Are the physiological and biochemical characteristics in dandelion plants growing in urban area (Pisa, Italy) indicative of soil pollution?

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Abstract. Physiological and biochemical characteristics were evaluated in dandelion plants (*Taraxacum officinale*) naturally growing in an urban environment. The study area was located in the town of Pisa, Italy, and 27 sites were chosen to assess biochemical and physiological features of dandelion plants and the trace metals content in the urban soil. Concentrations of elements including, Cr, Cu, Mn and Zn were analyzed in the soil and shoot and root tissues of dandelion collected from different sites. Chlorophyll *a* fluorescence analysis, pigment content, antioxidant power and phenols content were measured in dandelion. The results showed a widespread very limited soil pollution of trace metals in the urban sites. However, Zn was uptake and translocated by dandelion even though no damage was observed in plans. On the basis of the obtained results dandelion plant are able to survive in constrained environment thank to the high phenols that are useful to contrast the oxidative stress induced by heavy metals.

Keywords: Taraxacum officinale, trace element, photosynthetic pigments, antioxidant capacity

1 Introduction

The urban ecosystems are comprised by diverse land uses, including commercial, industrial, residential, transport, recreational, agricultural and natural areas. This determines different habitat for plants, animals and humans. The urban habitat quality comprises the integration of different abiotic and biotic components such as air, soil and water quality, microclimate and the presence of vegetation. Probably, pollution is the most significant characteristic of urban soil (Vrščaj et al. 2008) and this could

negatively affect the human health. Clearly, trace metals content plays a key role in the pollution (Biasioli et al. 2006).

Phytotoxic amounts of trace metals induce in plants an oxidative stress leading to cellular damage (Yadav 2010). In addition, plants accumulate metal ions that disturb cellular ionic homeostasis, the growth usually becomes inhibited and biomass production decreases (Moulis 2010). Trace metals are active in plant metabolic processes, but they can also be stored as inactive compounds in cell walls, so affecting the chemical composition of plants without causing any injury (Nagajyoti et al. 2010). For this reason, vascular plants are frequently used for biomonitoring (Korzeniowska and Panek 2010). However, native plants tolerant to trace metals characterized by a fast growing usually attract more attention as compared with slow growing plants (Massa et al. 2010). For a long time the dandelion (*Taraxacum officinale* Web.) has been proposed as a good bioindicator for trace elements polluted environment (Savinov et al. 2007; Bini et al. 2012).

Mossop et al. (2009) found a positive correlation between Cu and Pb concentrations in soil and in dandelion roots and leaves, whereas for Zn the relationship was found only for leaves. However, these authors considered the sum of the acid-exchangeable, reducible and oxidisable soil fractions of these elements a poor indicator of potential plant uptake. On the other hand, more recently, Gjorgieva et al. (2011), in a study conducted to assess trace metals pollution in Macedonia soils, classified *T. officinale* as a trace metals accumulator.

Dandelion has also a high antioxidant activity due to high content of secondary metabolites (Park et al. 2011; González-Castejón et al. 2012; Davaatseren et al. 2013). Being secondary metabolites important in plant adaptation to environmental stresses, it is presumable that plant species that possess a high content in secondary metabolites can efficiently counteract the environmental stress (Dixon and Paiva 1995).

Chlorophyll *a* fluorescence analysis provides a reliable measure of the photosynthetic performance of plants (Maxwell and Johnson, 2000) including trace metals (Küpper et al. 1996; Baumann et al. 2009; Nagajyoti et al. 2010; da Silva et al. 2012). Among different parameters achievable from this tool, certainly the F_v/F_m ratio has been extensively used to monitor photoinhibitory damage (Maxwell and Johnson, 2000). In the past, the dandelion has already been used for urban pollution monitoring by means of chlorophyll *a* fluorescence analysis and other photosynthetic parameters,

although the number of works on the subject is quite low (Lanaras et al. 1994; Sgardelis et al. 1994; Molina-Montenegro et al. 2010).

The novelty of this work is represented by the use of *T. officinale* as bioindicator assessing its healthy status by chlorophyll *a* fluorescence analysis. This is a first attempt to investigate the soil contamination in the town of Pisa (Italy) in order to establish correlation between trace metal pollution and their relative sources in an urban environment.

2 Materials and Methods

2.1 The study area

The study was conducted in the municipal area of the town of Pisa ((latitude 43°25'00N; longitudine 10°43'00E; Italy), an urban environment of approximately 187 km^2 and about 90,000 inhabitants. The artificialized surface (impermeable urban area) was approximately 27 km^2 (almost 15% of the total area), with a consumption of soil above the average compared with Regional and Provincial data. The built-up area has been steadily increasing, with the largest increases since the 50s (+260% increase from 1954 to 2003). Samples were collected at 31 sites around urban areas of the city of Pisa. Sites 1, 2, 8, 9, 10, 11, 12, 16, 25, 26, 28, 29, and 31 are public green areas utilized as playground; Sites 3, 5, 7, 14, 15, 19, 20, 21, 23, 24, 27, and 30 are traffic roundabouts; Sites 4 and 22 are city green squares; Sites 6 and 17 are public school gardens; Sites 13 and 18 are historic public gardens and at a control site (Site 0) nearby the city represented by the rural area of S. Rossore – Migliarino - Massaciuccoli Natural Regional Park (latitude 43°42'48N; longitude 10°21'44E). The control site is a green area, once cultivated and currently naturalized from more than twenty years. Really, the analyses on plants were carried out only in 27 sites because in 4 sites (sites 10, 17, 23 and 25) there were no plants that have reached the entire growth cycle.

2.2 Plant and soil sampling

Plants of *T. officinale* were collected at the flowering stage (from April to May) because in this phase the species was easily identifiable and reached the full development.

At each site, three soil samples were randomly collected from the topsoil (depth 0-20 cm). Each sample consisted of five sub-samples, each of which was taken within a 2×2 m square, four on the corners of the square and one in the middle. The five soil cores of each sub-sample were mixed to avoid local in-homogeneities. The sampling

was performed with a stainless steel hand auger and all the soil that was touched by the metallic digging tools were carefully eliminated with a porcelain putty knife, before soil packing in plastic bags, to avoid cross-contamination. In the laboratory, the samples were air-dried at room temperature (20°C), and, after manually removing any plant material, as roots and leaves, they were stored at 4°C until analysis. Most of the main properties of soils, including texture, pH (soil-water ratio 1:2,5), and limestone, were determined according to the standard methods (Sparks, 1996), while organic carbon content was obtained by the difference between total carbon (measured by dry combustion with an automatic C analyzer FKV induction furnace 900 CS, Eltra - F.K.V.) and inorganic carbon (limestone C).

2.3 Chlorophyll fluorescence measurements

The chlorophyll *a* fluorescence was measured with a PAM-2000 pulse-modulated fluorometer (Heinz Walz, Germany) connected with a leaf clip holder (2030-B Heinz Walz, Germany) as reported in Guidi et al. (2010). The F_v/F_m ratio $[(F_m-F_0)/F_m]$ was calculated as an indicator of the maximum quantum efficiency for PSII photochemistry. Estimates of photochemical (q_P) and non-photochemical (q_N) quenching coefficients were determined as reported by Schreiber et al. (1986) at an actinic illumination at 950 (±50) µmol photons $m^{-2}s^{-1}$. Actual quantum yield for linear electron transport through PSII, Φ_{PSII} was determined as $F_m'-F_s/F_m'$ where F_s represents the chlorophyll fluorescence yield in steady state conditions (Genty et al., 1989). The maximum electron transport rate was calculated according to Genty et al. (1990) at 950 µmol $m^{-2}s^{-1}$ PAR using equation: $ETR= \Phi_{PSII} \times PAR \times 0.5 \times 0.84$ where 0.5 corresponds to the two photosystems and 0.84 is an estimate of the fraction of the absorbed light and PAR is the value for the light intensity given.

2.4 Photosynthetic pigments content

Photosynthetic pigments were determined on three discs of leaf tissue (1.0 cm^2 of leaf area) extracted with acetone 80%. Chlorophylls concentrations were calculated from the equations proposed by Porra et al. (1989) whereas carotenoids concentration was calculated from the equation proposed by Lichtenthaler (1987).

2.5 Methanol extraction of plant tissues

According to Kang and Saltveit (2002), 1.0 g of sample tissue was homogenised, extracted in 2.5 mL of HPLC methanol and centrifuged at 15,000 *g* for 20 minutes at 4°C. The obtained supernatants were stored at -20°C until used for analysis of the antioxidant capacity, total phenolic compounds and metal chelating capacity.

2.6 Antioxidant capacity (DPPH)

The ability to scavenge DPPH (2,2-diphenyl-1-picrylydrazyl) free radicals was determined according to the method proposed by Brand-Williams et al. (1995) with minor modifications as reported in Landi et al. (2013). Results are expressed as Trolox equivalent antioxidant capacity (TEAC).

2.7 Total polyphenols (TP)

Total polyphenols were determined using the Folin-Ciocalteau method modified by Singleton and Rossi (1965). An aliquot of methanolic extracts was added to distilled water and Folin-Ciocalteau reagent. After 6 minutes, 7% sodium carbonate solution was added and then the mixtures keep at 25°C in the dark for 90 minutes. Total polyphenols content was calculated from a calibration curve, realized using gallic acid as standard. TP content is expressed as mg of gallic acid g^{-1} fresh weight (FW).

2.8 Metal chelating capacity (MCC)

Metal chelating capacity was determined by the method of Dinis et al. (1994) modified by Du et al. (2009). Briefly, methanolic extracts (1 mL) in 2.8 mL distilled water were mixed with 50 µL of 2 mM $FeCl_2x4H_2O$ and 150 µL of 5 mM ferrozine [3-(2-pyridyl)-5,6dyphenil-1,2,4triazine-p-p'-disulfonic acid monosodium salt hydrate, 97% ($C_{20}H_{13}N_4NaO_6S_2xnH_2O$)] and the mixtures were thoroughly shaken. After 10 minutes, Fe^{2+} was monitored by measuring the absorbance of ferrous ion–ferrozine complex at 562 nm. The metal chelating capacity was calculated as follows: MCC (%) = [(1-Absorbance of sample)/Absorbance of control] x100.

2.9 Plant trace metals content

The content of trace metals was determined on the aerial and root tissues of *T. officinale* plants, previously separated and dried in an oven at 60°C for 48 h. Briefly, 0.5 g samples of plant material were placed in microwave oven (Milestone Ethos labstation; Milestone, Italy) and digested with 65% HNO_3 and 30% H_2O_2. Samples

were diluted with distilled water (Baranowska et al. 2002). Digests were analysed for trace metals (Cr, Cu, Mn and Zn) by a flame atomic absorption spectrophotometer (Perkin Elmer Analyst 100, USA).

2.10 Soil trace metals content

The total content of trace metals was determined by the standard method ISO 11466 (International Organization for Standardization 1995). Briefly, 1 g of soil sieved to ≈ 80 mesh was treated with aqua regia. Pre-digestion was carried at room temperature for 16 hours with occasional manual agitation and, later on, digestion was performed for 2 hours at 130±2°C. The obtained suspension was then filtered (ashless Whatman 41 filter), the filtrate diluted with 0.17 M HNO_3 and stored at 4°C until analysis. The determinations of Cr, Cu, Mn and Zn were performed by inductively coupled plasma mass spectrometer (ICP-MS) by Acme Analytical Laboratories Ltd., Vancouver, Canada.

2.11 Indexes for contamination assessment

Geo-accumulation index (Igeo), enrichment factor (EF), and pollution index (PI) were calculated. Igeo was calculated according to the following equation (Müller 1969):

$$Igeo = \log_2 [Cx/1.5Bx]$$

where Cx is the measured concentration of the metal x and Bx is the geochemical background value of the element.

The EF calculation was expressed according to the equation (Lu et al. 2009):

$$EF = [Cx/Cref]sample/[Cx/Cref]background$$

where Cx is the concentration of the metal and Cref was the concentration of reference element for normalization. The reference element utilized for normalization was Fe (Lu et al. 2009).

The PI was calculated as the ratio between the metal concentration in the monitored area and the background content (Chen et al. 2005). The bioconcentration factor (BcF) or translocation factor was calculated, too. The BcF is the ratio of the metal concentration of shoots and root portion of dandelion to the metal concentration of the soil (Kim et al. 2003). The translocation ability of metal in dandelion plants was expressed as translocation index (TI) determined as the ratio of metal concentration in the above-ground tissue to its concentration in the root (Salt and Krämer 2000).

204 2.12 Statistical analysis

205 All of the performed analyses, were carried out in triplicate and the results were
206 presented as means ± SD. Analysis of variance was performed by ANOVA procedures
207 through CoStat program, 6.311 version (CoHort Software; Monterey, CA, USA). The
208 means were compared using Tukey's HSD test. Correlation analysis among
209 biochemical, physiological and chemical feature values was performed with NCSS
210 program, 2004 version (Number Cruncher Statistical Systems; Kaysville, UT, USA).

211

212 **3 Results and Discussion**

213 3.1 Trace metals content in soil

214 The general characteristics of the urban soils of Pisa are reported in **Table 1.** On the
215 basis of USDA classification, soils of Pisa showed a predominantly sandy-loam texture
216 (76% of the examined soils). Soil pH ranged from 7.2 to 8.3, with a mean of 7.6. On
217 the basis of the USDA terminology, most of soils (68%) were classified as slightly
218 alkaline, 13% were neutral soils while moderately alkaline soils accounted for 19%.
219 The organic C contents of the Pisa urban soils showed a marked variability, with values
220 ranging from 1.32 to 7.57 g C 100 g^{-1} dry soil and were considerably higher than that
221 of the control (0.90 g organic C 100 g^{-1} soil).

222 The amounts of Cr, Cu, Mn and Zn in soils of Pisa are summarized in **Table 2**.
223 The control site showed a content of 39.8 Cr mg kg^{-1} soil, a value statistically lower
224 than those observed in the remaining areas in which the total amount of Cr varied
225 between 46.4 and 227.5 mg kg^{-1} of dry soil with a mean values of 71.2 mg kg^{-1}. On the
226 basis of Igeo and EF indexes, approximately 90% of monitored sites showed a small
227 level of Cr pollution, while the remaining soils were classified as moderately polluted.
228 The PI, a more severe index, classified 94% of the examined areas as middle polluted
229 and the remaining as highly polluted.

230 Copper is one of the elements that mostly enrich urban soils. It is assumed that
231 its increase is due to civil and industrial activities, like coal and oil combustion, pesticide
232 and dyes use, but especially to vehicular traffic (US EPA 2006). The total content of
233 Cu ranged widely between 30.94 and 142.35 mg kg^{-1} whereas in the control site Cu
234 content was 32.24 mg Cu kg^{-1}, a value significantly similar to those found in a small
235 number of soils (about 10%). On average, Pisa urban soils showed Igeo of 0.40, typical
236 of unpolluted areas. On the contrary, the EF value of 1.55 indicated an anthropogenic
237 enrichment, and also PI showed a moderate and widespread pollution.

The total amount of Mn ranged between 509 and 1063 mg kg^{-1} while in the control site Mn amount was 526 mg kg^{-1}, a value significantly lower than those observed in the most of monitored areas. Both Igeo and EF classification showed no enrichment or soil pollution by Mn, while PI showed a medium-low pollution level.

The main source of Zn enrichment is attributable to the vehicular traffic but the metal may originate also from wearing of brake lining, types and road paved surfaces, losses of oil and cooling liquid, corrosion of galvanized steel safety fence and other road furniture (Blok 2005). The total content of Zn varied remarkably between 61.70 and 522.70 mg kg^{-1}. In the control site, Zn content was 51.00 mg kg^{-1}, a value statistically lower than those found in the most of monitored sites. The Igeo index classified most soils as not polluted, the EF indicated a moderate enrichment in 25% of the areas while PI classified 16% of soils as middle polluted and 6% as strong polluted.

Despite the pollution level of Pisa urban soils begins to be alarming, at least for some metals, neutral and slightly alkaline soil pH limits the mobility of these elements reducing their availability for plants.

3.2 Trace metals content in plant tissues

Trace metals contents in shoot and root dandelion plants collected in urban sites of Pisa are reported in **Table 3**. Cr contents of control were 2.7 and 8.7 mg kg^{-1} for shoot and root portions, respectively. In shoot of plants grown in urban soils Cr varied between 2.0 and 20.0 mg kg^{-1}, while in root it ranged between 4.0 and 14.7 mg kg^{-1}. Values significantly higher than control were recorded in shoot Cr concentration of plants collected in the sites 22 and 24, whereas at radical level the highest Cr concentrations were recorded in the sites 16 and 24.

Dandelion tissues of the control site showed Cu values of 18.1 and 19.3 mg kg^{-1} for shoot and root, respectively. The amounts of the metal did not vary in mostly of the monitored sites, with the exception of site 22 for shoot and sites 7 and 30 for the radical portion in which Cu concentrations were higher than control.

As compared to the control site, the level of Mn in shoot was significantly greater only in sites 21 and 22, while in roots Mn was higher than control in sites 7 and 30.

In the aerial part, Zn concentration varied between 63.1 and 196.8 mg kg^{-1}, while in the roots it ranged between 40.0 and 155.2 mg kg^{-1}. Zn contents of control plants

were 116.7 and 56.7 mg kg^{-1} for shoot and root, respectively and an increase in root was observed only in the sites 7 and 13.

The values of metals concentrations in dandelion tissues grown in sites of urban area of Pisa are similar to those reported by other authors (Finžgar and Leštan 2007; Massa et al. 2010; Ligocki et al. 2011). For the element Zn, dandelion was able to translocate it from roots to the aerial parts; however, for the other elements dandelion showed concentration of the elements similar or lower in shoot as compared to the concentration recorded in root. These results obtained for Zn confirm as dandelion represent an indicator plant (Baker and Brooks 1989). As already found by other authors (Ge et al. 2000), the difference between our results showed little changes of trace element concentrations in tissues of plants grown in urban sites with respect to control. These results are supported by both the bioconcentration factor (BcF) and the translocation index (TI) for Cr, Cu and Zn. In fact, the BcF values relative to both shoots and roots were always below the cut-value of 100, indicating the exclusion of these elements from the dandelion in respect to the soil matrix (**Table 4**). Also TI pointed out the low translocation of Cr, Cu and Mn from the radical part of the plant, being more concentrated in this plant portion (**Table 4**). Theonly exception was represented by Zn for which a active translocation from roots to shoot was observed indicating as Towards the shoot, on the other hand, the contents of such heavy metals in dandelion were within the range of non-toxic concentrations (Kabata-Pendias and Pendias 2001).

No significant correlations were found between trace metals in soil and in plant tissue with the exception of Mn (**Table 5**). For this element an increase in root dandelion tissues was found in relation to the increased level in soil. Ge et al. (2002) and Rosselli et al. (2006) reported no correlations between the contents of metals in soil and plant of dandelion grown in polluted soils. The obtained results confirm that dandelion, with respect to Cr, Cu and Mn, is an excluding plant and this is in contrast to what stated by Gjorgieva et al. (2011) who observed that dandelion was a metal accumulators.

Zinc was concentrated prevalently in the root portion but, differently to the other elements, its content was above the limits of toxicity (Kabata-Pendias and Pendias 2001). In fact, translocation index for Zn was in almost all the sites greater than 1 sometimes reaching the value of 2. These values testifying Zn translocation from roots towards the shoot (**Table 4**)**.** Consequently, the values of BcF of the aerial part, in various sites greater than 100, suggested the partitioning of the metal in aboveground

portion of the plant (**Table 4**). Similar results for Zn accumulation in dandelion plants growing in a polluted soil, were reported by Massa et al. (2010).

Results indicate that dandelion was an avoider for Cr, Cu and Mn, while, conversely, this species accumulated Zn in shoots even though without evident symptoms of damage such as chlorosis and/or necrosis.

3.3 Metal chelating capacity

Metal chelating capacity (MCC) of *T. officinale* plants collected from Pisa urban areas is reported in **Figure 1**. In aerial part, MCC varied in a wide range between 15.6% and 57.3% of the control (mean of 31.9%) while in roots it changed between 15.5% and 69.7% (mean of 48.9%). MCC of control were 15.2% and 40.3% for root and shoot portion, respectively. Differently from what reported by Seregin and Ivanov (2001), with very few exceptions, MCC did not change in different sites even though differences in MCC were found between shoot and root values. In fact, the latter showed, sometimes, a greater activity (**Figure 1**). The MCC of the dandelion shoots was not correlated with that of the roots, nor with any other biochemical or physiological parameters (**Table 6**), while the MCC of roots showed a correlation with the shoot and root phenols content, suggesting a possible involvement of these compounds in the mechanism of trace metals abatement.

3.4 Chlorophyll a fluorescence and pigment content

Maximum quantum yield of PSII (F_V/F_m), i.e. potential photosystem II efficiency for photochemistry, in leaves of dandelion grown in monitored site varied in a very narrow range between 0.808 and 0.838 (**Figure 2A**), value typical of leaves of "healthy" plants (Björkman and Demmig 1987). On the other hand, the actual quantum yield of PSII (Φ_{PSII}; **Figure 2B**) was meanly 0.602, similar to that of plants grown in control site (0.627). Leaves from site 11 showed the significant highest values of Φ_{PSII}, whereas the lowest values were recorded in sites 22, 24, 26 and 28 (**Figure 2B**).

The photochemical quenching coefficient, q_P, i.e. the proportion of open PSII centers, varied in a very narrow range between 0.802 and 0.940 (**Figure 2C**) and was similar to that recorded in control site. Non-photochemical quenching coefficient (q_N), i.e. the excitation energy dissipation through non-photochemical processes, like heat, varied in a range between 0.422 and 0.699 (mean of 0.559) (**Figure 2D**). Non-photochemical quenching coefficient was similar in dandelion leaves from different

monitored sites, with the only exception of sites 11 and 26. In fact, dandelion leaves from site 11 showed the lowest value of q_N and that from site 26 the highest. These results corroborate the values of Φ_{PSII}. In site 11 leaves showed the highest values of Φ_{PSII} and ETR (**Figure 2E**) and no activation of dissipation mechanisms, i.e. q_N, thus no alteration at photosynthetic level was recorded. On the other hand, in the other sites q_N increased, reaching the highest level in site 26 characterized by low levels of Φ_{PSII} and ETR (**Figures 2 B and E**).

These results are confirmed also by the significant correlation coefficients obtained from the regression analysis among Chl fluorescence parameters (**Table 6**). Values of Φ_{PSII} and ETR were significantly correlated with the others but the correlations were negative with q_N. The negative correlation between Φ_{PSII} and q_N reflects a reduction in the fraction of light energy that is used for photochemistry and an increase in dissipation mechanisms aimed to photoprotection of photosynthetic apparatus (Demmig-Adams and Adams 2006). A significant negative correlation was found between Zn concentration in the soils and F_v/F_m ratio indicating a negative influence of the increase in Zn in the soil and the PSII photochemical quantum yield (**Table 5**). Even though in experimental conditions, it has been already reported that Zn interacts with the donor side of PSII inhibiting the Hill reaction (Prasad and Strzalka 1999) limiting the PSII efficiency (Redondo-Gómez et al. 2011).

The total Chl content varied from 0.98 to 2.01 μg cm^{-2}, with a mean of 1.38 (**Figure 3A**), values significantly similar to that found in dandelion leaves grown in control site (1.20 μg cm^{-2}). A significant higher value of total Chl was found in site 28, while the lowest in site 13 (**Figure 3A**) as compared with the control. As regards carotenoids, their total content ranged between 0.22 and 0.33 μg cm^{-2}, values similar to that of the control site (**Figure 3B**). No statistical difference among leaves from different sites was detected. Pigment content was not related with the trace metals content in the soil (**Table 5**) while a strong correlation (0.547) between Chl content and carotenoid was found (**Table 6**). Conversely, no significant correlation among Chl content and Chl fluorescence parameters, suggesting that these last parameters were not influenced by Chl content.

Results from chlorophyll *a* fluorescence and content underline a low sensitivity of the dandelion photosynthetic mechanism to urban environment pollution. This was already reported by Lanaras et al. (1994) who investigating on the response of *T.*

officinale in an urban pollution of Thessaloniki (Greece) found no relation both between Chl amount and the F_v/F_m ratio and pollution levels. In a similar way, more recently, Massa et al. (2010), characterizing trace metals accumulating ability by autochtonous plants grown in an Italian multi-contaminated site, had shown in dandelion plants no significant changes in photochemical PSII efficiency.

Although there is evidence that the excess of trace metals inhibits directly the photosynthetic electron transport (Krupa and Baszyński 1995; Burzyński and Kłobus 2004; Nagajyoti et al. 2010) as well as the activities of Calvin-Benson cycle enzymes or the net assimilation of CO_2 (Prasad and Strzałka 1999), Sgardelis et al. (1994) showed that heavy metals did not influence PSII efficiency in *Sonchus* spp. and *Taraxacum* spp. plants grown in urban environment. Trace metal pollution has been shown to have no effect also on the chlorophyll content of some lichens species (Chettri et al. 1998). However different findings are available in literature. For example, Cu excess is demonstrated to cause *in vitro* photoinhibition of PSII, decrease of leaf chlorophyll concentration and reduction of the thylakoid membrane network in *Phaseolus vulgaris* L. (Pätsikkä et al. 2002). Furthermore, in two *Zea mays* L. cultivars, Cu was found to decrease the quantum efficiency of PSII, ETR and q_P, inducing also a drop of leaf chlorophyll and carotenoids contents (Tanyolaç et al. 2007).

3.5 Antioxidant capacity and phenol content

The antioxidant capacity in shoot varied in a wide range between 7.3 and 305.3 µM Trolox equivalent (TE) g^{-1} (mean of a 76.4), while in roots it ranged between 25.9 and 74.3 µM TE g^{-1} (**Figure 4A**). The antioxidant capacity of control was 132.6 and 40.5 µM TE g^{-1} for shoot and root portion, respectively. It is evident how the antioxidant capacity was higher in shoot portion than in root independently to the site where plants were grown (**Figure 4A**) and, with very few exceptions, it was similar in all monitored sites. The exception are represented by sites 6, 19, 28 and 31, in which the shoot portion of dandelion had a significantly high antioxidant capacity. On the other hand, in these sites the shoot portion contained a high phenols content (**Figure 4B**), that varied between 2.45 and 30.13 mg of gallic acid g^{-1}. In the same sites, root phenol amounts ranged between 2.80 and 6.97 mg of gallic acid g^{-1}. Phenolic substances of plants collected from the control site were 9.83 and 3.99 mg of gallic acid g^{-1} for shoot and root, respectively. Phenolic compounds in shoot and root portion of control were higher than those found in the same species by Zheng and Wang (2001), and were

similar to those found by Sengul et al. (2009) and by Hudec et al. (2007), which also found a slightly higher antioxidant activity in the root than in the leaves.

The antioxidant capacity and the phenol content in shoot portion of dandelion were positively correlated with Cu and Zn contents in soil (**Table 5**). The antioxidant capacities of both parts of the plant were strongly correlated each other and with their own phenolics content, as shown in **Table 6**. This because phenols are generally recognized as one of the most active groups of antioxidants (Bors and Michel 2002; Katalinic et al. 2006; Fraga et al. 2010). The antioxidant capacity of aerial and radical part is related with the photochemical efficiency of PSII, while antioxidant capacity of the aerial part showed a direct correlation also with the chlorophylls content.

4 Conclusions

The results reported in this work showed widespread very limited soil pollution of trace metals in the urban sites. Soil reaction close to neutral and slightly alkaline limits the mobility of the elements suggesting a scarce absorption by plants. On the other and, results of metal accumulation in shoot and root tissues of dandelion confirm these results, even though the ability of this species to exclude metals cannot be rejected. In fact, BcF values (below the cut-value of 100) indicate a scarce assimilation of the elements by dandelion, but also a poor translocation (TI values) from root to shoot. Results showed also that the trace metals concentration into the tissues is below the toxicity threshold. Zn that reached sometimes in root tissues the limits of toxicity represents the only exception. However, no visible symptoms of damage were recorded on leaf dandelion even if in plants in which Zn accumulated, the F_v/F_m ratio decreased significantly indicating a negative effect of this element on photosynthetic process. In addition, in these plants even an increase in phenols content and, consequently, in antioxidant capacity, was found. This could indicate that although dandelion accumulate Zn at level above toxicity (as confirmed also by the decrease in F_v/F_m ratio) the high antioxidant capacity of leaves represents a response to the oxidative load triggered by Zn.

Phenols play a key role as antioxidant moieties as evidenced by the their increase in tissues of plants in which higher Zn content was found.

In conclusion, dandelion shows a high physiological performance that induces in this species a high fitness in different environmental constrained conditions. This

seems attributable to the high phenols content that, in turn, determines a high antioxidant capacity.

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Legends of the Figures

Figure 1. Metal chelating capacity (MCC) of *Taraxacum officinale* shoot (white bar) and root (grey bar) from plants grown in different monitored sites. Each value represents the mean of three replicates. Different letters identify statistically different means in accordance with Tuckey HSD test (P=0.05).

Figure 2. Chlorophyll fluorescence parameters (maximal photochemical quantum yield of PSII, F_v/F_m; actual photochemical quantum yield of PSII, Φ_{PSII}; photochemical quenching coefficient, q_P; non-photochemical quenching coefficient, q_N; and electron transport rate, ETR) determined in leaves of *Taraxacum officinale* grown in monitored sites. Each value represents the mean of three replicates. Different letters identify statistically different means in accordance with Tuckey HSD test (P=0.05).

Figure 3. Pigment content in leaves of *Taraxacum officinale* collected in monitored sites. Each value represents the mean of three replicates. Different letters identify statistically different means in accordance with Tuckey HSD test (P=0.05).

Figure 4. Total antioxidant capacity (TEAC) and phenols content in shoot (white bar) and root (grey bar) of *Taraxacum officinale* collected in monitored area. Each value represents the mean of three replicates. Different letters identify statistically different means in accordance with Tuckey HSD test (P=0.05).

Table 1. General features of urban soils of Pisa as compared to rural area of San Rossore (Control). All parameters (n=3) are expressed as g 100 g^{-1} dry soil.

Soil property	Mean	±SD	Minimum	Maximum	Control
Sand	68.1	10.8	40.1	85.2	72.9
Silt	16.8	6.4	7.9	30.8	16.4
Clay	15.1	5.5	6.9	31.6	10.7
pH	7.6	0.3	7.2	8.3	8.0
$CaCO_3$	9.3	4.4	2.8	23.1	6.6
Organic C	3.3	1.2	1.3	7.6	0.9

Table 2. Heavy metals content (mg kg^{-1} soil) in urban soils of Pisa. Each values represents the mean of three replicates. Different letters identify different means in accordance with Tuckey HSD test (P=0.05).

Site	Heavy metals			
	Cr	**Cu**	**Mn**	**Zn**
0	39.8 n	32.2 pq	526 pq	51.0 r
1	115.0 c	124.9 b	668 ij	146.5 e
2	62.6 ef	69.3 hij	704 gh	100.9 jk
3	56.9 fgh	74.9 fg	765 ef	155.9 cde
4	48.5 lm	76.8 ef	746 f	133.7 f
5	52.4 hijklm	57.7 lmn	509 q	96.6 kl
6	49.4 klm	91.0 d	702 gh	96.7 kl
7	68.6 e	100.5 c	863 b	165.2 c
8	218 b	34.2 pq	632 lmn	90.9 klm
9	57.6 fghij	47.4 o	688 ghi	64.4 q
10	61.2 efg	61.2 klm	696 gh	117.3 hi
11	56.4 fghijk	77.7 ef	819 c	76.2 op
12	48.2 lm	74.1 fgh	606 no	84.3 mno
13	49.5 klm	70.0 ghi	687 ghi	160.0 cd
14	54.0ghijklm	62.5 kl	614 mno	110.2 ij
15	51.0 jklm	64.8 jk	658 jkl	131.2 fg
16	227.5 a	30.9 q	538 p	83.5 mno
17	56.8 fghijklm	56.2 mn	708 g	78.3 nop
18	51.7 ijklm	37.1 p	640 jkl	79.9 mnop
19	67.5 e	54.4 n	740 f	68.9 pq
20	58.9 fghi	54.1 n	704 gh	82.8 mno
21	59.2 fghi	62.6 kl	664 ijk	150.3 de
22	46.4 mn	57.2 mn	677 hij	205.3 b
23	61.5 efg	68.4 ij	804 cd	87.9 lmn
24	57.8 fghij	70.6 ghi	688 ghi	110.7 hij
25	94.4 d	80.8 e	1063 a	131.0 fg
26	55.9 fghijkl	63.0 k	700 gh	77.4 nop
27	52.9 hijklm	46.3 o	640 klm	90.3 klm
28	51.5 ijklm	44.7 o	589 o	61.7 qr
29	67.9 e	68.9 ij	798 cd	84.2 mno
30	57.1 fghijk	64.4 jk	782 de	121.5 gh
31	87.9 d	142.3 a	749 f	522.7 a
Average	**71.2**	**67.4**	**705**	**121.5**

716 **Table 3.** Heavy metals content (mg kg^{-1} dry weight) in shoot and root of *Taraxacum officinale* plants collected in monitored sites. Each
717 value represents the mean of three replicates. Different letters identify statistically different means in accordance with Tuckey HSD test
718 (P=0.05).-

Site	Shoot				Root			
	Cr	Cu	Mn	Zn	Cr	Cu	Mn	Zn
0	2.7 de	16.7 def	23.3 def	116.7 abcdefg	8.7 bcde	14.7 def	70.0 bc	56.7 fg
1	2.0 e	15.3 def	25.3 def	83.3 cdefg	7.3 bcde	16.7 def	28.0 def	40.0 g
3	2.0 e	20.0 bcdef	23.3 def	113.3 abcdefg	4.0 cde	15.3 def	24.7 def	53.3 fg
4	10.0 bcd	20.0 bcdef	50.0 cdef	100.0 bcdefg	7.7 bcde	23.1 bcd	53.8 cd	53.8 fg
6	2.0 e	16.0 def	43.3 cdef	130.0 abcdef	6.0 cde	15.3 def	42.0 cdef	66.7 efg
7	5.5 cde	20.8 bcdef	40.9 cdef	173.1 ab	9.1 bcde	33.3 a	90.9 ab	151.2 abcde
8	5.7 cde	15.3 def	34.1 def	162.9 abc	10.2 bcd	22.3 bcdef	29.2 def	77.7 cdefg
13	4.6 cde	13.9 f	41.4 cdef	87.6 bcdefg	10.3 bcd	20.7 bcdef	72.4 bc	155.2 abcd
14	8.8 bcde	20.5 bcdef	49.5 cdef	123.9 abcdefg	9.0 bcde	14.7 def	19.7 ef	63,7 fg
15	5.4 cde	16.9 def	34.4 def	105.0 bcdefg	8.7 bcde	18.5 cdef	35.7 def	119.6 abcdefg
16	4.0 cde	16.0 def	30.7 def	83.3 cdefg	14.7 ab	17.3 def	27.3 def	80.0 cdefg
21	5.3 cde	15.7 def	73.2 bc	121.5 abcdefg	9.2 bcde	17.1 def	55.6 cd	78.5 cdefg
22	20.0 a	28.2 ab	107.9 a	196.8 a	9.4 bcde	22.6 bcde	23.7 def	65.2 efg
24	11.2 bc	16.3 def	48.9 cdef	123.7 abcdefg	14.5 ab	14.5 ef	29.1 def	111.8 abcdefg
27	3.3 cde	18.7 cdef	32.0 def	103.3 bcdefg	6.4 cde	20.1 bcdef	27.6 def	87.5 bcdefg
30	5.7 cde	19.6 cdef	41.2 cdef	63.1 fg	7.8 bcde	26.5 abc	51.9 cde	76.7 cdefg
31	2.0 e	17.3 def	19.3 f	106.7 bcdefg	4.0 cde	14.7 def	24.7 def	70.0 defg
Average	**5.9**	**18.1**	**42.3**	**117.3**	**8.6**	**19.3**	**41.5**	**82.8**

719

720 **Table 4.** Indexes of heavy metals bioconcentration (BcF) or translocation (TI) in *Taraxacum officinale* plants collected in monitored sites.

	Cr			Cu			Mn			Zn		
Site	**BcF**		**TI**	**BcF**		**TI**	**BcF**		**TI**	**BcF**		**TI**
	Shoot	**root**		**shoot**	**root**		**shoot**	**root**		**shoot**	**root**	
0	6.7 bc	21.8 ab	0.3 b	51.7 a	45.5 bc	1.1 a	4.4 cdef	13.3 a	0.3 c	228.8 a	111.1 a	2.1 abc
1	1.7 c	6.4 cd	0.3 b	12.3 f	13.3 fg	0.9 a	3.8 def	4.2 c	0.9 bc	56.9 cd	27.3 bc	2.1 ab
3	3.4 c	6.7 cd	0.5 b	26.7 cdef	20.5 efg	1.3 a	3.1 ef	3.2 c	0.9 bc	72.7 cd	34.2 bc	2.1 ab
4	20.6 b	15.9 abc	1.3 ab	26.0 cdef	30.0 cdef	0.9 a	6.7 cde	7.2 bc	0.9 bc	74.8 cd	40.3 abc	1.9 abc
6	4.0 c	12.1 bcd	0.3 b	17.6 ef	16.8 efg	1.0 a	6.2 cdef	6.0 bc	1.0 bc	134.4 bc	68.9 abc	2.0 abc
7	8.0 bc	13.3 bcd	0.6 b	20.7 def	33.2 cde	0.6 a	4.7 cdef	10.5 ab	0.4 bc	104.8 bcd	91.7 ab	1.1 bc
8	2.6 c	4.7 d	0.6 b	44.7 ab	65.0 a	0.7 a	5.4 cdef	4.6 c	1.2 bc	179.3 ab	85.5 abc	2.1 abc
13	9.3 bc	20.9 ab	0.4 b	19.8 def	29.5 cdef	0.7 a	6.0 cdef	10.5 ab	0.6 bc	54.8 cd	97.0 ab	0.6 c
14	16.3 bc	16.7 abc	1.0 b	32.8 bcd	23.4 defg	1.4 a	7.8 bc	3.2 c	2.4 b	112.5 bc	57.8 abc	1.9 abc
15	10.6 bc	17.0 abc	0.6 b	26.1 cdef	28.6 cdefg	0.9 a	5.2 cdef	5.4 bc	1.0 bc	80.0 cd	91.2 ab	0.9 bc
16	1.8 c	6.4 cd	0.3 b	51.7 a	56.0 ab	0.9 a	5.7 cdef	5.1 bc	1.1 bc	99.8 bcd	95.8 ab	1.0 bc
21	9.5 bc	15.7 abc	0.6 b	25.1 def	27.4 cdefg	0.9 a	11.0 b	8.4 abc	1.3 bc	80.8 cd	52.2 abc	1.5 abc
22	43.1 a	20.3 ab	2.1 a	49.3 a	39.6 bcd	1.2 a	15.9 a	3.5 c	4.5 a	95.8 bcd	31.7 bc	3.0 a
24	19.4 b	25.2 a	0.8 b	23.1 def	20.6 efg	1.1 a	7.1 cd	4.2 c	1.7 bc	111.8 bc	101.0 ab	1.1 bc
27	6.3 bc	12.1 bcd	0.5 b	40.3 abc	43.5 bc	0.9 a	5.0 cdef	4.3 c	1.2 bc	114.4 bc	96.9 ab	1.2 bc
30	10.0 bc	13.8 bcd	0.7 b	30.4 bcde	41.2 bcd	0.7 a	5.3 cdef	6.6 bc	0.8 bc	52.0 cd	63.1 abc	0.8 bc
31	2.3 c	4.6 d	0.5 b	12.2 f	10.3 g	1.2 a	2.6 f	3.3 c	0.8 bc	20.4 d	13.4 c	1.5 abc

Average	**10.6**	**13.2**	**0.7**	**28.7**	**31.2**	**1.0**	**6.3**	**5.6**	**1.3**	**90.3**	**65.5**	**1.5**

721

Table 5. Correlation matrix among soil heavy metals content and heavy metals and some physiological and biochemical parameters in shoot and root of *Taraxacum officinale* collected in the monitored sites.**: P=0.01; *: P=0.05; ns.: P>0.05.

	Cr	Cu	Mn	Zn
F_v/F_m	ns	ns	ns	* -0.545
q_P	ns	ns	ns	ns
q_N	ns	ns	ns	ns
Φ_{PSII}	ns	ns	ns	ns
ETR	ns	ns	ns	ns
Chl *a*	ns	ns	ns	ns
Chl *b*	ns	ns	ns	ns
Total Chl	ns	ns	ns	ns
carotenoids	ns	ns	ns	ns
$TEAC_{shoot}$	ns	** 0.676	ns	* 0.513
$TEAC_{root}$	ns	ns	ns	ns
$Phenolics_{shoot}$	ns	** 0.637	ns	* 0.504
$Phenolics_{root}$	ns	ns	ns	ns
MCC_{shoot}	ns	ns	ns	ns
MCC_{root}	ns	ns	ns	ns
Cr_{shoot}	ns	ns	ns	ns
Cr_{root}	ns	* -0.544	ns	ns
Cu_{shoot}	ns	ns	ns	ns
Cu_{root}	ns	ns	* 0.544	ns
Mn_{shoot}	ns	ns	ns	ns
Mn_{root}	ns	ns	* 0.585	ns
Zn_{shoot}	ns	ns	ns	ns
Zn_{root}	ns	ns	ns	ns

Table 6. Correlation matrix among *Taraxacum officinale* features. The coefficient of correlation used to discriminate (30 *d.f*) within were: 0.449 for P=0.01 and 0.349 for P=0.05. **: P=0.01; *: P=0.05; ns.: P>0.05.

	F_v/F_m	q_P	q_N	Φ_{PSII}	ETR	Chl *a*	Chl *b*	Total Chl	carotenoids	$TEAC_{shoot}$	$TEAC_{root}$	$Phenol_{shoot}$	$Phenol_{root}$	MCC_{shoo}	MCC_{root}
F_v/F_m	1	-0.798	0.882	0.890	0.881	-0.227	-0.240	-0.260	0.025	0.444	0.579	0.467	0.487	-0.285	-0.089
q_P	**	1	-0.779	0.903	0.898	0.051	0.039	0.046	0.031	0.016	-0.150	-0.029	-0.220	0.286	0.343
q_N	**	**	1	-0.934	-0.912	0.054	0.025	0.052	0.152	0.123	0.257	0.135	0.258	-0.197	-0.154
$\Phi_{PS\ II}$	**	**	**	1	0.983	-0.040	0.014	-0.026	-0.099	-0.058	-0.210	-0.085	-0.198	0.235	0.313
ETR	**	**	**	**	1	-0.052	0.019	-0.031	-0.132	-0.054	-0.216	-0.083	-0.212	0.211	0.271
Chl *a*	ns	ns	ns	ns	ns	1	0.511	0.910	0.782	-0.448	-0.306	-0.406	-0.297	0.250	0.088
Chl*b*	ns	ns	ns	ns	ns	**	1	0.821	0.057	-0.355	-0.324	-0.291	-0.199	0.250	-0.021
Total Chl	ns	ns	ns	ns	ns	**	**	1	0.547	-0.467	-0.355	-0.407	-0.286	0.293	0.046
carotenoids	ns	ns	ns	ns	ns	**	ns	**	1	-0.009	0.081	-0.002	-0.110	0.191	0.310
$TEAC_{hoot}$	*	ns	ns	ns	ns	*	*	**	ns	1	0.795	0.965	0.435	-0.115	0.408
TEAChroot	**	ns	ns	ns	ns	ns	ns	*	ns	**	1	0.853	0.644	-0.126	0.337
$Phenol_{shoot}$	**	ns	ns	ns	ns	*	ns	*	ns	**	**	1	0.515	-0.017	0.382
$Phenol_{root}$	**	ns	ns	ns	ns	ns	ns	ns	ns	*	**	**	1	0.183	0.438
MCC_{hoot}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	1	0.219
MCCroot	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	*	ns	1