Water, Air, & Soil Pollution

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

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Corresponding Author:	Lucia Guidi University of Pisa Pisa, ITALY
Corresponding Author's Institution:	University of Pisa
First Author:	Giacomo Vanni
Order of Authors:	Giacomo Vanni
	Roberto Cardelli
	Fausto Marchini
	Alessandro Saviozzi
	Lucia Guidi
Abstract:	. Physiological and biochemical characteristics were evaluated in dandelion plants (Taraxacum officinale) growing naturally in an urban environment. The study area was located in Pisa, Italy, and 27 sites in the municipality were chosen to assess the biochemical and physiological features of dandelion plants and the trace metal content in the urban soil. Concentrations of elements including, Cr, Cu, Mn and Zn were analyzed in the soil together with dandelion shoot and root tissues collected from the various sites. Chlorophyll a fluorescence analysis the pigment content, antioxidant power and phenol content were determined in dandelion. The results showed very limited soil pollution due to trace metals in the urban sites. However, dandelion showed Zn uptake and translocation although no damage was observed in the plants. Our results highlight that dandelion plants are able to survive in a constrained environment thanks to the high phenol content which is effective in combatting the oxidative stress induced by heavy metals.
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Are the physiological and biochemical characteristics in dandelion plants growing in an urban area (Pisa, Italy) indicative of soil pollution?

G. Vanni, R. Cardelli, F. Marchini, A. Saviozzi, L. Guidi

Department of Agriculture, Food and Environment, Via del Borghetto, 80 – 56124 Pisa, Italy

Corresponding author: Lucia Guidi, Department of Agriculture, Food and Environment, Via del Borghetto, 80 – 56124 Pisa, Italy; Phone: +39 50 2216613; Email: luciaguidi@unipi.it

Abstract. Physiological and biochemical characteristics were evaluated in dandelion plants (*Taraxacum officinale*) growing naturally in an urban environment. The study area was located in Pisa, Italy, and 27 sites in the municipality were chosen to assess the biochemical and physiological features of dandelion plants and the trace metal content in the urban soil. Concentrations of elements including, Cr, Cu, Mn and Zn were analyzed in the soil together with dandelion shoot and root tissues collected from the various sites. Chlorophyll *a* fluorescence analysis the pigment content, antioxidant power and phenol content were determined in dandelion. The results showed very limited soil pollution due to trace metals in the urban sites. However, dandelion showed Zn uptake and translocation although no damage was observed in the plants. Our results highlight that dandelion plants are able to survive in a constrained environment thanks to the high phenol content which is effective in combatting the oxidative stress induced by heavy metals.

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

1 Introduction

Urban ecosystems are generally made up of commercial, industrial, residential, transport, recreational, agricultural and natural areas. This determines different habitats for plants, animals and humans. The quality of these urban habitats is affected by abiotic and biotic components such as air, soil and water quality, microclimate and vegetation. Pollution is probably the most significant characteristic of urban soil (Vrščaj et al. 2008) and this can negatively impact on human health. Clearly, trace metals content plays a key role in the pollution (Biasioli et al. 2006).

Phytotoxic amounts of trace metals induce an oxidative stress in plants leading to cellular damage (Yadav 2010). In addition, plants accumulate metal ions which disturb cellular ionic homeostasis, and growth usually becomes inhibited and biomass production decreases (Moulis 2010). Trace metals are active in plant metabolic processes; however they can also be stored as inactive compounds in cell walls, thus affecting the chemical composition of plants without causing any injury (Nagajyoti et al. 2010). Vascular plants are thus frequently used for biomonitoring (Korzeniowska and Panek 2010). However, native plants with trace metal tolerance characterized by rapid growth usually attract more attention compared to slow-growing plants (Massa et al. 2010). The dandelion (*Taraxacum officinale* Web.) has long been proposed as a good bioindicator for environments polluted by trace elements (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 only found for leaves. However, these authors considered the sum of the acid-exchangeable, reducible and oxidizable soil fractions of these elements as a poor indicator of potential plant uptake. On the other hand, in a study on trace metal pollution in Macedonia soils, Gjorgieva et al. (2011), classified *T. officinale* as a trace metal accumulator.

Dandelion also has a high antioxidant activity due to the high content of secondary metabolites (Park et al. 2011; González-Castejón et al. 2012; Davaatseren et al. 2013). As secondary metabolites are important in plant adaptation to environmental stress, it is likely that plant species that possess a high number of secondary metabolites efficiently counteract 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). With this tool, the F_v/F_m ratio has been extensively used to monitor photoinhibitory damage (Maxwell and Johnson, 2000). The dandelion has been previously used to monitor urban pollution by means of chlorophyll *a* fluorescence analysis together with other photosynthetic parameters, although there are only a few works on this topic (Lanaras et al. 1994; Sgardelis et al. 1994; Molina-Montenegro et al. 2010).

The novelty of our work consists in using *T. officinale* as a bioindicator to assess its healthy status by chlorophyll *a* fluorescence analysis. This is the 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 Pisa ((latitude 43°25’00N; longitude 10°43'00E; Italy), an urban environment of approximately 187 km^2 and about 90,000 inhabitants. The artificial surface (impermeable urban area) was approximately 27 km^2 (almost 15% of the total area), with an above average soil consumption compared with data for the surrounding areas. The built-up area has been steadily increasing, with the largest increases since the 1950s (+260% increase from 1954 to 2003). Samples were collected from 31 sites around the urban areas of Pisa. Sites 1, 2, 8, 9, 10, 11, 12, 16, 25, 26, 28, 29, and 31 are public green areas used as playgrounds; Sites 3, 5, 7, 14, 15, 19, 20, 21, 23, 24, 27, and 30 are traffic roundabouts; Sites 4 and 22 are green squares; Sites 6 and 17 are state school gardens; Sites 13 and 18 are public gardens.

Site 0 was the control site which is close to the city and 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, originally cultivated and now naturalized for more than twenty years. In reality the plant analyses were carried out only in 27 sites because four sites (sites 10, 17, 23 and 25) had no plants that had completed the entire growth cycle.

2.2 Plant and soil sampling

T. officinale plants were collected at the flowering stage (from April to May), which is when the species was easily identifiable and had reached 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 from the corners of the square and one in the middle. The five soil cores of each sub-sample were mixed to avoid local non-homogeneity. The sampling was performed with a stainless steel hand auger. To avoid cross-contamination, the soil that came into contact with the metal digging tools was carefully eliminated with a porcelain putty knife, before packing the soil into plastic bags. In the laboratory, the samples were air-dried at room temperature (20°C), and, after manually removing any plant material, such as roots and leaves, they were stored at 4°C until analysis. Most of the main properties of the soils, including texture, pH (soil-water ratio 1:2,5), and limestone, were determined according to the standard methods (Sparks, 1996), while the 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) with 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.

2.4 Photosynthetic pigment content

Photosynthetic pigments were determined on three discs of leaf tissue (1.0 cm^2 of leaf area) extracted with 80% acetone. Chlorophyll concentrations were calculated from the equations proposed by Porra et al. (1989), whereas carotenoid concentrations were calculated from Lichtenthaler's equation (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 supernatants obtained were stored at -20°C until 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 Brand-Williams et al. (1995) with minor modifications as reported in Landi et al. (2013). Results are expressed as the 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 six minutes, 7% sodium carbonate solution was added and then the mixture was kept at 25°C in the dark for 90 minutes. Total polyphenol content was calculated from a calibration curve, using gallic acid as the standard. TP content was expressed as mg of gallic acid g^{-1} fresh weight (FW).

2.8 Metal chelating capacity (MCC)

Metal chelating capacity was determined in accordance with 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 mixture were thoroughly shaken. After 10 minutes, Fe^{2+} was monitored by measuring the absorbance of the 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 metal content

The content of trace metals was determined in 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 a 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) using a flame atomic absorption spectrophotometer (Perkin Elmer Analyst 100, USA).

2.10 Soil trace metal content

The total content of trace metals was determined by ISO 11466 (1995). Briefly, 1 g of soil sieved to ≈ 80 mesh was treated with aqua regia. Pre-digestion was carried out at room temperature for 16 hours with occasional manual agitation and digestion was performed for 2 hours at 130±2°C. The suspension obtained 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

The 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 is the concentration of reference element for normalization. The reference element used 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 also calculated. The BcF is the ratio of the metal concentration of shoots and roots of dandelion to the metal concentration of the soil (Kim et al. 2003). The translocation ability of metal in dandelion was expressed as the 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).

The amounts of Pb found in the urban areas of Pisa were considerably lower than those measured in the coastal cities of Tuscany (the region where Pisa is located) (Bretzel and Calderisi, 2006). Most of the monitored areas showed statistically similar Ni and Cd contents to those observed in the control soil, and considerably lower than the limits established by Italian legislation for residential areas (Legislative Decree 152/2006). Contamination assessment indexes (Igeo and EF) Pb, Ni and Cd revealed negligible levels of pollution.

In addition, the most available chemical forms of the three metals, such as the soluble and oxidizable fractions, were present in very low values in all the monitored areas. Thus, the amounts of Pb, Ni and Cd were not reported.

2.12 Statistical analysis

All the analyses were carried out in triplicate and the results were presented as means ± SD. Analysis of variance was performed using CoStat, version 6.311 (CoHort Software; Monterey, CA, USA). The means were compared using Tukey’s HSD test. Correlation analysis of biochemical, physiological and chemical values was performed with NCSS, 2004 version (Number Cruncher Statistical Systems; Kaysville, UT, USA).

3 Results and Discussion

3.1 Trace metal content in soil

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

The amounts of Cr, Cu, Mn and Zn are summarized in **Table 2**. The control site showed a content of 39.8 Cr mg kg^{-1} soil, which is statistically lower than values observed in the other areas, in which the total amount of Cr varied between 46.4 and 227.5 mg kg^{-1} of dry soil with a mean value of 71.2 mg kg^{-1}. According to Igeo and EF indexes, approximately 90% of the monitored sites showed a low level of Cr pollution, while the remaining soils were classified as moderately polluted. The PI, which is a stricter index, classified 94% of the examined areas as medium polluted and the remaining areas as highly polluted.

Copper is one of the elements that enriches urban soils. It is assumed that its increase is due to civil and industrial activities, such as coal and oil combustion, pesticide and dye use, but especially to vehicular traffic (US EPA 2006). The total content of Cu ranged widely between 30.94 and 142.35 mg kg^{-1} whereas in the control site, the Cu content was 32.24 mg Cu kg^{-1}, which is similar to what was found in a small number of soils (about 10%). On average, Pisa urban soils showed an Igeo of 0.40, typical of unpolluted areas. However, the EF value of 1.55 indicated an anthropogenic enrichment, and also the PI revealed that pollution was moderate and widespread.

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

The main source of Zn enrichment is likely due to vehicular traffic however the metal may also originate from the wearing of brake linings, types of road surfaces, losses of oil and cooling liquids, corrosion of galvanized steel safety fences, and other road furniture (Blok 2005). The total content of Zn varied notably between 61.70 and 522.70 mg kg^{-1}. In the control site, the Zn content was 51.00 mg kg^{-1}, which was statistically lower than in the most of the monitored sites. The Igeo index classified most soils as unpolluted; the EF indicated a moderate enrichment in 25% of the areas, while the PI classified 16% of soils as averagely polluted, and 6% as strongly polluted.

Although the pollution levels in the urban soils in Pisa would seem to be beginning to reach alarming levels, at least for some metals, the neutral and slightly alkaline soil pH limits the mobility of these elements reduces their availability for plants.

3.2 Trace metal content in plant tissues

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

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

Compared to the control site, the Mn level of in shoots was significantly greater only in sites 21 and 22, while in roots, Mn was higher than the 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 this ranged between 40.0 and 155.2 mg kg^{-1}. Zn content of control plants was 116.7 and 56.7 mg kg^{-1} for shoots and roots, respectively and an increase in roots was observed only in sites 7 and 13.

The values of metal concentrations in dandelion tissues grown in the urban sites are similar to those reported by other authors (Finžgar and Leštan 2007; Massa et al. 2010; Ligocki et al. 2011). The dandelion was able to translocate Zn from the roots to the aerial parts; however, for the other elements, the dandelion showed similar or lower concentrations in shoot compared to the concentration recorded in root. These Zn results confirm that dandelion represents an indicator plant (Baker and Brooks 1989). As already found by other authors (Ge et al. 2000), the trace element concentrations in tissues of plants grown in urban sites were very similar to the 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 compared to the soil matrix (**Table 4**). The TI also highlighted the low translocation of Cr, Cu and Mn from the root portion of the plant, as these three elements were more concentrated in this plant portion (**Table 4**). The only exception was Zn which showed an active translocation from roots to shoots. On the other hand, the contents of these heavy metals in the 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 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 dandelion plants grown in polluted soils. The results confirm that dandelion, in terms of Cr, Cu and Mn, is an excluding plant, which is in contrast to findings by Gjorgieva et al. (2011) who observed that dandelion was a metal accumulator.

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

The results indicate that dandelion was an avoider species for Cr, Cu and Mn, while, conversely, it accumulated Zn in shoots despite evident symptoms of damage such as chlorosis and/or necrosis.

3.3 Metal chelating capacity

The metal chelating capacity (MCC) of *T. officinale* plants collected from the urban areas is reported in **Figure 1**. In the aerial part, MCC varied between 15.6% and 57.3% in the control (mean of 31.9%), while in roots it was between 15.5% and 69.7% (mean of 48.9%). The MCC of the control was 15.2% and 40.3% for root and shoot portions, respectively. Unlike the findings reported by Seregin and Ivanov (2001), with very few exceptions, MCC did not change in the various sites although differences in MCC were found between shoot and root values. In fact, the root values sometimes showed 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 the roots showed a correlation with the shoot and root phenol content, suggesting a possible involvement of these compounds in the mechanism of trace metal abatement.

3.4 Chlorophyll a fluorescence and pigment content

The maximum quantum yield of PSII (F_V/F_m), i.e. potential photosystem II efficiency for photochemistry, in leaves of dandelion growing in the monitored sites varied slightly between 0.808 and 0.838 (**Figure 2A**), which are values typical of the leaves of "healthy" plants (Björkman and Demmig 1987). On the other hand, the mean quantum yield of PSII (Φ_{PSII}; **Figure 2B**) was 0.602, similar to plants grown in the control site (0.627). Leaves from site 11 showed the highest significant 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 slightly between 0.802 and 0.940 (**Figure 2C**) and was similar to that recorded in the control site. The non-photochemical quenching coefficient (q_N), i.e. the excitation energy dissipation through non-photochemical processes, such as heat, varied between 0.422 and 0.699 (mean of 0.559) (**Figure 2D**). The 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 site 26 showed 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 active dissipation mechanisms, i.e. q_N, thus no photosynthetic alteration 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 also confirmed 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 used for photochemistry and an increase in dissipation mechanisms aimed at the photoprotection of the photosynthetic apparatus (Demmig-Adams and Adams 2006). A significant negative correlation was found between the Zn concentration in the soils and F_v/F_m ratio, thus indicating a negative influence of the increase in Zn in the soil and the PSII photochemical quantum yield (**Table 5**). Despite being conducted 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), thus 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**), very similar values to those found in dandelion leaves grown in the control site (1.20 µg cm^{-2}). A significantly higher value of total Chl was found in site 28, while the lowest in site 13 (**Figure 3A**) compared with the control. The total content of carotenoids ranged between 0.22 and 0.33 µg cm^{-2}, similar to the control site (**Figure 3B**). No statistical differences among leaves from different sites were detected. Pigment content was not related with the trace metal 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 was found between Chl content and Chl fluorescence parameters, suggesting that fluorescence was not influenced by Chl content.

Results from chlorophyll *a* fluorescence and content analysis underline the low sensitivity of the dandelion photosynthetic mechanism to urban environment pollution. This has already been reported by Lanaras et al. (1994) who, investigating the response of *T. officinale* to urban pollution in Thessaloniki (Greece) found no relation both between Chl amount and the F_V/F_m ratio and pollution levels. Similarly, characterizing the ability of trace metals to accumulate by autochthonous plants grown in a multi-contaminated site in Italy, Massa et al. (2010), showed no significant changes in photochemical PSII efficiency in dandelion plants.

Although there is evidence that an excess of trace metals directly inhibits 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 an urban environment. Trace metal pollution has also been shown to have no effect on the chlorophyll content of various lichens species (Chettri et al. 1998). However the findings in the literature vary. For example, Cu excess has been demonstrated to cause *in vitro* photoinhibition of PSII, a decrease in leaf chlorophyll concentration and reduction in the thylakoid membrane network in *Phaseolus vulgaris* L. (Pätsikkä et al. 2002). In addition, in two *Zea mays* L. cultivars, Cu was found to decrease the quantum efficiency of PSII, ETR and q_P, also inducing a reduction in leaf chlorophyll and carotenoid contents (Tanyolaç et al. 2007).

3.5 Antioxidant capacity and phenol content

The antioxidant capacity in shoots varied 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 the control was 132.6 and 40.5 µM TE g^{-1} for shoot and root portions, respectively. It is clear that the antioxidant capacity was higher in the shoot portion than in the roots irrespectively of the site where the plants were grown (**Figure 4A**) and, with very few exceptions, was similar in all the monitored sites. The exceptions were sites 6, 19, 28 and 31, in which the shoot portion had a significantly high antioxidant capacity. On the other hand, in these sites the shoot portion contained a high phenol content (**Figure 4B**), which varied between 2.45 and 30.13 mg of gallic acid g^{-1}. In the same sites, root phenol content ranged between 2.80 and 6.97 mg of gallic acid g^{-1}. Phenolic substances 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 portions of the 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), who also found a slightly higher antioxidant activity in the roots than in the leaves.

The antioxidant capacity and the phenol content in the shoot portion of dandelion were positively correlated with Cu and Zn contents in the soil (**Table 5**). The antioxidant capacities of both parts of the plant were strongly correlated with each other and with their own phenolic content, as shown in **Table 6**. This is 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 parts is related with the photochemical efficiency of PSII, while the antioxidant capacity of the aerial part also showed a direct correlation with the chlorophyll content.

4 Conclusions

The results reported in this work showed widespread but very limited soil pollution of trace metals in the urban sites. Soil reaction that is almost neutral and slightly alkaline limits the mobility of the elements, suggesting a scarce absorption by plants.

On the other hand, metal accumulation results in shoot and root tissues of dandelion confirm these results, although the ability of this species to exclude metals cannot be ruled out. 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 also showed that the trace metal concentration in the tissues was below the toxicity threshold. Zn in root tissues, which sometimes reached the limits of toxicity, was the only exception. However, no visible symptoms of damage were recorded on the dandelion leaves although in plants, in which Zn accumulated the F_v/F_m ratio significantly decreased, thus indicating a negative effect of this element on the photosynthetic process. In addition, in these plants an increase in phenol content and, consequently, in antioxidant capacity, was also found. This could indicate that although dandelion accumulates Zn at levels above toxicity (as also confirmed by the decrease in F_v/F_m ratio), the high antioxidant capacity of the leaves represents a response to the oxidative load triggered by Zn.

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

In conclusion, dandelion shows a good physiological performance which means that this species is particularly suited to different environmentally constrained conditions. This seems attributable to the high phenol content, which, in turn, determines a high antioxidant capacity.

References

Baker, A.M.J., Brooks, R.R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. *Biorecovery, 1,* 81-126.

Baranowska, I., Srogi, K., Włochowicz, A. & Szczepanik, K. (2002). Determination of heavy metal contents in samples of medicinal herbs. *Polish Journal of Environmental Studies, 11,* 467-471.

Baumann, H.A., Morrison, L. & Stengel, D.B. (2009). Metal accumulation and toxicity measured by PAM—Chlorophyll fluorescence in seven species of marine macroalgae. *Ecotoxicology and Environmental Safety, 72*, 1063-1075.

Biasioli, M., Barberis, R. & Ajmone-Marsan, F. (2006). The influence of a large city on some soil properties and metals content. *Science of the Total Environment, 356,* 154-164.

Bini, C., Wahsha, M., Fontana, S., Maleci, L. (2012). Effects of heavy metals on morphological characteristics of *Taraxacum officinale* Web growing on mine soils in NE Italy. *Journal of Geochemical Exploration, 123,* 101-108.

Björkman, O. & Demmig, B. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta, 170,* 489-504.

Blok, J. (2005). Environmental exposure of road borders to zinc. *Science of the Total Environment, 348,* 173-190.

Bors, W. & Michel, C. (2002). Chemistry of the antioxidant effect of polyphenols. *Annals of the New York Academy of Sciences, 957,* 57-69.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free-radical method to evaluate antioxidant activity. *Food Science and Technology – Lebensmittel, 28,* 25-30.

Bretzel, F., Calderisi, M. (2006). Metal contamination in urban soils of coastal Tuscany (Italy). *Environmental Monitoring and Assessment, 118,* 319-335.

Burzyński, M. & Kłobus, G. (2004). Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress. *Photosynthetica, 42,* 505-510.

Chettri, M.K., Cook, C.M., Vardaka, E., Sawidis, T. & Lanaras, T. (1998). The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis. Environmental and Experimental Botany, 39,* 1-10.

Chen, T.B., Zheng, Y.M., Lei, M., Huang, Z.C., Wu, H.T., Chen, H., Fan, K.K., Wu, X. & Tian, Q.Z. (2005). Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China*. Chemosphere, 60,* 542-551.

da Silva, A.J., Araújo do Nascimento, C.M., da Silva Gouveia-Neto, A. & Arcanjo da Silva-Jr E. (2012). LED-Induced chlorophyll fluorescence spectral analysis for the early detection and monitoring of cadmium toxicity in maize plants. *Water, Air and Soil Pollution, 223,* 3527-3533.

Davaatseren, M., Hur, H.J., Yang, H.J., Hwang, J.-T., Park, J.H., Kim, H.-J., Kim, M.J., Kwon, D.Y. & Sung, M.J. (2013). *Taraxacum officinale* (dandelion) leaf extract alleviates high-fat diet-induced nonalcoholic fatty liver. *Food Chemistry and Toxicoogy, 58,* 30-36.

Demmig-Adams, B. & Adams III, W.W. (2006). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytologist, 172,* 11-21.

Dinis, T.C.P., Madeira, V.M.C. & Almeida, L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics, 315,* 161-169.

Dixon, R.A. & Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell, 7,* 1085-1097.

Du, G., Li, M., Ma, F. & Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and Vitamin C in *Actinidia* fruits. Food Chem. 113, 557-562.

Finžgar, N., Leštan, D., 2007.’ Multi-step leaching of Pb and Zn contaminated soils with EDTA. *Chemosphere, 66,* 824-832.

Fraga, C.G., Galleano, M., Verstraeten, S.V. & Oteiza, P.I. (2010). Basic biochemical mechanisms behind the health benefits of polyphenols. *Molecular Aspects of Medicine, 31,* 435-445.

Ge, Y., Murray, P. & Hendershot, W.H. (2000). Trace metal speciation and bioavailability in urban soils. *Environmental Pollution, 107,* 137-144.

Ge, Y., Murray, P., Sauvé, S. & Hendershot, W. (2002). Low metal bioavailability in a contaminated urban site. *Environmental Toxicology and Chemistry, 21,* 954-961.

Genty, B., Briantais, J.-M. & Baker, N.R. (1989). The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta, 990,* 87-92.

Genty, B., Harbinson, J., Briantais, J.-M. & Baker, N.R. (1990). The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 photochemistry in leaves. *Photosynthesis Research, 25,* 249-257.

Gjorgieva, D., Kadifkova-Panovska, T., Bačeva, K. & Stafilov, T. (2011). Assessment of heavy metal pollution in Republic of Macedonia using a plant assay. *Archives of Environmental Contamination and Toxicology, 60,* 233-240.

González-Castejón, M., Visioli, F. & Rodriguez-Casado, A. (2012). Diverse biological activities of dandelion. *Nutrition Reviews, 70,* 534-547.

Guidi, L., Degl’Innocenti, E., Giordano, C., Biricolti, S. & Tattini, M. (2010). Ozone tolerance in *Phaseolus vulgaris* depends on more than one mechanism. *Environmental Pollution, 158,* 3164–3171.

Hudec, J., Burdová, M., Kobida, L., Komora, L., Macho, V., Kogan, G., Turianica, I., Kochanová, R., Ložek, O., Habán, M. & Chlebo, P. (2007). Antioxidant capacity changes and phenolic profile of *Echinacea purpurea*, nettle (*Urtica dioica* L.), and dandelion (*Taraxacum officinale*) after application of polyamine and phenolic biosynthesis regulators. *Journal of Agriculture and Food Chemistry, 55,* 5689-5696.

International Organization For Standardization (1995). ISO 11466 Soil quality. Extraction of trace elements soluble in aqua regia – International Organization for Standardization, Geneva, Switzerland, 6 pp. (available on web at site www-iso.ch)

Kabata-Pendias, A. & Pendias, H. (2001). Trace Elements in Soils and Plants (3rd ed.). CRC Press, Boca Raton, FL.

Kang, H.M. & Saltveit, M.E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agriculture and Food Chemistry, 50,* 7536-7541.

Katalinic, V., Milos, M., Kulisic, T. & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry, 94,* 550-557.

Kim, I.S., Kang, K.H., Johnson-Green, P. & Lee, E.J. (2003). Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. *Environmental Pollution, 126,* 235-243.

Korzeniowska, J. & Panek, E. (2010). Heavy metal (Cd, Cr, Cu, Ni, Pb, Zn) concentrations in Spruce *Picea abies* L. along the roads of various traffic density in the Podhale region, Southern Poland. *Geomatics and Environmental Engeneering, 4,* 89-96.

Krupa, Z. & Baszynski, T. (1995). Some aspects of heavy metals toxicity towards photosynthetic apparatus - direct and indirect effects on light and dark reactions. *Acta Physiologia Plantarum, 17,* 177-190.

Küpper, H., Küpper, F.C. & Spiller, M. (1996). Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. *Journal of Experimental Botany, 47,* 259-266.

Lanaras, T., Sgardelis, S.P. & Pantis, J.D. (1994). Chlorophyll fluorescence in the dandelion (*Taraxacum* spp.): a probe for screening urban pollution. *Science of the Total Environment, 149,* 61-68.

Landi, M., Pardossi, A., Remorini, D. & Guidi, L. (2013). Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess. *Environmental and .Experimental Botany, 5,* 64–75.

Lichtenthaler, H.K. (1987). Chlorophyll and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148,* 331-382.

Ligocki, M., Tarasewcz, Z., Zygmunt, A.& Anisko, M. (2011). The common dandelion (*Taraxacum officinale*) as an indicator of anthropogenic toxic metal pollution of environment. *Acta Scientiarum Poloronorum Zootechnica, 10,* 73-82.

Lu, X., Li, L.Y., Wang, L., Lei, K., Huang, J. & Zhai, Y. (2009). Contamination assessment of mercury and arsenic in roadway dust from Baoji, China. *Atmospheric Environment, 43,* 2489-2496.

Massa, N., Andreucci, F., Poli, M., Aceto., M., Barbato, R. & Berta, G. (2010). Screening for heavy metal accumulators amongst autochtonous plants in a polluted site in Italy. *Ecotoxicology and Environmental Safety, 73,* 1988-1997.

Maxwell, K. & Johnson, G.N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany, 51,* 659-668.

Molina-Montenegro, M.A., Atala, C. & Gianoli, E. (2010). Phenotypic plasticity and performance of *Taraxacum officinale* (dandelion) in habitats of contrasting environmental heterogeneity. *Biological Invasions, 12,* 2277-2284.

Mossop, K.F., Davidson, C.M., Ure, A.M., Shand, C.A. & Hillier, S.J. (2009). Effect of EDTA on the fractionation and uptake by *Taraxacum officinale* of potentially toxic elements in soil from former chemical manufacturing sites. *Plant and Soil, 320,* 117-129.

Moulis, J.-M. (2010). Cellular mechanisms of cadmium toxicity related to the homeostasis of essential metals. *BioMetals, 23,* 877-896.

Muller, G. (1969). Index of geoaccumulation in sediments of the Rhine River. *Geological Journal, 2,* 109-118.

Nagajyoti, P.C., Lee, K.D. & Sreekanth, T V.M. (2010). Heavy metals, occurrence and toxicity for plants: a review**.** *Environmental Chemistry Letters, 8,* 199-216.

Park, C.M., Park, J.Y., Noh, K.H., Shin, J.H. & Song, Y.S. (2011). *Taraxacum officinale* Weber extracts inhibit LPS-induced oxidative stress and nitric oxide production *via* the NF-κB modulation in RAW 264.7 cells. *Journal of Ethnopharmacology, 133,* 834-842.

Pätsikkä, E., Kairavuo, M., Šeršen, F., Aro, E.-M. & Tyystjärvi, E. (2002). Excess copper predisposes photosystem ii to photoinhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll. *Plant Physiology, 129,* 1359-1367.

Porra, R.J., Thompson, W.A. & Kriedemann, P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) – Bioenergetics, 975,* 384-394.

Prasad, M.N.V. & Strzałka, S. (1999). Impact of heavy metals on photosynthesis, in: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants, from Molecules to Ecosystems. Springer, Berlin, pp. 117-138.

Redondo-Gómez, S., Andrades-Moreno, L., Mateos-Naranjo, E., Parra, R., Valera-Burgos, J. & Aroca, R. (2011). Synergic effect of salinity and zinc stress on growth and photosynthetic responses of the cordgrass, *Spartina densiflora*. *Journal of Experimental Botany, 62, 5521-5530.*

Rosselli, W., Rossi, M. & Sasu, I. (2006). Cd, Cu and Zn contents in the leaves of *Taraxacum officinale*. *Forest Snow and Landscape Research, 80,* 361-366.

Salt, D.E. & Krämer, U. (2000). Mechanisms of metal hyperaccumulation in plants, in: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. Wiley, New York, pp. 231-246.

Savinov, A.B., Kurganova, L.N. & Shekunov, Y.I. (2007). Lipid peroxidation rates in *Taraxacum officinale* Wigg. and *Vicia cracca* L. from biotopes with different levels of soil pollution with heavy metals. *Russian Journal of Ecology, 38,* 174-180.

Schreiber, U., Schliwa, U. & Bilger, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research, 10,* 51-62.

Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z. & Ercisli, S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pakistan journal of pharmaceutical sciences, 22,* 102-106.

Seregin, I.V. & Ivaniov, V.B. (2001). Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian Journal of Plant Physiology, 48,* 606-630.

Sgardelis, S., Cook, C.M., Pantis, J.D. & Lanaras, T. (1994). Comparison of chlorophyll fluorescence and some heavy metal concentrations in *Sonchus* spp. and *Taraxacum* spp. along an urban pollution gradient. *Science of the Total Environment, 158,* 157-164.

Singleton, V.L. & Rossi jr., J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16,* 144-158.

Sparks, D.L. (1996). Methods of soil analysis. Prte 3: Chemical methods. ASA/SSSA Publisher Inc., Madison (W), USA.

Tanyolaç, D., Ekmekçi, Y. & Ünalan, S. (2007). Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. *Chemosphere, 67,* 89-98.

U.S. EPA. 2010/15 PFOA Stewardship Program. 2006. Available: http://www.epa.gov/oppt/pfoa/pubs/pfoastewardship.htm.

Vrščaj, B., Poggio, L. & Ajmone Marsan, F. (2008). A method for soil environmental quality evaluation for management and planning in urban areas. *Landscape and Urban Planning, 88,* 81-94.

Yadav, S.K. (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany, 76,* 167-179.

Zheng, W. & Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agriculture and Food Chemistry, 49,* 5165-5170.

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**

Table 3. Heavy metals content (mg kg^{-1} dry weight) in shoot and root of *Taraxacum officinale* plants 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).-

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**

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**

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

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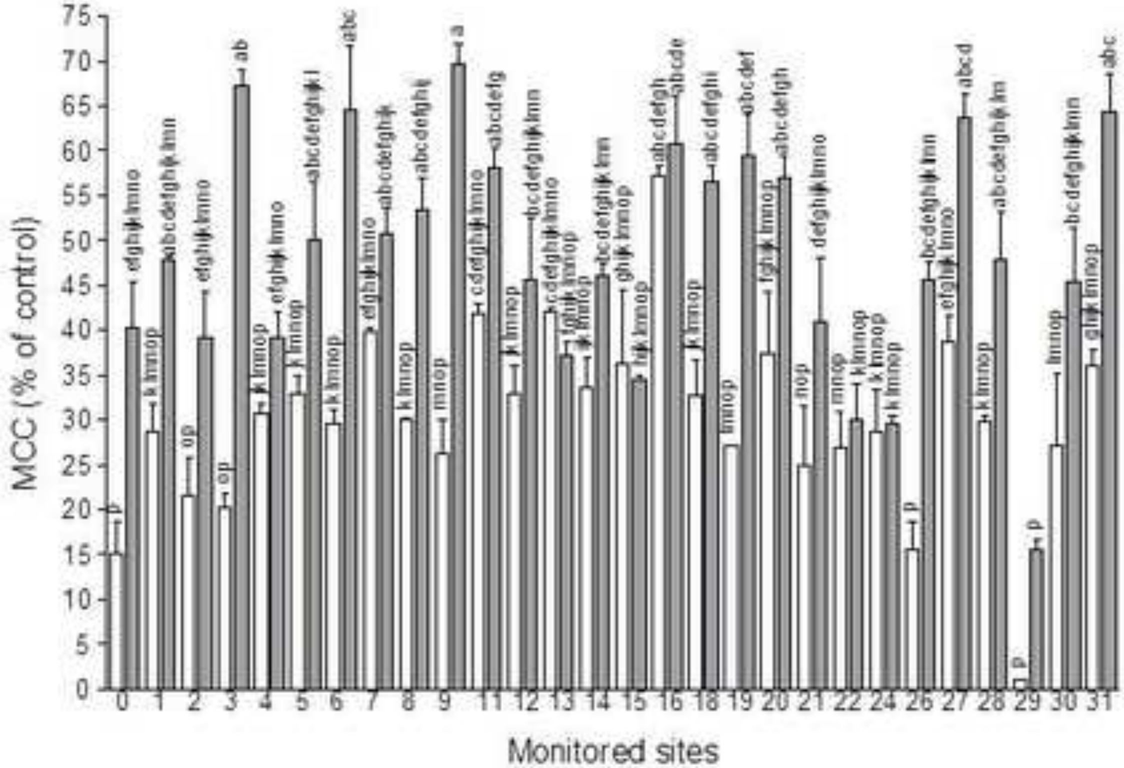

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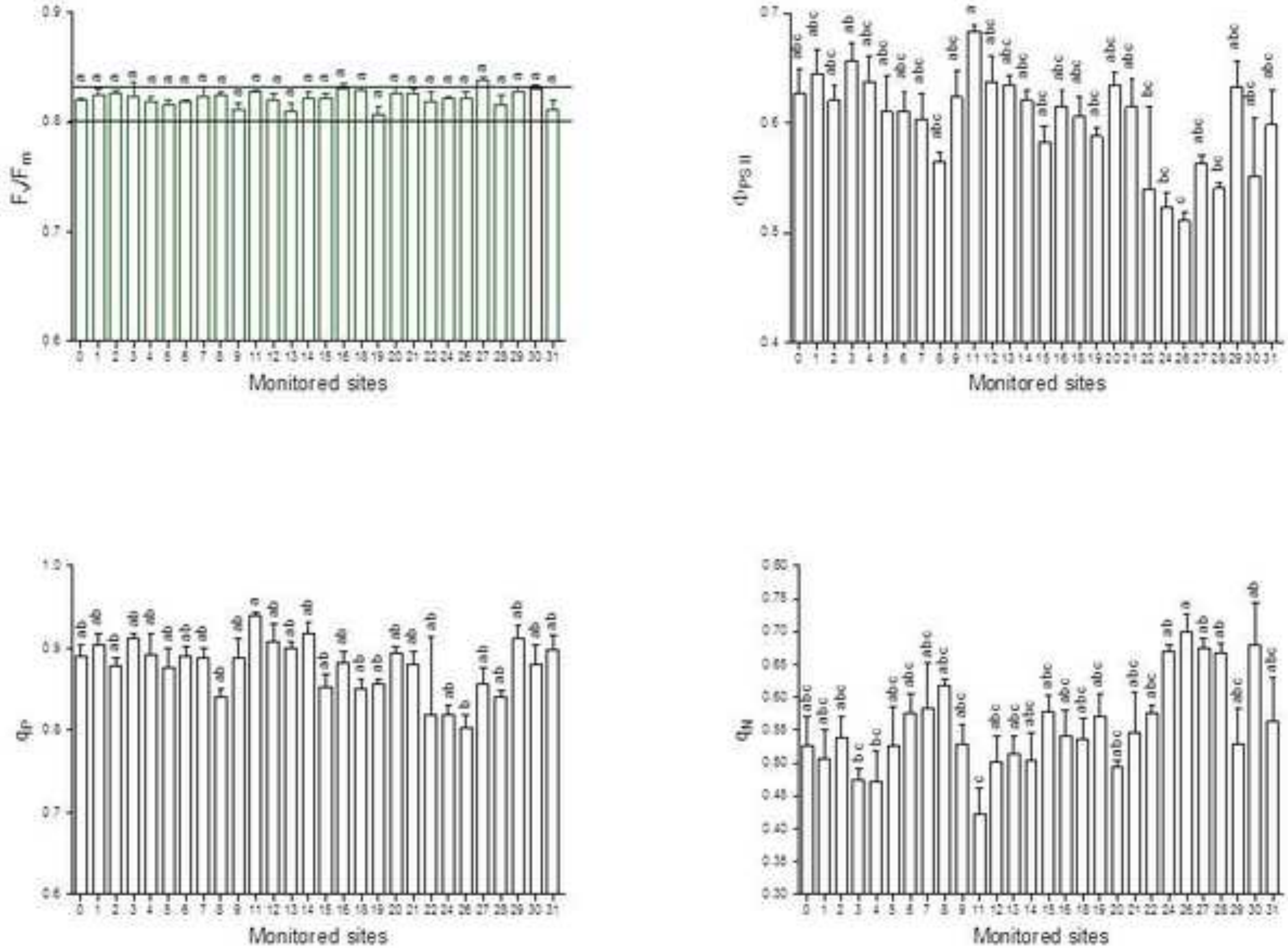

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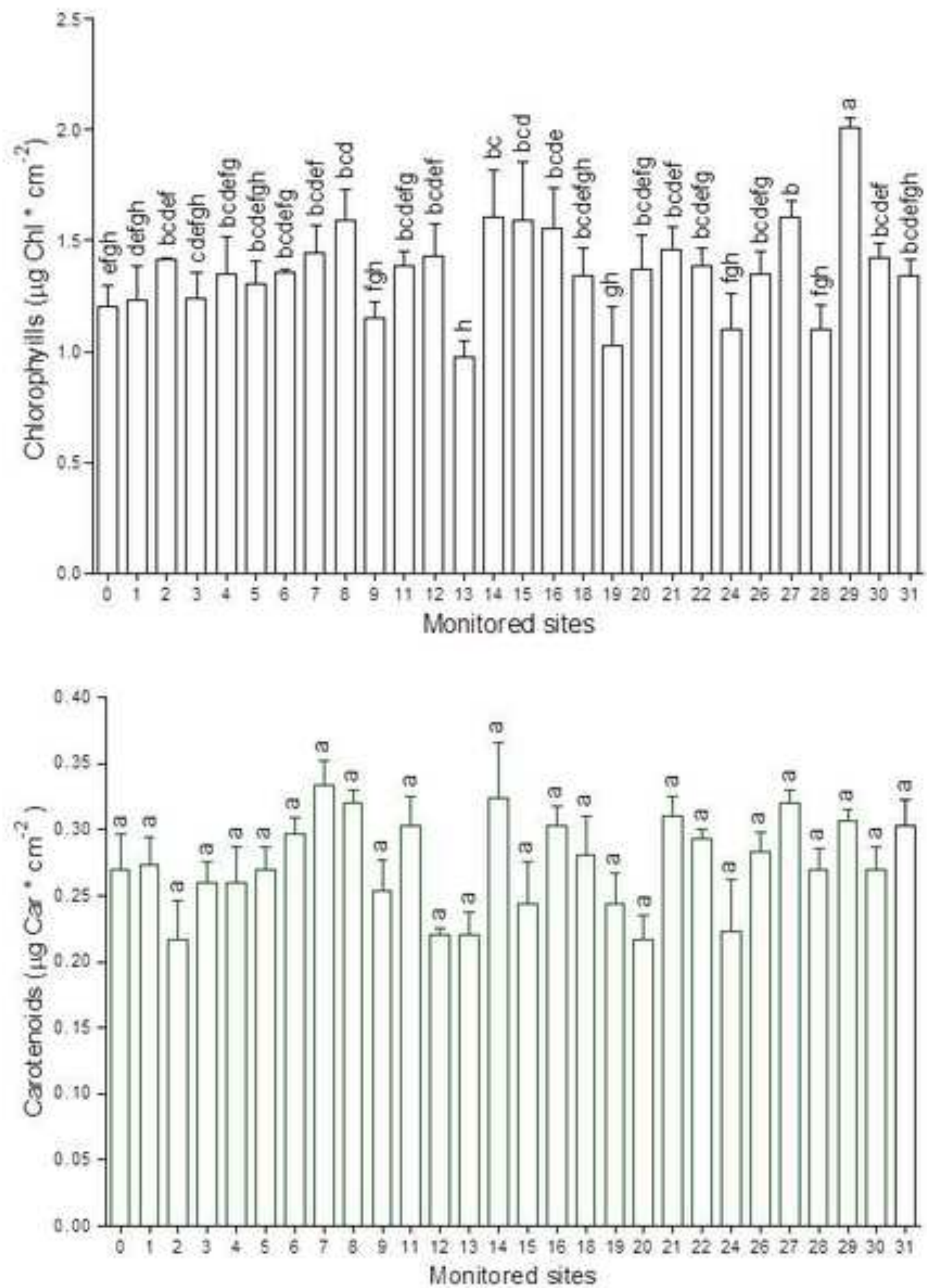

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