. We evaluated the effect of the addition of vermiculite by measuring metal uptake by plants, using lettuce and spinach as test crops, and by the first two fractions of Tessier's protocol, that provide assessment of potential metal availability to plants. In this way we also evaluated the suitability of the extractants used in such fractions in predicting the phytoavailability of metal pollutants.

3.2.1. Effect of amendment on the uptake of metals by lettuce and spinach

Heavy metals, when present in excess, disturb plant metabolism, affecting respiration, photosynthesis, stomata opening and growth.

The extent of assimilation of heavy metals from soil depends on whether they are present in a form that can be absorbed by plants. For example, Pb can be strongly absorbed by soil particles and, thus, it is scarcely translocated to plants, while Cd ions are relatively mobile in soil and can be more easily absorbed by vegetation. Plants accumulate heavy metals from soils through different mechanisms such as: absorption, ion exchange, redox reactions, precipitation – dissolution, etc. In addition to these accumulation mechanisms, the solubility of trace elements in soils depends on the minerals present in them (carbonates, oxides, hydroxides, etc.), on the level of soil organic matter (humic acids, fulvic acids, polysaccharides and organic acids), soil pH, redox potential, temperature and humidity (Tarradellas et al., 1996). Only the portions of elements which present availability are transferred into plants (Smical et al., 2008).

In order to evaluate the effectiveness of treatment with vermiculite for the reduction of the phytoavailability of the pollutants, green salad (*Lactuca sativa*) and spinach (*Spinacia oleracea*) were selected as test plants. The first was chosen because it is easily available, it assimilates all relevant toxic metals, and common people have some experience in growing it worldwide. As early as 1957, lettuce seedlings were proposed as a means to investigate the amount of available nutrients in test soils, as an alternative to chemical extraction methods. In fact, lettuce is a general indicator for heavy metals because it can easily accumulate high metal concentrations per plant biomass. Spinach was chosen because, like lettuce, is a very popular and commonly seen leafy vegetable and, moreover, many researchers showed that it can be easily contaminated by Cd from soil (Wang et al., 2009; Dheri et al., 2007; Zupan et al., 1995).

Figure 3a demonstrates that metal uptake by lettuce and spinach is much higher in the contaminated soil than in the control soil, due to the high percentages of available elements present in it (see section 3.2.2.). Moreover, it is evident that the addition of vermiculite strongly influences the amount of metals assimilated by plants, in particular for Cr, Cu and Ni, which have a higher concentration in Borgomanero site, and for lettuce, known as bioaccumulator plant (Sager et al., 2007). In particular, comparing the metal concentrations in the leaf vegetables grown in the untreated polluted soil and in the same amended with vermiculite, a percentage decrease from 62 to nearly 100 % for pollutant (Cd, Cr, Cu, Ni, Pb, Zn) uptake was observed.

As soil pH is the most important factor which governs the solid-solution equilibria of metals in soil (Hooda and Alloway, 1998), the effect of addition of vermiculite on soil pH was studied. The application of this clay raised the soil pH by approximately 2 units, from 4.17 to 5.99. Therefore, the influence of vermiculite on metal availability is first of all related to the increase in pH brought about by the addition of this amendment. Many researchers (Hooda and Alloway, 1998; Naidu et al., 1994; Paulose et al., 2007) evidenced an increase of metal sorption in soils with increasing pH. The reasons advanced for this behaviour are: 1) an increase in negative surface charge, resulting in an increase in cation adsorption; 2) a higher probability of formation of hydroxy species of cation metals that have a greater affinity for adsorption sites than the aqueous metal cations and, finally, 3) a higher possibility of precipitation of metal hydroxides. Moreover, previous studies (Malandrino et al., 2006; Abollino et al., 2008) demonstrated that, in this pH condition, vermiculite presents a high uptake total capacity toward the considered metals. Since the pH_{zpc} of this clay is 8.63, at pH ~ 6 most of the silanol and aluminol groups on edges of the clay are protonated, hence the main mechanisms responsible for retention of these metals are sorption by reaction with the planar sites of the clay and consequent formation of outer-sphere complexes and introduction inside the lamellar spaces of this clay.

Metal concentrations in lettuce and spinach plants grown in the unpolluted soil decreased in the order Zn > Mn > Cu > Ni ≈ Cr > Pb ≈ Cd. This behaviour suggests that, in natural conditions, these vegetables have a similar trend of assimilation capability for these elements. In the vegetables cultivated in the contaminated soil the order of heavy metal concentrations was different: Cu > Cr > Ni > Zn > Pb > Mn > Cd. This probably occurs because Cu, Cr and Ni were present at high concentrations in the contaminated soil and, hence, they were preferentially assimilated by plants even if the vegetables considered do not have higher assimilation capacity towards these elements in comparison to the other ones. The addition of vermiculite caused variation in the concentration order of heavy metals in lettuce and spinach relative to that observed in untreated contaminated soil. In detail, the concentrations in lettuce and spinach decreased, respectively, in the order Mn > Ni > Zn > Cu > Cr > Pb ≈ Cd and Zn > Ni > Cu > Mn > Cr > Pb ≈ Cd after the first harvest, while the sequences observed after the second harvest were respectively Mn > Zn > Cu > Cr > Ni > Pb ≈ Cd and Zn > Mn > Cu > Ni > Cr> Pb ≈ Cd. Therefore, it is evident that the addition of vermiculite caused a decrease in the phytoavailability of the pollutants such as Cr, Cu and Ni and that, increasing the contact time between contaminated soil and clay, the order of metal assimilation for both vegetables verges on that found in control soil, i.e. in natural conditions. Nevertheless the metal concentrations found in vegetables grown in soil treated with vermiculite were higher than the values usually reported for leaf vegetables grown in unpolluted soils (Gaw et al., 2008; Li et al., 2010) and in our control soil. Further studies are necessary to find out whether, increasing the contact time between polluted soil and vermiculite, the metal concentrations in lettuce and spinach would fall again in the natural ranges for these vegetables.

The TF values of heavy metals from soil to vegetables are shown in Figure 3b.

Figure 3. a) Metal uptake by lettuce and spinach grown on uncontaminated soil, untreated contaminated soil and contaminated soil amended with vermiculite (metal uptake by: LNC = lettuce grown on uncontaminated soil; SNC = spinach grown on uncontaminated soil; VC = vegetables (lettuce + spinach) grown on untreated contaminated soil; LCV I = lettuce grown on amended

contaminated soil (first harvest); LCV II = lettuce grown on amended contaminated soil (second harvest); SCV I = spinach grown on amended contaminated soil (first harvest); SCV II = spinach grown on amended contaminated soil (second harvest). b) Translocation factors (TF) of metals from soil to plants (data for Cd not shown because the concentrations in plants were lower than the ICP detection limit).

These values are higher than the TFs usually reported for field-grown vegetables (Smical et al., 2008; Li et al., 2010). In fact it must be borne in mind that the uptake of metals from soils is greater in plants grown in pots than in the field from the same soil. This is probably due to differences in microclimate and soil moisture, and mainly to the fact that the roots of container-cultivated plants grow solely in contaminated soil and more closely near from each other, whereas those of field-cultivated plants may reach down to less contaminated soil layers (Smical et al., 2008; Benzarti et al., 2008). The soil-to-plant TF values decrease in the order Zn > Mn > Ni > Cu > Cr > Pb > Cd. These values are similar for both vegetables but they differ significantly among control soil, contaminated soil and the same amended with vermiculite. The difference in TFs between control and contaminated soils may be related both to their metal content and to general soil properties. Actually, the control soil, besides being characterised by lower metal content, is also higher in organic matter and clay content and, hence, it has a higher uptake capacity towards metals. Compared to the very high content of Pb and Cd in the polluted soil, the transfer of these metals to the plants is always quite smaller than the uptake of the other metals. This could be due to the absorption of a higher quantity of Pb and Cd by the clay – humic complex in the soil (Smical et al., 2008) or to the low affinity of plants for these elements, which have no role in their metabolism. The metal uptake of the two vegetables grown in contaminated soil added with vermiculite changed over time, i.e. from the first to the second harvest, with an opposite trend, showing an increase in the lettuce for all elements with the exception of Ni and a decrease in the spinach for all elements with the exception of Mn. This suggests that these plants have different capacity to absorb and eliminate toxic elements, but the detailed mechanism needs to be further investigated. Spinach seems to have a higher assimilation capacity since it absorbs in greater extent all elements with the exception of Cr but it is likely also less inhibited by these metals since the metal content in this plant decreases over time in correlation with an increase in biomass.

The values of TF clearly show that the addition of vermiculite to the contaminated soil greatly decreases the translocation of toxic metals to the plants rendering it similar to that one found in the control soil for all elements with the exception of Mn. The different behaviour of this element is explicable considering that it is essential for plants at higher concentrations and it is present as exchangeable ion in the interlayer of vermiculite.

Altogether it is evident that the contaminated soil is not suitable for lettuce and spinach growth, whereas the treatment with vermiculite effectively reduces the phytoavailability of the pollutants to the two plants, even if the metal uptake in the two crops significantly differs over time.

3.2.2. Effect of amendment on mobility and reactivity of metals in soil

The percentages of metals in the fractions obtained by sequential extraction for contaminated soil, contaminated soil amended with vermiculite and the control soil before and after plant growth are reported in Figure 4. Cd was not considered in this part of the study because it was always lower than the instrumental detection limit (10 µg L^{-1}) in the uncontaminated soil and it was not absorbed in significant amounts in the two vegetables considered.

Figure 4. Metal percentages extracted into the first four fractions according to Tessier's procedure for: untreated contaminated soil before (a) and after (d) plant growth; contaminated soil amended by vermiculite before (b) and after (e) plant growth; uncontaminated soil before (c) and after (f) plant growth.

The percentages of metals extracted into the first two fractions were higher in the contaminated soil than in the control pot with the exception of Mn, that was characterised by a opposite trend, likely because it is not a pollutant in Borgomanero site and it is present at higher percentage as carbonate in the control soil. The application of vermiculite was effective in reducing the exchangeable and carbonate bound fractions of Cu, Ni, Pb and Zn in soil, that is of the elements present as pollutants in Borgomanero site with the exception of Cr.

In the case of Ni, Pb and Zn, the plant growth caused a pronounced variation in the fractionation pattern for the contaminated samples, since the percentages extracted into the first two fractions increased whereas those extracted into fractions III and IV decreased. The most striking effect of plant growth on metal fractionation in the control soil was the increase of the amounts of Cr, Cu, Ni, Pb and Zn bound to organic matter and sulphides. These changes in fractionation can be explained with the interaction of root exudates with the surroundings solids. Excretion of root exudates, which contain organic acids, amino acids, sugars and high molecular weight compounds, increase the solubility and bioavailability of metals in the rhizosphere (Mench and Martin, 1991; Sager et al., 2007).

The rhizosphere is the zone about 1 – 2 mm wide between plant roots and the surrounding soil. It receives appreciable amounts of organic material from the roots, including exudates, mucilage, sloughed-off cells and their lysates. These organic compounds give rise to intense microbiological and biochemical activity in the rhizosphere which enables roots to mobilise some of the metals which are adsorbed in the soil, by acidification, redox changes, or the formation of organic complexes. It is possible that in the control soil, which has a higher organic matter content, the metals mobilised by roots in the rhizosphere are mainly present as organic complexes, while in the contaminated soil amended by vermiculite they are characterised by higher solubility.

Metal percentages extracted in the first two fractions are increased in all samples following plant growth. It is necessary to specify that in any case the exchangeable and carbonate bound fractions of all elements in the contaminated soil treated with vermiculite were always lower than in the untreated contaminated soil. However, the effect of plant growth on fractions III and IV extracted from the untreated and amended contaminated samples goes in different directions, namely the metal percentages extracted into these fractions are increased in the former and decreased in the latter. Our findings agree with those of other researchers (Sager et al., 2007; Mench and Martin, 1991) who found a significantly enhanced metal solubility in the rhizosphere of different plants and evidenced that the mobilization processes seem to be more important on moderately contaminated soils than on strongly contaminated soils.

It must be borne in mind that the plant roots, when growing in pots, are in much closer contact with one another and with the adjacent soil than in the field and, for this reason, fractionation changes are more probable to be detected from pot experiments. Hence, it is not sure that the same fractionation changes would be found in field conditions.

Finally, the sum of the first two fractions of Tessier’s protocol for Cu, Ni and Zn were significantly correlated with their concentrations in plants ($r = 0.842$ for Cu, $r = 0.846$ for Ni, $r = 0.942$ for Zn; $p < 0.05$), while a similar correlation was not found for Cr and Mn. Indeed, we found that the content of Mn in plants was negatively correlated with the sum of the amounts extracted into the first two fractions ($r = - 0.959$; $p < 0.05$). This probably occurs because Mn is not a pollutant in Borgomanero soil and it is an essential element for plants; a low correlation was found for Cr, probably due to its feature of inert metal that gives rise to its low mobility. Altogether in this study we found that the sum of first two fractions of Tessier’s protocol is suitable in predicting the phytoavailability of Cu, Ni and Zn but fails in the case of Cr and Mn.

4. Conclusions

From this study, it can be concluded that:

1) High concentrations of Cd, Cr, Cu, Ni, Pb and Zn were found in Borgomanero site. In particular, the contents of Cr, Cu and Ni exceeded Italian “B” limits, and those of Cd, Pb and Zn were higher than “A” limits.
2) The percentages of metals extracted into the first two fractions of Tessier’s protocol were higher in the samples collected in the centre than in those taken at the border of the site. In particular, Cd, Cu, Ni, Pb and Zn were easily extracted from the soil, suggesting that they can be readily transferred to other environmental compartments, such as water and plants.
3) The vermiculite is a good candidate as amendment for chemical stabilization of contaminated soils, because it demonstrated to reduce significantly the uptake of metal pollutants by lettuce and spinach. Its effectiveness increases with increasing contact time with the polluted soil.
4) The plant growth had different effects on the metal fractionation pattern in the contaminated and in the control soils, namely giving rise to an increase in extractability into the first two fractions for the former and to an increase in the percentages released into the fourth fraction for the latter. Surely the excretion of root exudates, which contain organic acids, amino acids, sugars and high molecular weight compounds, plays an important role in increasing the mobility of metals in the soils.
5) The sum of the metal percentages extracted into the first two fractions of Tessier’s protocol is suitable in predicting the phytoavailability of the pollutants present in the investigated soil (Cu, Ni and Zn) with the exception of Cr, owing to its features of inert metal.

The knowledge of the soil-plant relationships in contaminated sites is an important issue because the use of polluted soils in urban and peri-urban areas for agriculture is increasing, and the consumption of plants grow in such soils could have a harmful effect to humans.

In this scenario it is essential to individuate a simple and cost-effective remediation technique that allows the reduction of metal assimilation from contaminated soils by edible plants. The chemical stabilization by vermiculite proposed in this study demonstrated to meet all these requirements.

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References

Abollino, O., Aceto, M., Malandrino, M., Mentasti, E., Sarzanini, C., Barberis, R., 2002. Distribution and mobility of metals in contaminated sites. Chemometric investigation of pollutant profiles. Environ. Pollut.119, 177-193.

Abollino, O., Aceto, M., Malandrino, M., Mentasti, E., Sarzanini, C., Petrella, F., 2002. Heavy metals in agricultural soils from Piedmont, Italy. Distribution, speciation and chemometric data treatment. Chemosphere 49, 545–557.

Abollino, O., Giacomino, A., Malandrino, M., Mentasti, M., Aceto, M., Barberis, R., 2006. Assessment of metal mobility in a contaminated soil by sequential extraction. Water Air Soil Poll. 137, 315–338.

Abollino, O., Giacomino, A., Malandrino, M., Mentasti, E., 2008. Interaction of metal ions with montmorillonite and vermiculite. Appl. Clay Sci. 38, 227–236.

Alloway, B.J., 1990. Heavy metals in soils. Blackie, John Wiley and Sons Inc., Glasgow, London, New York.

Bartlett, R.J., James, B.R., 1988. Mobility and bioavailability of chromium in soils, in: Nriagu, J.O., Nieboer, E. (Eds.), Chromium in the Natural and Human Environment. John Wiley and Sons Inc., New York, pp. 267-304.

Benzarti, S., Mohri, S., Ono, Y., 2008. Plant response to heavy metal toxicity: Comparative study between the hyperaccumulator Thlaspi caerulescens (ecotype Ganges) and nonaccumulator plants: Lettuce, radish, and alfalfa. Environ. Toxicol. 23, 607-616.

Bermond, A.P., Yousfi, I., 1997. Reliability of comparisons based on sequential extraction procedures applied to soil samples: The thermodynamic point of view. Environ. Technol. 18, 219-224.

Bolan, N.S., Duraisamy, V.P., 2003. Role of inorganic and organic soil amendments on immobilization and phytoavailability of heavy metals: a review involving specific case studies. Aust. J. Soil Res. 41, 533–555.

Burt, R., Wilson, M.A., Keck, T.J., Dougherty, B.D., Strom, D.E., Lindahl, J.A., 2003. Trace element speciation in selected smelter-contaminated soils in Anaconda and Deer Lodge Valley, Montana, USA. Adv. Environ. Res. 8, 51–67.

Cary, E.E., Allaway, W.H., Olsen, O.E., 1977. Control of Chromium Concentration in Food Plants: 2. Chemistry of Chromium in Soils and Its Availability to plants. J. Agric. Food Chem. 25, 305-309.

Chhonkar, P.K., Datta, S.P., Joshi, H.C., Pathak, H., 2000a. Impact of industrial effluents on soil health and Agriculture – Indian experience. Part I – Distillery and paper mill effluents. J. Sci. Indust. Res. 59, 350–361.

Chhonkar, P.K., Datta, S.P., Joshi, H.C., Pathak, H., 2000b. Impact of industrial effluents on soil health and Agriculture – Indian experience. Part II – Tannery and textile industrial effluents. J. Sci. Indust. Res. 59, 446–454.

Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qiu, Y., Liang, J.Z., 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environ. Int. 30, 785–791.

Davidson, C.M., Duncan, A.L., Littlejohn, D., Ure, A.M., Garden, L.M., 1998. A critical evaluation of three-stage BCR sequential extraction procedures to assess the potential mobility and toxicity of heavy metals in industriallycontaminated land. Anal. Chim. Acta 363, 45–55.

Delmas, C., Larpin, L., Legret, M., Astruc, M., 2002. Mobility and adsorption capacity of Pb and Zn in a polluted soil from a road environment: Laboratory batch experiments. Environ. Technol. 23, 381–390.

Gaw, S.K., Kim, N.D., Northcott, G.L., Wilkins, A.L., Robinson, G., 2008. Uptake of ΣDDT, Arsenic, Cadmium, Copper, and Lead by Lettuce and Radish Grown in Contaminated Horticultural Soils. J. Agric. Food Chem. 56, 6584–6593.

Gomez-Ariza, J.L., Giraldez, I., Sanchez-Rodas, D., Morales, E., 1999. Metal readsorption and redistribution during the analytical fractionation of trace elements in oxic estuarine sediments. Anal. Chim. Acta. 399, 295-307.

Greger, M., Malm, T., Kautsky, L., 2007. Heavy metal transfer from composted macroalgae to crops. Eur. J. Agron. 26, 257–265.

Gupta, U.C., Gupta, S.C., 1998. Trace element toxicity relationships to crop production and livestock and human health: Implications for menagement. Commun. Soil Sci. Plant Anal. 29, 1491–1522.

Gworek, B., 1992. Lead inactivation in soils by zeolites. Plant Soil 143, 71–74.

Hooda, P.S., Alloway, B.J., 1998. Cadmium and Lead sorption behaviour of selected English and Indian soils. Geoderma 84, 121-134.

Irvine, K.N., Perrelli, M.F., Ngoen-klan, R., Droppo, I.G., 2009. Metal levels in street sediment from an industrial city: spatial trends, chemical fractionation, and management implications. J. Soils Sediments. 9, 328-341.

Khattak, R.A., Page, A.L., 1992. Mechanism of manganese adsorption on soil constituents. In: Adriano, D.C. (Ed.), Biogeochemistry of Trace Metals. Lewis Publ., Boca Raton, FL, pp. 383–400.

Li, Q.S., Cai, S.S., Mo, C.H., Chu, B., Peng, L.H., Yang, F.B., 2010. Toxic effects of heavy metals and their accumulation in vegetables grown in a saline soil. Ecotox. Environ. Safe. 73, 84–88.

Li, X., Coles, B.J., Ramsey, M., Thornton, I., 1995. Chemical partitioning of the new National Institute of Standards and Technology Standard Reference Materials (SRM 2709–2711) by sequential extraction using Inductively Coupled Plasma Atomic Emission Spectrometry. Analyst. 120, 1415–1419.

LopezSanchez, J.F., Rubio, R., Samitier, C., Rauret, G., 1996. Trace metal partitioning in marine sediments and sludges deposited off the coast of Barcelona (Spain). Water Res. 30, 153-159.

Lu, Y., Gong, Z.T., Zhang, G.L., Burghardt, W., 2003. Concentrations and chemical speciations of Cu, Zn, Pb and Cr of urban soils in Nanjing, China. Geoderma 115, 101-111.

Lua, Y., Gonga, Z., Zhanga, G., Burghardtc, W., 2003. Concentrations and chemical speciations of Cu, Zn, Pb and Cr of urban soils in Nanjing, China. Geoderma**.** 115, 101–111.

Malandrino, M., Abollino, O., Giacomino, A., Aceto, M., Mentasti, E., 2006. Adsorption of heavy metals on vermiculite: Influence of pH and organic ligands. J. Colloid Interface Sci. 299, 537–546.

McGrath, S.P., Smith, S., 1990. Chromium and nickel, in: Alloway, B.J., (Ed.), Heavy metals in soils. Blackie, John Wiley and Sons Inc., Glasgow, London, New York, 125-150.

McGrath, S.P., Zhao, F.J., Lombi, E., 2002. Phytoremediation of metals, metalloids, and radionuclides. Adv. Agron. 75, 1–56.

Mench, M., Martin, E., 1991. Mobilization of cadmium and other metals from two soils by root exudates of Zea mays L., Nicotiana tabacum L., and Nicotiana rustica L. Plant Soil 132, 187–196.

Ministerial Decree, 2006, Norme in materia ambientale. Italian Official Gazzette no. 152.

Naidu, R., Bolan, N.S., Kookana, R.S., Tiller, K.G., 1994. Ionic-strength and pH effects on the sorption of cadmium and the surface charge of soils. Eur. J. Soil Sci. 45, 419-429.

Paulose B., Datta, S.P., Rattan, R.K., Chhonkar, P.K., 2007. Effect of amendments on the extractibility, retention and plant uptake of metals on a sewage-irrigated soil. Env. Pollut. 146, 19-24.

Pierzynski, G.M., Sims, J.T., Vance, G.F., 2000. Soil phosphorus and environmental quality. Soils and Environmental Quality. CRC Press, Boca Raton. 155–207.

Rattan, R.K., Datta, S.P., Chandra, S., Saharan, N., 2002. Heavy metals and environmental quality, Indian scenario. Fertil. News 47, 21–26.

Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K., Singh, A.K., 2005. Long-term impact of sewage irrigation with sewage effluents on heavy metal content in soils, crops and groundwater – A case study. Agric. Ecosyst. Environ. 109, 310–322.

Rico, M.I., Alvarez, J.M., Lopez-Valdivia, L.M., Novillo, J., Brador, A., 2009. Manganese and zinc in acidic agricultural soils from central Spain: distribution and phytoavailability prediction with chemical extraction tests. Soil Sci. 174, 94-104.

Sager, M., Park, J.H., Chon, H.T., 2007. The effect of soil bacteria and perlite on plant growth and soil properties in metal contaminated samples. Water Air Soil Pollut. 179, 265-281.

Smical, A.I., Hotea, V., Oros, V., Juhasz, J., Pop, E., 2008. Studies on transfer and bioaccumulation of heavy metals from soil into lettuce. Environ. Eng. Manag. J. 7, 609-615.

Sposito, G., 1989. The Chemistry of soils. Oxford University Press, Inc., New York.

Tarradellas, J., Bitton, G., Russel, D., 1996. Soil Ecotoxicology, CRC Lewis Publisher, New York.

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.

Tessier, A., Campbell, P., Bisson, M., 1980. Trace metal speciation in the Yamaska and St. Francois Rivers (Quebec). Can. J. Earth Sci. 17, 90–105.

Turekian, K.K., Wedepohl, K.H., 1961. Distribution of the elements in some major units of the Earth’s Crust. Geol. Soc. Am. Bull. 72, 175–92.

USDA Forest Service, Soil Conservation Service, 1983. Soil taxonomy: A basic system of soil classification for making & interpreting soil surveys, Wiley, New York.

U.S. Environmental Protection Agency, 1991. ROD annual reports FY 1990. USEPA report 540/8 – 91/067. USEPA, Washington, DC.

Vangronsveld, J., Van Assche, F., Clijsters, H., 1990. Immobilization of heavy metals in polluted soils by application of a modified alumino-silicate: Biological evaluation. In: Barcelo, J. (Ed.), Environmental Contamination. Proc. 4th Int. Conf. Barcelona. CEP Consultants, Edinburgh, UK, pp. 283–285.

Yukselen, M.A., Gokyay, O., 2006. Leachability of metals from soil contaminated by mining activities. Environ. Eng. Sci. 23, 125-132.

Wong, S.C., Li, X.D., Zhang, G., Qi, S.H., Min, Y.S., 2002. Heavy metals in agricultural soils of the Pearl River Delta, South China. Environ. Pollut. 119, 33–44.

Zupan, M., Hudnik, V., Lobnik, F., Kadunc, V. 1995. Accumulation of Pb, Cd, and Zn from contaminated soil to various plants and evaluation of soil remediation with indicator plant (Plantago lanceolata). In: Prost, R. (Ed.) Contaminated Soils: Third International Conference on the Biogeochemistry of Trace Elements, INRA Editions, Paris.

Table 1. General characteristics of the investigated soils

		Contaminated Soil	Uncontaminated Soil
Organic Carbon (% w/w)		17.14	34.00
Organic Matter (% w/w)		29.55	58.62
CEC (cmol/kg)		31.30	56.82
Particle Size Distribution			
	% Sand	19.30	49.03
	% Fine Sand	56.88	31.37
	% Silt	10.10	5.88
	% Fine Silt	8.35	6.00
	% Clay	5.37	7.72

Table 2. Total metal concentrations (mg kg^{-1}), standard deviations and pH in soils at Borgomanero site

	B1	B2	B3	B4	B6	B7
pH	5.00	4.06	5.17	4.51	5.80	6.08
Al	24,000 ± 1,300	27,840 ± 904	23,214 ± 984	30,060 ± 978	29,542 ± 4,352	20,964 ± 2,860
Cd	5.93 ± 0.05	5.97 ± 0.09	4.81 ± 0.11	3.37 ± 0.22	1.07 ± 0.04	1.19 ± 0.13
Cr	3,056 ± 106	3,332 ± 172	3,300 ± 18	3,408 ± 20	4,617 ± 200	2,174 ± 183
Cu	4,424 ± 180	5,245 ± 206	5,340 ±287	4,680 ± 140	7,022 ± 282	1,576 ± 148
Fe	30,000 ± 610	26,504 ± 1,225	29,152 ± 442	26,630 ± 183	28,390 ± 1,440	25,416 ± 1,223
La	6.66 ± 0.21	8.09 ± 1.09	7.57 ± 0.90	8.33 ± 0.90	14.7 ± 0.8	6.25 ± 0.94
Mn	268 ± 8	276 ± 16	270 ± 5	240 ± 4	296 ± 16	340 ± 8
Ni	760 ± 18	1,345 ± 8	1,990 ± 40	1,106 ± 13	1,969 ± 89	904 ± 60
Pb	847 ± 36	648 ± 39	641 ± 7	620 ± 22	1,017 ± 59	345 ± 6
Sc	2.70 ± 0.34	3.08 ± 0.24	2.61 ± 0.24	2.77 ± 0.24	3.45 ± 0.50	2.39 ± 0.31
Ti	5,737 ± 120	4,664 ± 253	4,513 ± 79	4,360 ± 138	6,873 ± 173	4,717 ± 254
V	31.9 ± 1.8	27.5 ± 1.6	29.2 ± 0.2	26.6 ± 0.7	30.4 ± 2.1	20.3 ± 2.1
Y	9.20 ± 0.45	7.32 ± 0.94	9.02 ± 0.94	8.07 ± 0.94	8.34 ± 1.21	8.34 ± 1.23
Zn	379 ± 13	552 ± 18	1112 ± 10	573 ± 5	951 ± 45	545 ± 25
Zr	47.1 ± 2.9	45.3 ± 1.4	41.5 ± 1.2	39.1 ± 2.0	49.5 ± 2.1	52.3 ± 3.2

Table 3. Mean metal concentrations in soil samples at Borgomanero site, means and typical ranges in soil and earth's crust, admissible levels in soil according to the Italian Legislation (mg kg^{-1})

	Borgomanero site	Soil[a, b]	Earth's crust[c]	"A" Limit[d]	"B" Limit[e]
Al	25,937	72,000	55,320; 4,200 – 88,000		
Cd	3.72	0.35; 0.01 – 2.0	0.20; 0.035 – 0.42	2	15
Cr	3,315	54; 5 – 1,500	204; 2 – 1,600	150	800
Cu	4,715	25; 2 – 250	52.33; 4 – 250	120	600
Fe	27,680	26,000	39,610; 3,800 – 94,300		
La	8.60	37	54; 10 – 115		
Mn	282	550; 20 – 10,000	1,617; 390 – 6,700		
Ni	1,346	19; 2 – 750	252; 2 – 2,000	120	500
Pb	686	19; 2 – 300	17.80; 1 – 80	100	1,000
Sc	2.83	8.9	10.50; 1 – 30		
Ti	5,144	2,900	3,407; 300 – 13,800		
V	26.80	80; 3 – 500	76.2; 20 – 250	90	250
Y	6.90	25	38.22; 20 – 90		
Zn	685	60; 1 – 900	71.5; 16 – 165	150	1,500
Zr	103	230	157; 19 – 500		

[a] Sposito, 1989

[b] Alloway, 1990

[c] Turekian and Wedepohl, 1961

[d] Limit established by Italian Legislation for public and private green areas and residential sites

[e] Limit established by Italian Legislation for industrial areas

CAPTIONS TO FIGURES

Figure 1. Geographical map of Piedmont and detail of the area investigated (grey square).

Figure 2. Percentages extracted into the first four fractions according to Tessier’s procedure (untreated contaminated soil) for: a) Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn and b) Al, La, Sc, Ti, V, Y and Zr.

Figure 3. a) Metal uptake by lettuce and spinach grown on uncontaminated soil, untreated contaminated soil and contaminated soil amended with vermiculite (metal uptake by: LNC = lettuce grown on uncontaminated soil; SNC = spinach grown on uncontaminated soil; VC = vegetables (lettuce + spinach) grown on untreated contaminated soil; LCV I = lettuce grown on amended contaminated soil (first harvest); LCV II = lettuce grown on amended contaminated soil (second harvest); SCV I = spinach grown on amended contaminated soil (first harvest); SCV II = spinach grown on amended contaminated soil (second harvest). b) Translocation factors (TF) of metals from soil to plants (data for cadmium not shown because the concentrations in plants were lower than the ICP detection limit).

Figure 4. Metal percentages extracted into the first four fractions according to Tessier’s procedure for: untreated contaminated soil before (a) and after (d) plant growth; contaminated soil amended by vermiculite before (b) and after (e) plant growth; uncontaminated soil before (c) and after (f) plant growth.

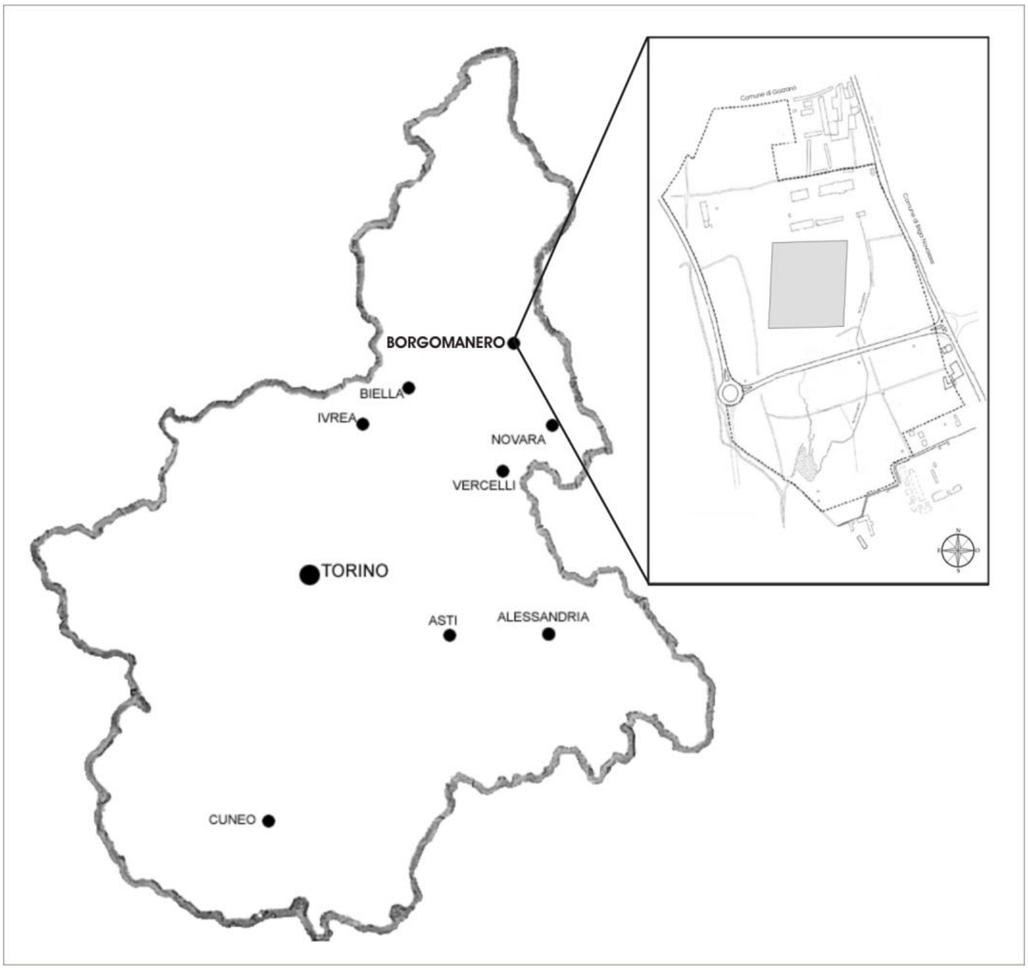
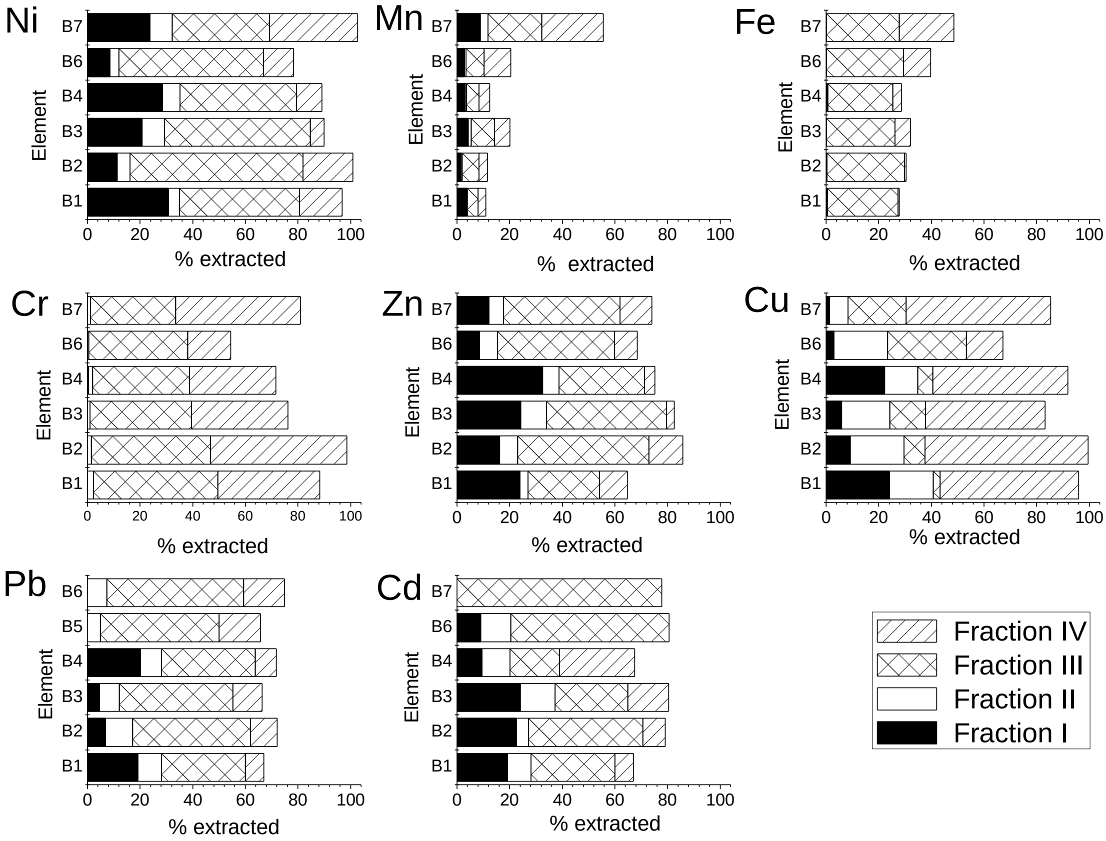

BORGOMANERO
BIELLA
IVREA
NOVARA
VERCELLI
TORINO
ASTI
ALESSANDRIA
CUNEO
Comune di Gozzano

Figure 1

a)


Ni
Mn
Fe
Cr
Zn
Cu
Pb
Cd
B1
B2
B3
B4
B5
B6
B7
Element
% extracted
0
20
40
60
80
100
Fraction IV
Fraction III
Fraction II
Fraction I

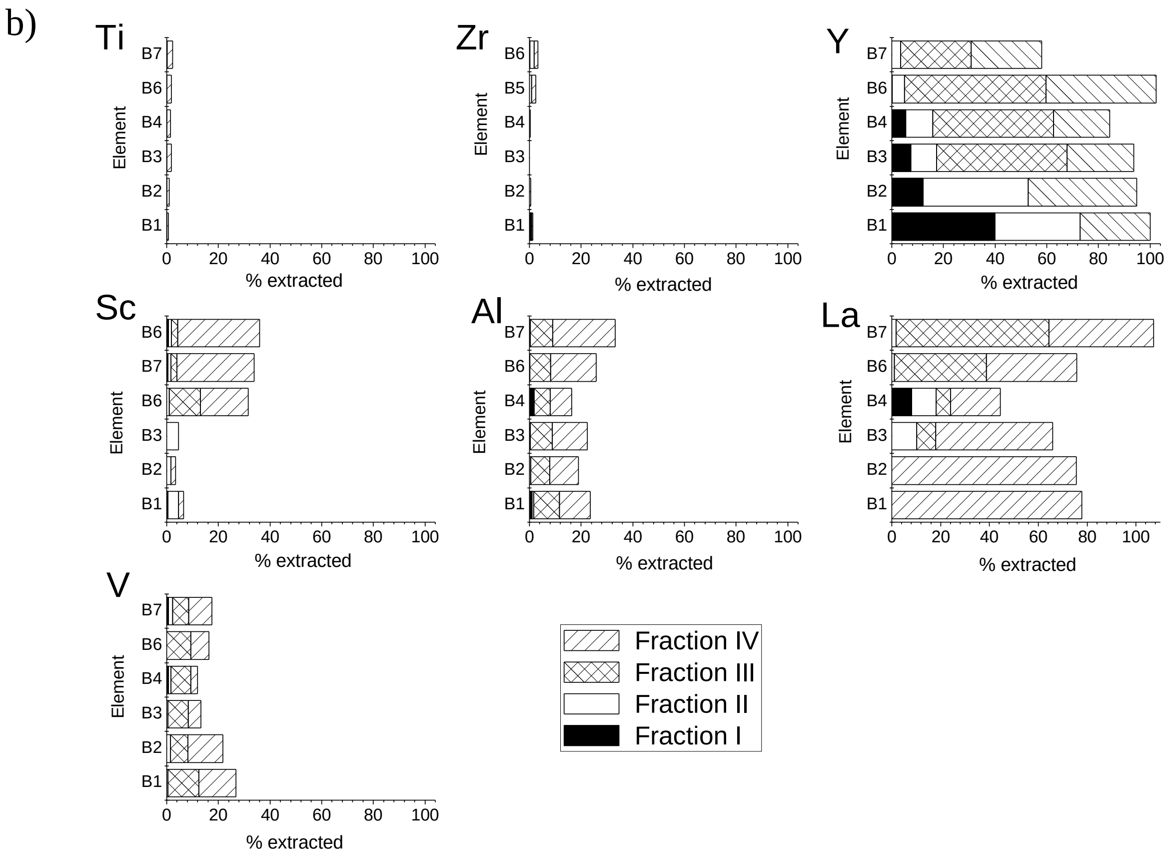

b)
Ti
B7
B6
B4
B3
B2
B1
Element
0
20
40
60
80
100
% extracted
Zr
B6
B5
B4
B3
B2
B1
Element
% extracted
Y
B7
B6
B4
B3
B2
B1
Element
% extracted
Sc
B6
B7
B6
B3
B2
B1
Element
% extracted
Al
B7
B6
B4
B3
B2
B1
Element
% extracted
La
B7
B6
B4
B3
B2
B1
Element
% extracted
V
B7
B6
B4
B3
B2
B1
Element
% extracted
▨ Fraction IV
▩ Fraction III
□ Fraction II
■ Fraction I

Figure 2

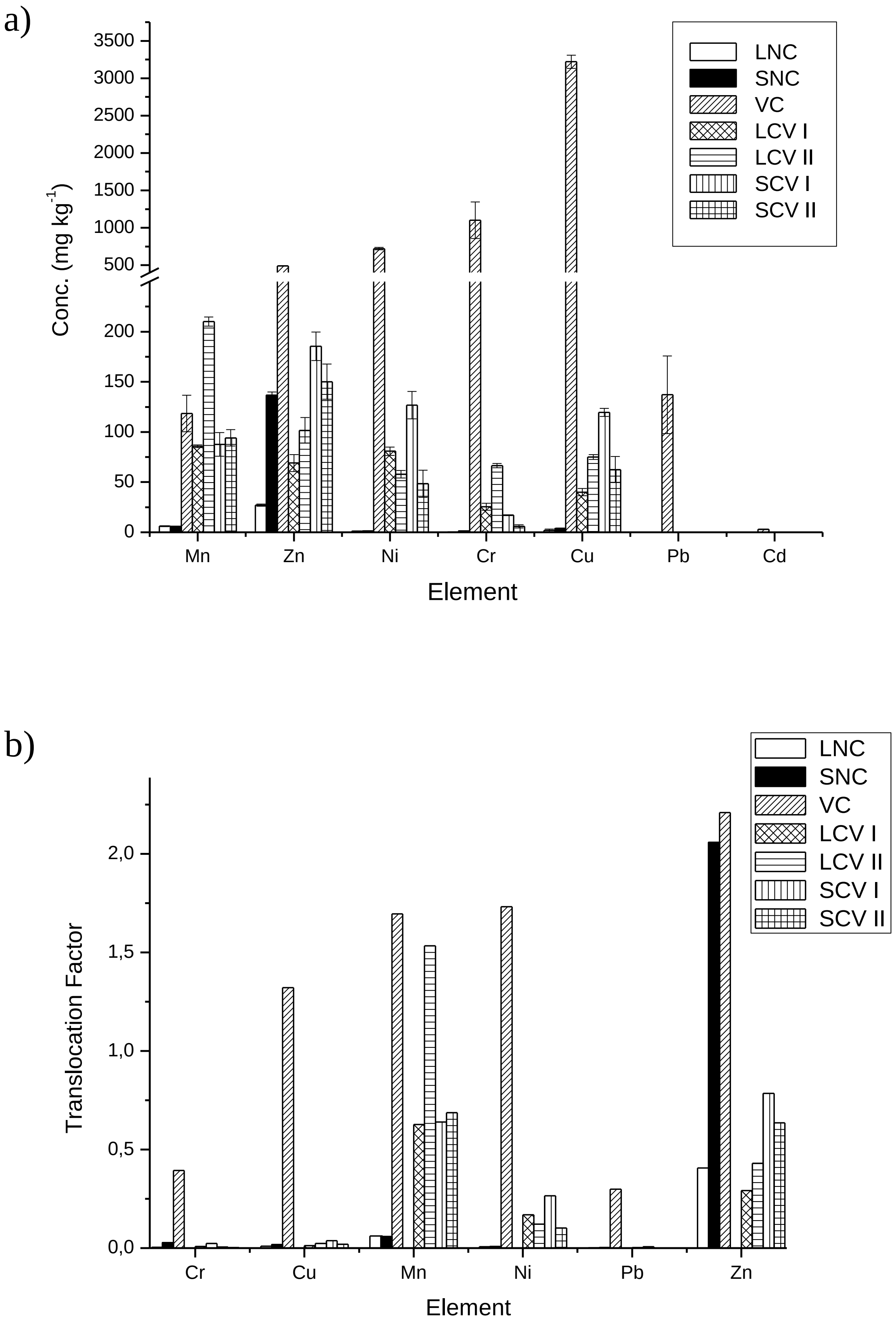

a)
Conc. (mg kg^{-1})
0
50
100
150
200
500
1000
1500
2000
2500
3000
3500
Mn
Zn
Ni
Cr
Cu
Pb
Cd
Element
LNC
SNC
VC
LCV I
LCV II
SCV I
SCV II
b)
Translocation Factor
0,0
0,5
1,0
1,5
2,0
Cr
Cu
Mn
Ni
Pb
Zn
Element
LNC
SNC
VC
LCV I
LCV II
SCV I
SCV II

Figure 3

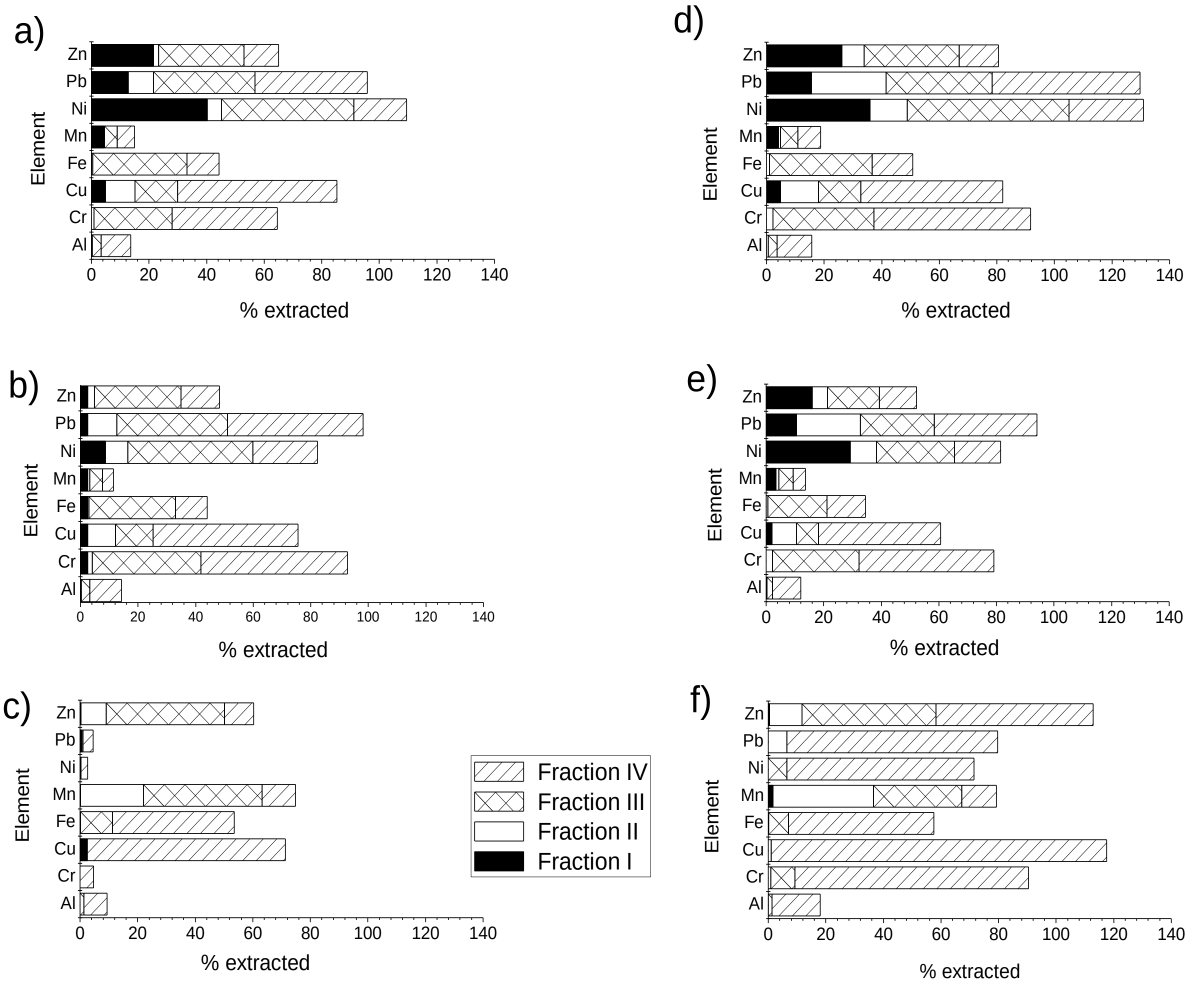

a)
b)
c)
d)
e)
f)
Element
Zn
Pb
Ni
Mn
Fe
Cu
Cr
Al
0
20
40
60
80
100
120
140
% extracted
Fraction IV
Fraction III
Fraction II
Fraction I

Figure 4