Common plants as alternative analytical tools to monitor heavy metals in soil

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From CMA4CH 2010: Multivariate Analysis and Chemometry to Cultural Heritage and Environment
Taormina, Italy. 26-29 September 2010

Abstract

Background: Herbaceous plants are common vegetal species generally exposed, for a limited period of time, to bioavailable environmental pollutants. Heavy metals contamination is the most common form of environmental pollution. Herbaceous plants have never been used as natural bioindicators of environmental pollution, in particular to monitor the amount of heavy metals in soil. In this study, we aimed at assessing the usefulness of using three herbaceous plants (Plantago major L., Taraxacum officinale L. and Urtica dioica L.) and one leguminous (Trifolium pratense L.) as alternative indicators to evaluate soil pollution by heavy metals.

Results: We employed Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to assess the concentration of selected heavy metals (Cu, Zn, Mn, Pb, Cr and Pd) in soil and plants and we employed statistical analyses to describe the linear correlation between the accumulation of some heavy metals and selected vegetal species. We found that the leaves of Taraxacum officinale L. and Trifolium pratense L. can accumulate Cu in a linearly dependent manner with Urtica dioica L. representing the vegetal species accumulating the highest fraction of Pb.

Conclusions: In this study we demonstrated that common plants can be used as an alternative analytical tool for monitoring selected heavy metals in soil.

Background

Heavy metals contamination is one of the major kind of environmental pollution in urbanized cities due to emissions from heating, transport, industry and other human activities. In the past, the main contribution to heavy metals contamination has been due to lead used as anti detonating agent in fuels. At the end of 1998, the European Parliament and Council with the Directive 98/70/EC prohibited the marketing of leaded petrol within their territory. Since that date, the contribution of lead to heavy metal pollution have to depend from other anthropogenic sources (i.e., exausted batteries, paintings and other industrial wastes). Cadmium, zinc and nickel originate from oils, pneumatics and old car pieces in general, copper from cars and other electric vehicles and manganese prevalently from natural sources. Accumulation (and distribution) of anthropogenic heavy metals in soil may depend on wet and dry depositions that convey particles from air to soil. Heavy metals may impair plant physiology by reducing respiration and growth, interfering with photosynthetic processes and inhibiting fundamental enzymatic reactions if accumulated at high concentrations. When these toxic metals are present in soil at a low concentration, plants continue to grow uniformly despite accumulating these metals. The ability of plants to accumulate heavy metals into their organs may hence be used to monitor soil pollution, and in particular the amount of heavy metals.

In the past, several authors investigated the distribution of heavy metals in roadside soil [1-4], grass [5] and leaves [6,7] emphasizing lead accumulation in soils and vegetation [8-10], near highways [11], in small mammals [12,13], humans [14] and invertebrates [15,16]. Other authors focused their attention on heavy metals accumulation by...
higher plants in order to study the urban pollution [17-20].

One interesting study on the air pollution by vehicular traffic in Rome was reported [21], but only higher plants have been considered as environmental pollution markers.

In this study, common plants have been considered for two reasons. First, they are ephemeral: they live for a short time and thus they are exposed only for a very specific period of time to bioavailable pollutants. Second, they can be picked up more easily than other higher plants. Therefore, we studied three herbaceous plants (Plantago major, Linnaeus, Taraxacum officinale, Linnaeus and Urtica dioica, Linnaeus) and one leguminous (Trifolium pratense, Linnaeus) and we compared the heavy metals accumulation in roots and leaves. Together with Cu, Zn, Mn, and Pb we decided to consider also Cr and Pd to investigate if a significant release from vehicles components or from catalytic converters can occur. Our study is therefore aimed at finding simple and reliable vegetal indicators to monitor environmental pollution and in particular soil pollution by heavy metals.

Experimental
Reagents
Concentrated HNO₃ (65%) was purchased by Sigma-Aldrich. Standard reference materials (SRM No. 2587 and 2711) were from the National Institute of Standards and Technology, Gaithersburg, USA.

Apparatus
Analytical determination and data elaboration
The concentration of selected heavy metals (Cu, Mn, Zn, Pb, Cr and Pd) were determined by means of ICP-AES spectrophotometer (Varian Vista MPX CCD. Simultaneous ICP–OES) equipped with a US5000 AT+ nebulizer (Cetac Technologies). In order to maximize the element sensitivity and to avoid interferences, wavelengths were accurately chosen (324.754 nm for Cu, 257.610 nm for Mn, 206.200 nm for Zn, 220.353 for Pb, 267.716 for Cr and 340.458 for Pd) and two spectral regions were investigated. To assure a correct calibration of the instrument, at least one standard sample has been run every 10 test samples. Concentrations have been reported as mean values of three replicates. We found that all analytical determinations performed by ICP-MS are affected by an error equal to 5%. Data and graphics were elaborated with SigmaPlot Ver. 8.0 and Excel.

Methods and procedures
Soil and plants sampling
For this study we considered four different vegetal species (Plantago major L, Taraxacum officinale L, Urtica dioica L. and Trifolium pratense L.) collected in spring (mid-March), in summer (at the end of June) and in autumn (beginning of October) of year 1999. Five sampling areas (SAs) in the city of Rome have been chosen according to their different level of anthropogenic pollution. In particular, two of these sites (SA1 and SA2) are located close to high-traffic roads (Muro Torto and Olimpica), other two near medium- and low- traffic (SA3 and SA4) roads (Ostienose and Eur) and the last (SA5) from a large park (Pamphilii). The latter was assumed as the reference (uncontaminated) site.

Surface soils and plants samples (each weighing about 500 g) were taken in triplicate, at the same distance from the street across a 1x1 m² area by employing a stainless steel trowel to a 20 cm depth from the surface. After classification, plants and surface soil samples have been put in suitable plastic containers on the same occurrence.

Sample preparation and digestion procedure
Soil samples coming from the same site were pooled together, air-dried up to dryness, then sieved by passing through a 1 mm nylon sieve; fractions less than 1 mm size were further ground in an agate mortar, till all the sample was homogenized. Soil samples (particle size around 0.2 mm) were sealed in polyethylene bottles and stored.

The roots and leaves of the collected plants, suitably separated, were repeatedly washed first with tap water then with deionized water and finally air-dried. Roots samples from each of the three plants (of the same species) were pooled together, oven dried (105 °C, 48 h) homogenized and grinded in a metal free mill to obtain a fine powder. The same protocol was applied also to leaves.

For analysis, 350-400 mg (exactly weighted) of soil, roots or leaves were digested with 10 ml of concentrated HNO₃ (65%) for 24 h at 130 °C in 25 ml round bottomed flasks equipped with reflux condensers. The vessels were cooled, and stock solutions were obtained by transferring samples in 25 ml volumetric flasks and made up to the mark with deionized water (0.05 µScm⁻¹). The solution was filtered through a Whatman 541 paper and stored in glass bottles. Working solutions were obtained by diluting 1:10 (v:v) the correspondent stock solutions. Moreover, we performed also the analysis of blanks (clean mineralization solution) and standard reference materials (SRM) from the National Institute of Standards and Technology, Gaithersburg, USA (SRM No. 2587 and No. 2586 - Trace Elements in Soil containing lead from paint) in the same experimental conditions and by using the same protocol. The recovery varied from 95 to 98% and all the obtained values ±3σ were within the range of certified values.
## Results

### Analytical determinations

The mean concentration of Cu, Mn, Zn, Pb, Cr and Pd from surface soil, *Plantago major* L., *Taraxacum officinale* L., *Urtica dioica* L. and *Trifolium pratense* L. (both roots and leaves) have been summarized in Tables 1, 2, 3, 4.

### Heavy metals in soil

We found that Cu, Mn, Zn, Pb, Cr and Pd amount in soil varies with the order SA1 ≈ SA2 > SA3 > SA4 > SA5, being SA1 the most polluted area and SA5 the less contaminated one. Heavy metals concentration we found, is therefore closely linked to the level of contamination of the different sampling areas. The trend observed is independent on vegetal species considered and/or seasons. In every sampling site, among the heavy metals taken into consideration, Mn and Pb are the two most abundant whereas, Cr and Pd display the lowest concentrations.

The results of the heavy metals determined in soil seems to evidence a seasonal dependence. Fig. 1 reports an indicative example of the seasonal variation of heavy metal concentration for Cu and Pb in *Plantago major* L.

### Table 1 Heavy metals concentrations in *Plantago major* L. Cu, Mn, Zn, Pb, Cr and Pd soil, roots and leaves concentrations (ppm) in *Plantago major* L.

| Metal | Season | Soil | Roots | Leaves | Soil | Roots | Leaves | Soil | Roots | Leaves | Soil | Roots | Leaves |
|-------|--------|------|-------|--------|------|-------|--------|------|-------|--------|------|-------|--------|
| Cu    | SPRING | SA1  | 111 ± 5.6 | 53 ± 2.7 | 17 ± 0.9 | 199 ± 10.0 | 124 ± 6.2 | 60 ± 3 | 188 ± 9.4 | 67 ± 3.4 | 37 ± 1.9 |
|       | SUMMER | SA1  | 126 ± 6.3 | 62 ± 3.1 | 35 ± 1.8 | 214 ± 10.7 | 57 ± 2.9 | 26 ± 1.3 | 195 ± 9.8 | 104 ± 5.2 | 20 ± 1 |
|       | AUTUMN | SA1  | 52 ± 2.6  | 20 ± 1.1 | 10 ± 0.5 | 137 ± 6.9  | 93 ± 4.7 | 36 ± 1.8 | 129 ± 6.5 | 90 ± 4.5 | 29 ± 1.5 |
| Mn    | SPRING | SA1  | 773 ± 38.7| 129 ± 6.5| 51 ± 2.6  | 570 ± 28.5 | 160 ± 8  | 84 ± 4.2 | 627 ± 31.4| 62 ± 3.1 | 31 ± 1.6 |
|       | SUMMER | SA1  | 730 ± 36.5| 72 ± 3.6 | 29 ± 1.5  | 509 ± 25.5 | 94 ± 4.7 | 34 ± 1.7 | 534 ± 26.7| 106 ± 5.3 | 21 ± 1.1 |
|       | AUTUMN | SA1  | 784 ± 39.2| 72 ± 3.6 | 49 ± 2.5  | 579 ± 29.0 | 147 ± 7.4| 44 ± 2.2 | 579 ± 29.0| 36 ± 1.8 | 29 ± 1.5 |
| Zn    | SPRING | SA1  | 206 ± 10.3| 106 ± 5.3| 57 ± 2.9  | 303 ± 15.2 | 199 ± 10.0| 95 ± 4.8 | 342 ± 17.1| 62 ± 3.1 | 31 ± 1.6 |
|       | SUMMER | SA1  | 226 ± 11.3| 158 ± 7.9| 91 ± 4.6  | 321 ± 16.1 | 167 ± 8.4| 75 ± 3.8 | 334 ± 16.7| 156 ± 7.8 | 75 ± 3.8 |
|       | AUTUMN | SA1  | 104 ± 5.2 | 72 ± 3.6 | 29 ± 1.5  | 290 ± 14.5 | 134 ± 6.7| 76 ± 3.8 | 368 ± 18.4| 181 ± 9.1 | 95 ± 4.8 |
| Pb    | SPRING | SA1  | 578 ± 28.9| 54 ± 2.7 | 12 ± 0.6  | 840 ± 42  | 35 ± 1.8 | 11 ± 0.6 | 686 ± 34.3| 28 ± 1.4 | 8 ± 0.4 |
|       | SUMMER | SA1  | 488 ± 24.4| 38 ± 1.9 | n.d.      | 792 ± 39.6 | 6 ± 0.3  | n.d. | 596 ± 298 | 15 ± 0.8 | 3 ± 0.2 |
|       | AUTUMN | SA1  | 276 ± 13.8| n.d.     | n.d.      | 546 ± 27.3 | 26 ± 1.3 | n.d. | 523 ± 262 | 18 ± 0.9 | 3 ± 0.2 |
| Cr    | SPRING | SA1  | 40 ± 2    | 8 ± 0.4 | 2 ± 0.1  | 24 ± 12   | 3 ± 0.2 | 1 ± 0.1 | 26 ± 13   | 6 ± 0.3 | 1 ± 0.1 |
|       | SUMMER | SA1  | 43 ± 2.2  | 9 ± 0.5 | 3 ± 0.2  | 21 ± 1.1  | 3 ± 0.2 | 1 ± 0.1 | 30 ± 1.5  | 3 ± 0.2 | n.d.   |
|       | AUTUMN | SA1  | 33 ± 1.7  | 7 ± 0.4 | 2 ± 0.1  | 14 ± 0.8  | 2 ± 0.1 | 1 ± 0.1 | 14 ± 0.8  | 3 ± 0.2 | n.d.   |
| Pd    | SPRING | SA1  | 71 ± 3.6  | 7 ± 0.4 | 3 ± 0.2  | 74 ± 3.7  | 3 ± 0.2 | 1 ± 0.1 | 72 ± 3.6  | 5 ± 0.3 | 1 ± 0.1 |
|       | SUMMER | SA1  | 70 ± 3.5  | 7 ± 0.4 | 2 ± 0.1  | 72 ± 3.6  | 4 ± 0.2 | 2 ± 0.1 | 77 ± 3.9  | 6 ± 0.3 | 1 ± 0.1 |
|       | AUTUMN | SA1  | 73 ± 3.7  | 4 ± 0.2 | 1 ± 0.1  | 70 ± 3.5  | 4 ± 0.2 | 1 ± 0.1 | 74 ± 3.7  | 5 ± 0.3 | 1 ± 0.1 |
|       |         | SA5  | 67 ± 3.4  | 7 ± 0.4 | 2 ± 0.1  | 73 ± 3.7  | 5 ± 0.3 | 2 ± 0.1 | 67 ± 3.4  | 7 ± 0.4 | 2 ± 0.1 |
|       |         | SA5  | 41 ± 2.1  | 2 ± 0.1 | 1 ± 0.1  | 41 ± 2.1  | 3 ± 0.2 | 1 ± 0.1 | 44 ± 2.2  | 3 ± 0.2 | 1 ± 0.1 |
Concentrations of Cu and Pb reach the maximum value during summer while Mn reaches the minimum value. Zn concentration increases from spring to autumn while Cr and Pd concentrations remain relatively constant. Other factors can influence the local concentration of heavy metals in soil: temperature, rainfall, evapotranspiration, soil pH and redox potential. To correlate heavy metals concentration with the level of precipitation, we collected the rainfall data for the city of Rome from the Meteorological Centre of Rome. Superimposing the precipitation records with heavy metals concentrations we were able to observe some characteristic trends. In particular, during spring and autumn when the first and the third sampling occurred, moderate to abundant precipitation were registered whilst in summer rains are rare. The higher temperature and reduced rainfall may hence favour the water evaporation in soils leading to a higher accumulation of metals with respect to spring or autumn. Cu, and Pb seem to follow such a behaviour, with a maximum concentration during summer (214 ppm and 1266 ppm, respectively), while Zn concentration reaches a maximum during autumn (742 ppm). On the contrary, Mn follows the opposite trend showing the lowest value during summer (449 ppm). Cr and Pd seem not to be influenced by atmospheric conditions and their concentration remain relatively low and constant all over the year (between 15 and 45 ppm for Cr and between 37 and 77 ppm for Pd).

### Heavy metals in plants

Heavy metals found in roots and leaves of the three herbaceous plants (*Plantago major L.*, *Taraxacum officinale L.* and *Urtica dioica L.*) and the leguminous *Trifolium pratense L.*, allowed us to conclude that the content of heavy metals in roots is higher than in leaves and that accumulation process of herbaceous plants does not significantly...

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### Table 2

**Heavy metals concentrations in Taraxacum officinale L. Cu, Mn, Zn, Pb, Cr and Pd soil, roots and leaves concentrations (ppm) in *Taraxacum officinale L.***

|     | SPRING |     |     |     |     | SUMMER |     |     |     |     | AUTUMN |     |     |     |     |
|-----|--------|-----|-----|-----|-----|--------|-----|-----|-----|-----|--------|-----|-----|-----|-----|
|     | Soil   | Roots| Leaves| Soil | Roots| Leaves| Soil | Roots| Leaves| Soil | Roots| Leaves|     |     |     |     |
| Cu  |        |      |       |     |      |       |     |      |       |     |      |       |     |     |     |
| SA1 | 126 ± 6.3 | 36 ± 1.8 | 35 ± 1.8 | 136 ± 6.8 | 95 ± 4.8 | 51 ± 2.6 | 131 ± 6.6 | 97 ± 4.9 | 52 ± 2.6 |
| SA2 | 116 ± 5.8 | 46 ± 2.3 | 39 ± 2.0 | 144 ± 7.2 | 64 ± 3.2 | 42 ± 2.1 | 142 ± 7.1 | 71 ± 3.6 | 45 ± 2.3 |
| SA3 | 110 ± 5.5 | 31 ± 1.6 | 37 ± 1.9 | 155 ± 7.8 | 55 ± 2.8 | 39 ± 2.0 | 99 ± 5.0 | 27 ± 1.4 | 34 ± 1.7 |
| SA4 | 54 ± 2.7 | 40 ± 2 | 23 ± 1.2 | 127 ± 6.4 | 31 ± 1.6 | 26 ± 1.3 | 74 ± 3.7 | 30 ± 1.5 | 24 ± 1.2 |
| SA5 | 32 ± 1.6 | 15 ± 0.8 | 15 ± 0.8 | 104 ± 5.2 | 31 ± 1.6 | 24 ± 1.2 | 55 ± 2.8 | 25 ± 1.3 | 10 ± 0.5 |
| Mn |        |      |       |     |      |       |     |      |       |     |      |       |     |     |     |
| SA1 | 809 ± 40.5 | 49.2 ± 2.5 | 39 ± 2.0 | 546 ± 27.3 | 61 ± 3.1 | 41 ± 2.1 | 624 ± 31.2 | 43 ± 2.2 | 19 ± 1.0 |
| SA2 | 720 ± 36 | 115 ± 5.8 | 64 ± 3.2 | 576 ± 28.8 | 112 ± 5.6 | 35 ± 1.8 | 602 ± 30.1 | 61 ± 3.1 | 42 ± 2.1 |
| SA3 | 755 ± 37.8 | 76 ± 3.8 | 74 ± 3.7 | 571 ± 28.6 | 83 ± 4.2 | 58 ± 2.9 | 583 ± 29.2 | 65 ± 3.3 | 38 ± 1.9 |
| SA4 | 788 ± 39.4 | 91 ± 4.6 | 98 ± 4.9 | 600 ± 30 | 66 ± 3.3 | 51 ± 2.6 | 580 ± 29 | 104 ± 5.2 | 54 ± 2.7 |
| SA5 | 646 ± 32.3 | 45 ± 2.3 | 44 ± 2.2 | 631 ± 31.6 | 86 ± 4.3 | 59 ± 3.0 | 624 ± 31.2 | 59 ± 3.0 | 32 ± 1.6 |
| Zn |        |      |       |     |      |       |     |      |       |     |      |       |     |     |     |
| SA1 | 229 ± 11.5 | 155 ± 7.8 | 133 ± 6.7 | 374 ± 18.7 | 211 ± 10.6 | 148 ± 7.4 | 742 ± 37.1 | 227 ± 11.4 | 90 ± 4.5 |
| SA2 | 220 ± 11 | 157 ± 7.9 | 121 ± 6.1 | 426 ± 21.3 | 234 ± 11.7 | 150 ± 7.5 | 678 ± 33.9 | 265 ± 13.3 | 137 ± 6.9 |
| SA3 | 215 ± 10.8 | 119 ± 6.0 | 109 ± 5.5 | 263 ± 13.2 | 152 ± 7.6 | 105 ± 5.3 | 694 ± 34.7 | 187 ± 9.4 | 64 ± 3.2 |
| SA4 | 101 ± 5.1 | 94 ± 4.7 | 79 ± 4.0 | 254 ± 12.7 | 72 ± 3.6 | 80 ± 4 | 393 ± 19.7 | 73 ± 3.7 | 55 ± 2.8 |
| SA5 | 61 ± 3.1 | 59 ± 3.0 | 70 ± 3.5 | 93 ± 4.7 | 70 ± 3.5 | 80 ± 4 | 140 ± 7 | 73 ± 3.7 | 40 ± 2 |
| Pb |        |      |       |     |      |       |     |      |       |     |      |       |     |     |     |
| SA1 | 627 ± 31.4 | 68 ± 3.4 | 8 ± 0.4 | 796 ± 39.8 | 109 ± 5.5 | 22 ± 1.1 | 730 ± 36.5 | 84 ± 4.2 | 11 ± 0.6 |
| SA2 | 588 ± 29.4 | 75 ± 3.8 | n.d. | 769 ± 38.5 | 155 ± 7.8 | 28 ± 1.4 | 730 ± 36.5 | 108 ± 5.4 | 22 ± 1.1 |
| SA3 | 206 ± 10.3 | n.d. | n.d. | 644 ± 32.2 | 26 ± 1.3 | 12 ± 0.6 | 560 ± 28 | 15 ± 0.8 | 3 ± 0.2 |
| SA4 | 244 ± 12.2 | n.d. | n.d. | 371 ± 18.6 | 11 ± 0.6 | 8 ± 0.4 | 107 ± 5.4 | 9 ± 0.5 | 2 ± 0.1 |
| SA5 | 148 ± 7.4 | n.d. | n.d. | 174 ± 8.7 | 6 ± 0.3 | 4 ± 0.2 | 89 ± 4.5 | 4 ± 0.2 | 1 ± 0.1 |
| Cr |        |      |       |     |      |       |     |      |       |     |      |       |     |     |     |
| SA1 | 35 ± 1.8 | 7 ± 0.4 | 3 ± 0.2 | 29 ± 1.5 | 8 ± 0.4 | 2 ± 0.1 | 30 ± 1.5 | 3 ± 0.2 | 1 ± 0.1 |
| SA2 | 38 ± 1.9 | 10 ± 0.5 | 5 ± 0.3 | 34 ± 1.7 | 10 ± 0.5 | 3 ± 0.2 | 35 ± 1.8 | 4 ± 0.2 | 1 ± 0.1 |
| SA3 | 40 ± 2 | 4 ± 0.2 | 3 ± 0.2 | 31 ± 1.6 | 7 ± 0.4 | 1 ± 0.1 | 32 ± 1.6 | 3 ± 0.2 | 1 ± 0.1 |
| SA4 | 45 ± 2.3 | 7 ± 0.4 | 3 ± 0.2 | 29 ± 1.5 | 4 ± 0.2 | 2 ± 0.1 | 21 ± 1.1 | n.d. | n.d. |
| SA5 | 33 ± 1.7 | 3 ± 0.2 | 3 ± 0.2 | 28 ± 1.4 | 2 ± 0.1 | 1 ± 0.1 | 28 ± 1.4 | n.d. | n.d. |

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Table 3 Heavy metals concentrations in Urtica dioica L. Cu, Mn, Zn, Pb, Cr and Pd soil, roots and leaves concentrations (ppm) in *Urtica dioica L*.

|         | Soil  | Roots | Leaves | Soil  | Roots | Leaves | Soil  | Roots | Leaves | Soil  | Roots | Leaves |
|---------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|
| **Cu**  |       |       |        |       |       |        |       |       |        |       |       |        |
| SA1     | 104 ±5.2 | 42 ±2.1 | 21 ±1.1 | 186 ±9.3 | 106 ±5.3 | 41 ±2.1 | 162 ±8.1 | 51 ±2.6 | 28 ±1.4 |
| SA2     | 92.5 ±4.7 | 22 ±1.1 | 19 ±1.0 | 156 ±7.8 | 100 ±5 | 34 ±1.7 | 169 ±8.5 | 55 ±2.8 | 39 ±2.0 |
| SA3     | 85 ±4.3 | 18 ±0.9 | 14 ±0.7 | 105 ±5.3 | 40 ±2 | 15 ±0.8 | 117 ±5.9 | 61 ±3.1 | 33 ±1.7 |
| SA4     | 38 ±1.9 | 15 ±0.8 | 17 ±0.9 | 101 ±5.1 | 56 ±2.8 | 18 ±0.9 | 105 ±5.3 | 46 ±2.3 | 23 ±1.2 |
| SA5     | 26 ±1.3 | 17 ±0.9 | 13 ±0.7 | 47 ±2.4 | 30 ±1.5 | 20 ±1 | 87 ±4.4 | 51 ±2.6 | 30 ±1.5 |
| **Mn**  |       |       |        |       |       |        |       |       |        |       |       |        |
| SA1     | 651 ±32.6 | 158 ±7.9 | 41 ±2.1 | 580 ±29 | 147 ±7.4 | 71 ±3.6 | 626 ±31.3 | 163 ±8.2 | 99 ±2.0 |
| SA2     | 603 ±30.2 | 182 ±9.1 | 37 ±1.9 | 514 ±25.7 | 104 ±5.2 | 29 ±1.5 | 554 ±27.7 | 130 ±6.5 | 34 ±1.7 |
| SA3     | 626 ±31.3 | 121 ±6.1 | 70 ±3.5 | 532 ±26.6 | 110 ±5.5 | 63 ±3.2 | 640 ±32 | 169 ±8.5 | 48 ±2.4 |
| SA4     | 587 ±29.4 | 111 ±5.6 | 54 ±2.7 | 496 ±24.8 | 86 ±4.3 | 77 ±3.9 | 620 ±31 | 160 ±8 | 78 ±3.9 |
| SA5     | 553 ±27.7 | 98 ±4.9 | 31 ±1.6 | 518 ±25.9 | 121 ±6.1 | 69 ±3.5 | 620 ±31 | 142 ±7.1 | 55 ±2.8 |
| **Zn**  |       |       |        |       |       |        |       |       |        |       |       |        |
| SA1     | 185 ±9.3 | 97 ±4.9 | 46 ±2.3 | 255 ±12.8 | 144 ±7.2 | 128 ±6.4 | 336 ±16.8 | 198 ±9.9 | 124 ±6.2 |
| SA2     | 137 ±6.9 | 34 ±1.7 | 19 ±1.0 | 224 ±11.2 | 103 ±5.2 | 100 ±5 | 428 ±21.4 | 210 ±105 | 152 ±7.6 |
| SA3     | 152 ±7.6 | 49 ±2.5 | 36 ±1.8 | 150 ±7.5 | 75 ±3.8 | 65 ±3.3 | 374 ±18.7 | 172 ±86 | 130 ±6.5 |
| SA4     | 160 ±8 | 58 ±2.9 | 41 ±2.1 | 185 ±93 | 91 ±4.6 | 69 ±3.5 | 223 ±112 | 130 ±6.5 | 98 ±4.9 |
| SA5     | 92 ±4.6 | 26 ±1.3 | 14 ±0.7 | 147 ±7.4 | 112 ±5.6 | 95 ±4.8 | 204 ±102 | 145 ±73 | 120 ±6 |
| **Pb**  |       |       |        |       |       |        |       |       |        |       |       |        |
| SA1     | 528 ±26.4 | 75 ±3.8 | 21 ±1.1 | 888 ±44.4 | 81 ±4.1 | 23 ±1.2 | 710 ±35.5 | 95 ±4.8 | 30 ±1.5 |
| SA2     | 452 ±22.6 | 73 ±3.7 | 19 ±1.0 | 971 ±48.6 | 60 ±3 | 21 ±1.1 | 854 ±42.7 | 85 ±4.3 | 23 ±1.2 |
| SA3     | 215 ±10.8 | 44 ±2.2 | 13 ±0.7 | 548 ±27.4 | 46 ±2.3 | 14 ±0.7 | 434 ±21.7 | 59 ±3.0 | 16 ±0.8 |
| SA4     | 152 ±7.6 | 23 ±1.2 | 11 ±0.6 | 294 ±14.7 | 37 ±1.9 | 13 ±0.7 | 230 ±11.5 | 43 ±2.2 | 14 ±0.7 |
| SA5     | 136 ±6.8 | 32 ±1.6 | 9 ±0.5 | 202 ±10.1 | 34 ±1.7 | 10 ±0.5 | 198 ±9.9 | 37 ±1.9 | 12 ±0.6 |
| **Cr**  |       |       |        |       |       |        |       |       |        |       |       |        |
| SA1     | 28 ±1.4 | 5 ±0.3 | 3 ±0.2 | 36 ±1.8 | 10 ±0.5 | 5 ±0.3 | 28 ±1.4 | 6 ±0.3 | 2 ±0.1 |
| SA2     | 26 ±1.3 | 7 ±0.4 | 4 ±0.2 | 40 ±2 | 12 ±0.6 | 4 ±0.2 | 30 ±1.5 | 7 ±0.4 | 3 ±0.2 |
| SA3     | 22 ±1.1 | 5 ±0.3 | 3 ±0.2 | 43 ±2.2 | 10 ±0.5 | 4 ±0.2 | 29 ±1.5 | 4 ±0.2 | 2 ±0.1 |
| SA4     | 21 ±1.1 | 7 ±0.4 | 5 ±0.3 | 41 ±2.1 | 11 ±0.6 | 4 ±0.2 | 27 ±1.4 | 5 ±0.3 | 2 ±0.1 |
| SA5     | 23 ±1.2 | 5 ±0.3 | 2 ±0.1 | 25 ±1.3 | 7 ±0.4 | 3 ±0.2 | 21 ±1.1 | 4 ±0.2 | 1 ±0.1 |
| **Pd**  |       |       |        |       |       |        |       |       |        |       |       |        |
| SA1     | 66 ±3.3 | 12 ±0.6 | 5 ±0.3 | 65 ±3.3 | 9 ±0.5 | 3 ±0.2 | 68 ±3.4 | 13 ±0.7 | 3 ±0.2 |
| SA2     | 71 ±3.6 | 16 ±0.8 | 9 ±0.5 | 72 ±3.6 | 10 ±0.5 | 3 ±0.2 | 72 ±3.6 | 13 ±0.7 | 3 ±0.2 |
| SA3     | 76 ±3.8 | 16 ±0.8 | 7 ±0.4 | 70 ±3.5 | 10 ±0.5 | 2 ±0.1 | 65 ±3.3 | 10 ±0.5 | 2 ±0.1 |
| SA4     | 67 ±3.4 | 18 ±0.9 | 9 ±0.5 | 77 ±3.9 | 11 ±0.6 | 3 ±0.2 | 70 ±3.5 | 13 ±0.7 | 3 ±0.2 |
| SA5     | 55 ±2.8 | 8 ±0.4 | 4 ±0.2 | 57 ±2.9 | 5 ±0.3 | 1 ±0.1 | 59 ±3.0 | 6 ±0.3 | 1 ±0.1 |

differ from that of leguminous plants: the higher the metal concentration in the soil, the higher the concentration in roots and consequently in leaves (Fig. 2).

We further analyzed the correlation between heavy metals content in soil and in leaves of the various vegetal species. We calculated the mean value of heavy metals concentrations in soil and leaves taking into account the values obtained in the three seasons. In this calculation we also considered all the sampling areas in order to analyze different levels of pollution. We calculated the correlation coefficients (Pearson’s correlation) between these two set of data and we considered only those metals with $r \geq 0.95$. We therefore found that for *Plantago major L*. Mn has a correlation coefficient of 0.950, in *Taraxacum officinale L*. Cu has a coefficient of 0.984, in *Urtica dioica L*. Pb has a correlation of 0.952 while in *Trifolium pratense L*. Cu and Pb have coefficients of 0.956 and 0.962, respectively (Table 5 and Fig. 3).

**Discussion**

**Heavy metals in soil**

The amount of heavy metals in soil is extremely variable and these differences are more clearly emphasized if we...
consider different sampling areas. Different anthropogenic activities may locally alter the amount of some heavy metals, especially of those sites located near high-traffic roads. We found that the amount of some of these metals can be very high (higher than 1000 ppm for some metals) while in the control site (a non-polluted park) the concentrations are relatively low. In this study we did not evaluate the effect of the various vegetal species in determining a different ‘local environment’ that we selected for analytical determination. We did not considered also the various effects of pH, temperature and other physicochemical parameters that can influence the relative heavy metals concentration. However, we found that heavy metals concentration directly correlate with the degree of pollution and, as a consequence, of anthropogenic activity in agreement with previous authors that reported that the principal source of heavy metals pollution (96% for Pb, 66% for Zn and 56% for Cu) originates from human activities [22].

### Seasonal variation of heavy metals in soil

We found a seasonal variation of heavy metals concentration in soil, that we ascribed to a different level of metal dissolution due to rainfall. In fact, during summer...
the rainfalls are reduced if compared to spring or autumn and high temperatures (or an increase in evapotranspiration) favour an increase of metals concentrations.

Manganese has been found almost equally distributed in all the sampling areas and this indicates that the presence of this metal in soil was not only due to anthropogenic sources (as in most polluted areas) but also to
some other sources, most likely of natural origin. In fact, it has been reported that Mn present in soil comes from 89% from natural sources and only for the 11% from human activities [22]. Moreover, Mn gives rise to quite complex acid-base and redox equilibrium reactions in soil, depending on conditions (temperature, soil pH and structure, humidity, etc.) leading to a bio-distribution and bio-availability difficult to analyze in details without a widespread investigation that is beyond the scope of this work.

Taking into account the seasonal distribution of heavy metals in soil and the rainfall in Rome (Fig. 1) we can hypothesize that higher temperatures and reduced rainfalls may determine a higher water evaporation leading to a higher accumulation (as dry weight) of metals with respect to spring or autumn. Cu, and Pb seem to follow such behaviour, with a maximum concentration during summer (214 ppm and 1266 ppm, respectively). Zn reaches a maximum during autumn (742 ppm) and Mn follows an opposite trend showing the lowest value during summer (449 ppm). Cr and Pd do not seem to be influenced by atmospheric conditions and their concentration remain relatively low and constant all over the year (between 15 and 45 ppm for Cr and between 37 and 77 ppm for Pd). Owing to the low Pd concentration and the almost equal distribution in all the sampling areas considered, we may conclude that the eventual release of this metal from catalytic converters is therefore negligible, at least in our study. Interestingly, we also noticed the same correlation between heavy metals accumulation in soil and the concentration of some selected metals found by Cardarelli et al. in lichens collected in Rome in the same periods [23].

Table 5 Heavy metals mean concentration for selected herbaceous plants. Concentrations of heavy metals contained in selected common plants. Data have been reported together with correlation coefficients in Figure 3.

| Plant species          | Heavy metals | Mean concentration (ppm) | Standard Deviation |
|------------------------|--------------|--------------------------|-------------------|
| Urtica dioica L.       | Pb           | Soil: 708.7              | Leaves: 24.7      |
|                        |              | Soil: 180                | Leaves: 4.7       |
|                        | Cu           | Soil: 131                | Leaves: 46        |
|                        |              | Soil: 5                  | Leaves: 9.5       |
| Taraxacum officinale L.| Pb           | Soil: 759                | Leaves: 21        |
|                        |              | Soil: 277.2              | Leaves: 2         |
|                        | Cu           | Soil: 121.3              | Leaves: 36.7      |
|                        |              | Soil: 29.7               | Leaves: 2.5       |
| Plantago major L.      | Mn           | Soil: 656.7              | Leaves: 55.3      |
|                        |              | Soil: 104.7              | Leaves: 26.8      |
| Trifolium pratense L.  | Cu           | Soil: 126.6              | Leaves: 43        |
|                        |              | Soil: 33.6               | Leaves: 34        |
|                        | Pb           | Soil: 970.7              | Leaves: 11        |
|                        |              | Soil: 242.7              | Leaves: 4.6       |

Heavy metals accumulation in plants

In our study we have considered four different vegetal species (three herbaceous and one leguminous plants) in order to investigate the feasibility of employing them as useful and simple tools to monitor environmental pollution, and in particular soil pollution by heavy metals. We therefore investigated if these plants can be selective toward specific heavy metal and in order to minimize
variability in the analytical determination, we assessed the heavy metals concentration in three different seasons over the course of one solar year. From our extensive study, we found some direct correlations between the amount of heavy metals in soil and in the leaves of the selected plants (Fig. 3). Only Cu, Mn and Pb display a good linear dependence on metal concentration in soil. In particular, both *Taraxacum officinale* L. and *Trifolium pratense* L. can accumulate Cu in their leaves in a linearly dependent manner respect to soil content. Additionally, the fraction of Cu accumulated by these two species is quite high (25-40%) if compared to the amount present in soil. On the other hand, *Plantago major* L. can accumulate only small fractions of Mn (5-10%) in their leaves. *Urtica dioica* L. and *Trifolium pratense* L. are both able to accumulate Pb in their leaves even if at different percentages (10-20% for *Trifolium pratense* L. and 30-60% for *Urtica dioica* L.). For the latter two species, *Urtica dioica* L. represents the vegetal species that can accumulate the highest fraction of a dangerous heavy metal such as Pb. The higher amount of Pb in the most polluted sampling areas (near trafficked roads) is a direct consequence of anthropogenic contribution, since in 1999 Pb was still added into fuels as an additive agent.

**Conclusions**

Our results demonstrate that common herbaceous and leguminous plants can be used as alternative and simple analytical tools that can be employed to monitor environmental pollution and in particular soil pollution by heavy metals. Other physicochemical parameters such as soil pH, temperature, humidity, soil texture analysis, microbiological composition and soil redox potential, to cite only a few, have to be considered in order to deeply study the metal accumulation mechanisms by plants and employ them as efficient indicators of environmental pollution. Moreover, increasing the number of vegetal species it will be possible to find better indicators for different heavy metals, and suggest a panel of common plants to employ routinely in analytical determinations for environmental pollution monitoring.

**Acknowledgments**

Authors thank the Meteorological Centre of Rome, the Meteorological Centre of Milan (Centro Meteorologico Lombardo) and the Italian Meteorological Society Onlus of Turin for having provided atmospheric data, Dr. Lorenzo Ciccarese for helpful suggestions and discussions and revision of the first manuscript, Dr. Fabiana Console for collection and revision of bibliography, and the Italian Ministry of University and Research (MIUR) for financial support. This article has been published as part of *Chemistry Central Journal* Volume 6 Supplement 2, 2012: Proceedings of CMA4CH 2010: Application of Multivariate Analysis and Chemometry to Cultural Heritage and Environment. The full contents of the supplement are available online at http://journal.chemistrycentral.com/content/6/S2.

**Authors’ contributions**

DM collected soil and plants samples for ICP-MS analysis, prepared them and helped AG to perform the analytical determinations, AG acquired and analyzed data, GO contributed to the writing of the manuscript and revision.
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Published: 2 May 2012