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Elemental characterization of wild edible plants from countryside and urban areas

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Abstract

Wild edible plants (WEP) represent a nutritious and important food source in many countries. In this study the content of 13 elements (Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Cr, Co, Cd, Ni and Pb) in 11 different genotypes of WEP was determined by inductively coupled plasma-optical emission spectroscopy. Each genotype was collected from the inner countryside and from fields near the highways of the metropolitan area of Bari (Apulia region). The elements intake by the consumption of potential serving sizes of WEP was also evaluated and discussed.

Independently from the harvesting area, *Borago officinalis* and *Papaver rhoeas* could be considered good sources of Mn and Fe, respectively. *Amaranthus retroflexus* and *Sinapis arvensis* may contribute to an adequate intake for Ca, while *Portulaca. oleracea* may be a good source of Mg. In contrast, the Pb content in *Plantago lagopus* (1.40 mg kg^{-1} FW) and *A. retroflexus* (0.33 mg kg^{-1} FW) - both harvested from the inner part of the countryside (IPC) areas - was over the maximum level fixed by the in EC regulation 1881/2006. The Cd content of *A. retroflexus* (12.7 µg 100 g^{-1} FW) was over the fixed level too. Finally, the consumption of *Diplotaxis tenuifolia* harvested from the IPC areas could result in a Ni intake higher than the toxicity level for nickel-sensitized persons.

In conclusion, WEP may give a substantial contribution to the elements intake for consumers, but in a few case their consumption may supply high level of elements potentially toxic for human health. Anyway, both ANOVA and PCA analyses have highlighted the low influence of the harvesting site on the elements content.

Keywords: Nutritional value**,** essential elements, ICP-OES, local habit**,** food risk**,** EC regulation.

1. Introduction

Wild edible plants (WEP) are a favorite delicacy in many countries and have always represented an important food source for the rural communities of the Mediterranean basin (Hadjichambis et al., 2008). Several studies have demonstrated their relevant role in the traditional Mediterranean diets (Heinrich et al., 2005) and their nutritional value even after cooking processes (Boari, Cefola, Di Gioia, Pace, Serio & Cantore, 2013). Therefore, a lot of people harvest WEP also because of their substantial contribution to the diet in terms of healthy compounds such as minerals, antioxidants and vitamins. So, in Italy and other European countries, the tradition of eating spontaneous plants is not only still alive but is increasing since the WEP are considered natural and healthy foods (Pereira, Barros, Carvalho & Ferreira, 2011; Renna & Gonnella, 2012; Sánchez-Mata et al., 2012).

In Apulia region (Southern Italy) there are about 2500 wild herbaceous species of which over 500 may be consumed as food (Bianco, Mariani & Santamaria, 2009). Thus, inhabitants of Apulia harvest WEP as a local habit and many people pick plants from both countryside and near highway of urban areas.

The WEP represent an extraordinary source of food that may be used to diversify and enrich modern diet with many colours and flavours, while providing essential elements such as Ca, K, Mg and Fe.

Nevertheless, the information on elements content of WEP are scarce especially as regard the presence of potentially toxic ones. As reported by some Authors (Alloway, 2004; Clark, Brabander & Erdil 2006; Shinn, Bing-Canar, Cailas, Peneff & Binns, 2000), vegetables grown in urban and peri-urban areas are generally exposed to a higher level of pollutants including heavy metals. Thus, the traditional practice of Apulia inhabitants to harvest WEP not only in the countryside but also near highway of urban areas could increase human health risks. Effectively, various studies have revealed that consuming vegetables from polluted sites can lead to serious public health problems (Hough et al., 2004; Kachenko & Singh, 2006; Pruvot, Douay, Hervé & Waterlot, 2006; Qadir, Ghafoor & Murtaza, 2000; Sharma, Agrawal & Marshall, 2007). In this context, the potential contemporary presence in the WEP of beneficial and toxic elements could lead to doubt about their dietary value and health benefits. However, while many Authors have reported the elements content of wild mushrooms harvested from different sites (Gençcelep, Uzun, Tunçtürk & Demirel 2009; Mendil, Uluözlü, Hasdemir & Çaglar, 2004; Ouzouni, Veltsistas, Paleologos & Riganakos, 2007; Yamaç, Yıldız, Sarıkürkcü & Halil Solak, 2007), to our best knowledge the literature lacks information with regard to the WEP.

With almost one million inhabitants, the metropolitan area of Bari (Apulia region) represents a big share of the population potentially susceptible both to the risks and benefits of an indiscriminate harvesting of WEP. In this area the harvesting of WEP is a time-honored custom, moreover several species represent the essential ingredient to prepare traditional dishes (Bianco et al., 2009).

Starting from these remarks the aims of the present study were: i) to assess the concentration of selected elements in several WEP collected from the inner countryside and from fields near the highways of the metropolitan area of Bari (Apulia region); ii) to understand the benefits and risks of consuming the WEP coming from countryside sites, perceived safe, and from sites close to the highways, considered potentially polluted.

2. Materials and methods

2. 1. Plant material and harvesting sites

The WEP selected for the investigation are reported in Table 1. The choice of the 11 WEP was made according to the local habit and to several ethnobotanical surveys conducted in the Mediterranean area (Bianco, Santamaria & Elia, 1998; Bianco et al., 2009).

The plants were collected from the metropolitan area of Bari, Apulia region, Italy, between winter and spring of 2011. The harvesting area included countryside sites and urban sites, the second ones normally exposed to the vehicular traffic (Fig. 1). Therefore, the collected samples were classified and evaluated into two harvesting areas: near road (NR) area and inner part of countryside (IPC) area. The distances from the road were 0–20 m for samples from the NR area and beyond 1000 m for samples from the IPC area. The plants were harvested manually and a minimum of 30 samples for each replication were pooled to form a single bulk. Each sample was immediately preserved in a portable refrigerator and transported to the laboratory within 2 h from harvest. Samples of each species were gathered according to local consumers practices and preferences in the Apulia region in the season when WEP are most suitable for consumption.

2. 2. Sample preparation and dry matter determination

The collected plants were gently cleansed and separated into the edible and the waste portion. The latter generally consisted of the older leaves and stems that are removed during the normal dish preparation. The processed sample of each species for each site was divided into two equal portions of 300 g each. One portion (subdivided into three replicates of 100 g each) was dried in a forced air oven at 105 °C until reaching a constant mass for the determination of the dry weight (DW) content. Results were expressed as g 100 g^{-1} fresh weight (FW). The other portion (equally in triplicate of 100 g each) was dried at room temperature and gently ground in an agate mortar to be used for the elemental analysis.

2. 3. Elemental analysis

Major and trace elements of the selected WEP were analyzed in 132 samples (11 species x 2 harvesting area x 6 replications).

Approximately 0.3 g of each homogenized sample were weighted into a Teflon digestion tube. A mixture of high purity grade concentrated HNO_3, HCl and H_2O_2 (6:1:1) was added and the tube was heated in an Anton Paar Multiwave 3000 microwave oven. The samples digestion occurred in four steps: the first one started raising the oven power to 800 W in 8 min and keeping it for another 8 min; the second step occurred increasing the power to 1000 W in a period of 8 min and keeping it for another 7 min; during the third phase, the power of 1200 W was reached in 6 min and kept constant for another 6 min; finally, the cooling phase occurred in 25 min. Several blanks were obtained with each batch of samples.

Digested samples were transferred into 50 mL volumetric flasks, diluted with deionized water, filtered through Whatman 42 and stored in polypropylene tubes. All glassware and plastic were cleaned using a 6 M HCl solution and then rinsed with ultra-pure water (18.2 $M\Omega\ cm^{-1}$). The latter was obtained from a Milli – Q Element system (Millipore, Molsheim, France) and used to prepare all solutions.

The concentration of the elements (K, Ca, Mg, Na, Fe, Mn, Cu, Zn, Cd, Cr, Co, Ni and Pb) in the WEP was assessed by inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements using an iCAP 6000 Series ICP-OES Spectrometer, Thermo Electron Corporation. Table 2 provides the detection limits of the ICP spectrometer.

2. 4. Statistical analysis

The analysis of variance (ANOVA) was performed using the GLM procedure (SAS software, Version 9.1) applying a completely randomized design with wild species and picking areas as main factors. The separation of means was obtained by the Student-Newman-Keuls (SNK) test.

For a visual analysis of the data, Principal Component Analysis (PCA) (PRINCOMP procedure, SAS software, Cary, NC, USA) was performed on mean centered and standardized data (unit variance scaled) prior to analysis. The data matrix submitted to PCA was made up of 22 observations (11 WEP picked in two areas, NR and IPC) and 14 variables (dry matter, K, Ca, Mg, Na, Fe, Mn, Cu, Zn, Cd, Cr, Co, Ni and Pb). The PCA was applied to obtain an overview of the whole data variability simplified in a few main information. The results of the PCA are shown as biplots of scores (species) and loadings (variables).

3. Results and discussions

3.1. Dry matter

The Figure 2 shows the DW content of the WEP harvested from the NR and the IPC sites. The mean values ranged from 7.78 g 100 g^{-1} FW in *P. oleracea* to 17.91 g 100 g^{-1} FW in *P. lagopus*, both harvested from the NR areas. The DW content of *Sonchus* spp. and *P. lagopus* was significantly higher in samples harvested from the NR sites, while *A. retroflexus* and *P. oleracea* showed higher DW in samples harvested from the IPC areas. For the other species the harvest site did not significantly affect the DW content, probably for a lower influence of the environmental factors on this parameter.

3.2. Elements content in the selected WEP

Table 3 shows the elements content in the WEP harvested from different sites. The mean value of Na ranged from 0.15 mg g^{-1} DW in *A. retroflexus* harvested from the NR areas to 8.88 mg g^{-1} DW in *B. officinalis* from IPC area. The average Na content was high also in *Sonchus* spp., *G. coronaria* and *C. intybus*, confirming what stated by Bianco et al. (1998), that is plants belonging to the *Asteraceae* family generally show a higher Na content.

The mean value of K ranged from 12.20 mg g^{-1} DW in *P. lagopus* to 38.26 mg g^{-1} DW in *P. oleracea,* both harvested from NR area. Nevertheless, the average K content was high also in *P. oleracea* from IPC area as well as in *P. rhoeas* and *A. retroflexus* from both area. Calcium mean value ranged from 8.62 mg g^{-1} DW in *P. oleracea* to 27.44 mg g^{-1} DW in *S. arvensis,* both from IPC area. Respect to what reported for Na, the variability of these two elements among the plants was lower. In fact, the K and Ca content variation between minimum and maximum value was of about three times, in comparison to about sixty times for Na. That is possibly related to the functions of K, Ca and Na in the plants: in fact, K is cofactor of a lot of enzymes and is involved in the osmotic regulation and the electroneutrality of the cells, while Ca is a constituent of the middle lamella and cofactor of several enzymes. In contrast, Na is extremely important for the regeneration of the phosphoenolpyruvate in C4 and CAM plants and can substitute the K in the osmotic regulation.

Magnesium mean value ranged from 1.36 mg g^{-1} DW in *S. arvensis* from NR area to 12.28 mg g^{-1} in *P. oleracea* harvested on IPC area. In this case the variability among the species was higher respect to K and Ca but was lower respect to Na.

The Fe concentration ranged from 132.7 mg kg^{-1} DW in *F. vulgare* to 1732 mg kg^{-1} DW in *P. rhoeas,* both from IPC area. Manganese mean value ranged from 35.60 mg kg^{-1} DW in *A. retroflexus* to 155.92 mg kg^{-1} DW in *B. officinalis,* both harvested from NR area. The Cu content ranged from 3.70 mg kg^{-1} DW in *B. officinalis* harvested from NR area to 27.19 mg kg^{-1} DW of the *P. lagopus* of the IPC area. The mean value of the Zn concentration ranged from 21.11 mg kg^{-1} DW in *F. vulgare* harvested from the IPC area to 121.50 mg kg^{-1} DW in *P. roheas* from NR area. Chromium mean value ranged from 0.59 mg kg^{-1} DW in *G. coronaria* harvested on IPC area to 2.56 mg kg^{-1} DW in *F. vulgare* from NR area. The Co concentration was below the detection limit in *F. vulgare*, *Sonchus* spp., *D. tenuifolia*, *S. arvensis* and *P. lagopus* regardless their harvesting area, while the highest content was detected in *P. rhoeas* harvested on IPC area. Generally, for these elements the differences between minimum and maximum value, ranged from about 4 times for Mn and Zn to about 13 times for Fe.

The Cd content ranged from 0.01 mg kg^{-1} DW in *P. lagopus* harvested from NR areas to 0.47 mg kg^{-1} DW in *S. arvensis* from IPC area. The Ni concentration was below the detection limit in *Sonchus* spp., regardless the harvesting areas, and in *G. coronaria* only from IPC area, while *P. roheas* showed the highest content when harvested from NR sites. Finally, Pb mean value ranged from 0.01 mg kg^{-1} DW in *B. officinalis* to 9.76 mg kg^{-1} DW in *P. lagopus,* both harvested from the IPC areas.

The concentration of each element studied changed among the WEP regardless their harvesting sites suggesting: i) a different uptake of those elements by each species; ii) an indistinct separation between the NR and IPC sites possibly due to diffuse anthropogenic activities such as fertilization and atmospheric fall out.

3.3. Intake of elements on the basis of the fresh weight

Considering the local custom to use the WEP to prepare dishes, may be interesting to evaluate the intake of each element for serving size.

3.3.1. Major elements

Sodium mean value was highest in *G. coronaria*, *Sonchus* spp. and *B. officinalis*, while was lowest in *A. retroflexus, D. tenuifolia, S. arvensis, P. rhoeas* and *P. oleracea*. The interaction between species and harvesting site was not significantly different (Fig. 3A). For human beings, this element plays a vital role in the regulation of fluid balance, blood pressure and transmembrane gradients (Thomas, 2001). However, the ingestion of less than 2 g of sodium per day is recommended in order to prevent heart diseases attributable to high blood pressure (World Health Organization, 2006). Several foods in our diet contain small amounts of sodium in their natural state, although its content can increase significantly when foods are processed or cooked. In agreement to this, the results of the present study showed that all the samples analyzed may be considered as low contributors to the daily supply of sodium. Effectively, 100 g of FW of all species supply less than 100 mg of sodium, which represents about 7% of the daily intake (1.5 g of sodium per day) recommended by the European Food Safety Authority (European Food Safety Authority, 2006).

As regards K content, the highest values were shown by *A. retroflexus, P. oleracea* and *F. vulgare* in descending order. All other species did not show significant differences among them (Fig. 3B); the interaction between species and harvesting site was not significant too. In the human body, K is predominantly an intracellular cation and plays a fundamental role in acid–base regulation, fluid balance, muscle contraction and nerve conduction (Thomas, 2001). The Institute of Medicine of the National Academies (Food and Nutritional Board, 2005) has set an intake of 4.7 g K per day from food as an adequate intake. So, the WEP analyzed in this study could satisfy from 4.25 to 8.25% of the daily adequate intake, approximately.

The mean value of Ca was highest in *A. retroflexus* and *S. arvensis* followed by *D. tenuifolia* and then by *B. officinalis* and *P. lagopus*. All the other remaining species showed significant lower levels of Ca (Fig. 3C). Moreover, as previously reported for Na and K, the interaction between species and harvesting site was not significant for Ca too. Calcium is the fifth most abundant element in the human body, it is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes, blood clotting, and provides rigidity to the skeleton (World Health Organization & Food and Agriculture Organization, 2004). As reported by Albuquerque et al. (2013) the calcium dietary reference intake has been estimated in 1000 mg per day. Therefore, 100 g of fresh *A. retroflexus* or *S. arvensis* supply between 350 and 400 mg of calcium, providing about the 35-40% of the daily intake of this element. Instead, the others WEP analyzed may provide approximately 10-30% of the daily intake.

The highest value of Mg was found in *P. oleracea* harvested from the IPC areas (154.23 mg 100 g^{-1} FW) followed by *A. Retroflexus,* independently from the harvesting site (108.05 mg 100 g^{-1} FW, on average), and *P. oleracea* harvested from the NR areas (94.30 mg 100 g^{-1} FW). In the other cases, the Mg concentration ranged from 20 to 30 mg 100 g^{-1} FW without significant differences among species and sites (data not showed). It is well know that magnesium is the fourth most abundant cation in the human body and is second only to potassium in intracellular concentration. Therefore Mg is critical for a great number of cellular functions: it is a cofactor for more than 300 enzymes in the body and also has functions that affect membrane properties and thus the nerve conduction (Nielsen, 2008). For males and females between 19-30 years old the recommended dietary Mg intake has been estimated in 400 and 310 mg per day, respectively; man and women over 30 years old should intake 420 and 320 mg per day, respectively (Food and Nutrition Board, 1997). Considering the Mg content of the fresh matter of the WEP analyzed, *P. oleracea* harvested from the IPC areas may provide about 38% and 48% of the daily intake for males and females, respectively. However, even *A. retroflexus,* harvested from both areas, and *P. oleracea,* harvested from the NR ones, may contribute to an adequate intake of Mg because both species provide about 25 and 33% of the Mg recommended daily intake (for a serving size of 100 g) for men and women, respectively.

3.3.2. Essential trace elements

The highest content of Mn was found in *B. officinalis* followed by *G. coronaria* and *Sonchus* spp. All other species did not show significant differences among them as well as no interaction between species and harvesting site (Fig. 3D). Manganese plays an important role in several physiological functions of the human body, in fact the Food and Nutritional Board (2001) recommended an adequate intake of 2.3 and 1.8 mg day^{-1} for males and females, respectively. Therefore, a *B. officinalis* serving size of 100 g may abundantly contribute to the daily requirements of Mn (about 100% in case of females and 80% for males). However, even *G. coronaria* and *Sonchus* spp. may contribute to an adequate intake for manganese, although to a lesser extent.

Zinc contents in *A. retroflexus* and *P. lagopus* were significantly higher respect to *F. vulgare, C. intybus, G. coronaria, B. officinalis, D. tenuifolia* and *S. arvensis*, but not significantly different from *Sonchus* spp., *P. rhoeas* and *P. oleracea*. The interaction between species and harvesting site was not significant (Fig. 3E). Zinc is an essential trace element for human beings since it has structural, regulatory or catalytic role in many enzymes. It plays important role in growth and development, immune response, neurological function, and reproduction (Vallee & Galdes, 1984; Hambridge, Casey & Krebs, 1986). With regards to the Recommend Dietary Allowances, the European Population Reference Intake (PRI) for Zn is 9.5 and 7.0 mg day^{-1} for adult males and females, respectively (Scientific Committee for Food, 1993). Considering the content of this element in the FW of the WEP analyzed, 100 g of *A. retroflexus, Sonchus* spp*., P. lagopus, P. oleracea* and *P. rhoeas* may supply about 8 and 11% of the requirements for males and females, respectively.

The mean value of Cr in *S. arvensis* was significantly higher with respect to *G. coronaria, Sonchus* spp.*, B. officinalis, P. lagopus* and *P. oleracea*, but non significantly different from all other species. The interaction between species and harvesting site was not significant (Fig. 3F). Chromium is an essential nutrient that potentiates insulin action and thus it influences carbohydrate, lipid and protein metabolism. Currently, there is no formal Recommended Dietary Allowance (RDA) for Cr, nevertheless the US Food and Nutrition Board (2001) derived Adequate Intakes (AI) for this element (e.g. 35 µg day^{-1} and 25 µg day^{-1} for 19 to 50 year old men and women, respectively). Accordingly, 100 g of *S. arvensis* could supply about 100 and 77% of Cr AI for women and men, respectively. In contrast, the same serving size of *Sonchus* spp. or *P. oleracea* could supply only 44 and 31% of Cr AI for women and men, respectively.

Regarding Fe content, the highest mean level was found in *P. rhoeas* followed by *A. retroflexus* (Fig. 4A). In the human body, Fe acts as oxygen carrier in hemoglobin in blood and myoglobin in muscle. Iron deficiency is the most common and widespread nutritional disorder in the world, affecting a large number of children and women in developing countries, and it is the only nutrient deficiency which is also significantly prevalent in industrialized countries (World Health Organization, 2008). For adult males and females after menopause the recommended dietary iron intake has been estimated between 8 and 10 mg per day, while for women in reproductive age is recommended an intake of 15-20 mg per day (Food and Nutritional Board, 2001). Considering the content of this element in the FW of the analyzed WEP, *P. rhoeas* may provide about 100 and 200% of the daily intake for females in reproductive age and adult males and females after menopause, respectively. However, even *A. retroflexus, C. intybus*, *S. arvensis*, *P. rhoeas* and *D. tenuifolia* may contribute to an adequate Fe intake. In fact, these WEP may provide at least 100% of Fe recommended daily intake (for a serving size of 100 g) for adult males and women after menopause.

The highest mean value of Cu was found in *P. lagopus* harvested from the IPC areas, followed by *G. coronaria* and *P. oleracea* harvested from the IPC areas too. All other species showed Cu concentration lower and without significant differences among species (Fig. 4B). Copper is an essential trace element for living organisms, including humans, being a vital component of several enzymes and proteins (European Food Safety Authority, 2006). It has been recommended that adult males and females should have a dietary intake of 900 µg day^{-1} (Scientific Committee for Food, 1993). Therefore, 100 g of fresh *P. lagopus* harvested from the IPC areas may supply about 43% of the daily requirement of this element. The Cu content in *P. oleracea* and *G. coronaria* harvested from the IPC areas is even interesting since they supply about one fourth of daily intake with a serving size of 100 g (Fig. 4A).

The highest value of Co was found in *P. rhoeas* harvested from the IPC areas (6.17 µg 100 g^{-1} FW) followed by *B. officinalis* independently of the harvesting site (4.33 µg 100 g^{-1} FW on the average), *A. retroflexus* and *C. intybus* belonging to the IPC and NR areas, respectively (3.33 µg 100 g^{-1} FW), *P. rhoeas* (2.67 µg 100 g^{-1} FW) and *A. retroflexus* (2.17 µg 100 g^{-1} FW) harvested from the NR areas, *C. intybus* harvested from the IPC areas (1.83 µg 100 g^{-1} FW), *P. oleracea* (1.67 µg 100 g^{-1} FW) and *G. coronaria* (1.50 µg 100 g^{-1} FW) harvested from the IPC areas and, finally, *P. oleracea* (1.33 µg 100 g^{-1} FW) and *G. coronaria* (0.17 µg 100 g^{-1} FW) harvested from the NR areas. In all other species, Co was below the detection limit of the instrumentation. Cobalt is a transition metal with physical and chemical properties similar to Fe and serves as an integral part of vitamin B_{12}. However only a very low quantity (about 0.1 µg per day) is required by the human body as vitamin B_{12} (Food and Nutritional Board, 1998). Therefore, in all samples in which the cobalt has been determined, 100 grams of fresh product may satisfy the daily requirement of the human body.

3.3.3. Elements potentially toxic for human health

The highest content of Cd (> 12 µg 100 g^{-1} FW) was found in *A. retroflexus*, while in all other cases it was ≤ 6.50 µg 100 g^{-1} FW (Fig. 4C). According to the EC regulation 1881/2006 (European Commission, 2006), the content of this element is over the legal threshold (0.10 mg kg^{-1} FW) only in *A. retroflexus*, while in all other cases the Cd content is below the established limit. This element is considered relatively toxic because its intake by food can result in kidney and liver dysfunction (Godt et al., 2006) as well as osteoporosis on long term. Therefore, the Joint FAO/WHO Expert Committee on Food Additives (World Health Organization, 1993) recommended that 65 µg per day of Cd should be regarded as the maximum tolerable intake (for a 65-kg man). Considering these remark, the Cd content for a serving size of 100 grams is always lower with respect to the mentioned intake independently of the harvesting site for all the WEP.

The highest Ni mean value was determined in *D. tenuifolia* from the IPC areas (984.8 µg 100 g^{-1} FW), while in all other cases the values were significantly lower and anyway not higher than 383.3 µg 100 g^{-1} FW (data not showed). Nickel is essential for the catalytic activity of some plant and bacterial enzymes, while biochemical functions of Ni have not been demonstrated in the human body. Nevertheless, it is considered a micro-nourishing for human beings at low concentration but large doses may be toxic when taken orally (Zicari, Russo, Rivetti & Soardo, 2011). The nutritional requirements or RDA for this element have not been established. However, the finding that an oral intake of about 500 µg per day has been reported to aggravate hand eczema in Ni sensitized subjects (European Food Safety Authority, 2006) suggests that the threshold level for toxicity can be quite low in specific situations, and could thus be set at less than 500 µg per day. Considering the content of this element in the FW of the WEP analyzed, the consumption of only 100 g of *D. tenuifolia* harvested from the IPC areas supplies an amount of nickel about 2-fold higher respect the threshold level for toxicity. In contrast, the contribution of Ni from all other WEP is always lower than the previously mentioned limit, independently of the harvesting site.

As regards Pb content, the highest mean level was found in *P. lagopus* harvested from the IPC areas (1.54 mg kg^{-1} FW), followed by *A. retroflexus* harvested from the same area (0.33 mg kg^{-1} FW). In all other cases values were less than 0.24 mg kg^{-1} FW (data not showed). The content of this element in the fresh matter of *A. retroflexus* and *P. lagopus* harvested both from IPC areas was over the maximum level (0.30 mg kg^{-1} FW) defined by EC regulation 1881/2006 (European Commission, 2006). In fact, the Pb concentration was 13% higher than the legal threshold for *A. retroflexus,* while for *P. lagopus* it was over 5-fold higher. The Scientific Committee on Food reported that the mean level of Pb in foodstuffs does not seem to be a cause of immediate concern (European Commission, 2006), considering a tolerable weekly intake between 1.5 and 175 mg (World Health Organization, 1993). The results of the present study are, generally, in agreement with this statement, with the exception of *P. lagopus* harvested from the ICP areas, since the Pb content per kg of fresh vegetable potentially consumable in seven days amounts almost to this tolerable weekly intake limit.

3.4. Principal Component Analysis

For a better interpretation of the several variables of the 11 WEP harvested in two macroareas (NR and IPC), data were analyzed using multivariate data analysis. The eigenvalues of the correlation matrix showed that the first four Principal Components (PCs) explained 75% of the total variance. The first three PCs represented 27.5, 20.3 and 14.7%, respectively. Figure 5 shows the PC1 vs PC2 and the PC1 vs PC3 bi-plots, since some variables (such as dry matter, Fe, Co) had a relevant influence on PC3. Actually, dry matter, Ca, Mn and Ni showed a notable load on PC4 but this Principal Component did not explain a great portion of variability. For this reason it was not included in the figures.

The PCA would be able to separate some groups of data giving an immediate idea of the most relevant weight of each factor on the whole variability. However, PCA results showed that the harvesting areas were not so critical in the differentiation of the chemical properties as it could have been expected (Fig. 5). On the contrary, species were a more discriminating factor since a relative grouping of data based on genotypes of the same species or the same botanical family was possible. Two main groups can be distinguished: the *Brassicaceae* (*D. tenuifolia* and *S. arvensis*) and the *Asteraceae* (*G. coronaria*, *Sonchus* spp. and *C. intybus*) families, but most of the examined species placed in the same portion of the graphical space independently from the original collection areas (Fig. 5).

Sodium content was the parameter that was the highest correlated to PC1. Cadmium, K, Fe, Ca, Mg were negatively correlated to PC1 (Fig. 5A). Copper was responsible for most of the variance explained by PC2, but also Cr, Pb, Zn, Mg were highly correlated to PC2, even if only Cr correlated negatively (Fig. 5A). The extreme position of *A. retroflexus* (especially harvested from the IPC areas) in both PCA biplots is explained by its very high Cd content (Fig. 4) and its very low Na content (actually for both areas), that collocates this species in the left part of the plots (inversely correlated to PC1) (Fig. 5). *P. lagopus* was positively correlated to PC2 for its high content of Cu, Zn and Pb, as well as *P. oleracea* for its content of Cu and Mg, when both species were collected from the IPC areas (Fig. 5A). Probably the position of the *Asteraceae* group was determined mainly by Na; while Ca and Cr influenced positioning of the *Brassicaceae* species (Fig. 5A). Dry matter, Co and Fe were highly correlated to PC3, with dry matter negatively (Fig. 5B). In particular the high Co content determined the separation of *P. rhoeas* in Figure 5B.

4. Conclusions

In this study the concentration of elements in WEP harvested from countryside and urban areas was assessed, as well as the evaluation of elements intake by their consumption.

WEP may give a substantial contribution to the elements intake of consumers, but the ANOVA has highlighted the low influence of the harvesting sites on elements concentration in the WEP. In contrast, significant differences were found between several species as regards elements content. This result was confirmed by Principal Component Analysis which allowed to visualize a null effect of the environment on the WEP chemical composition possibly due to diffuse anthropogenic activities. Primarily, *B. officinalis* and *P. rhoeas* could be considered a good sources of Mn and Fe, respectively. *A. retroflexus* and *S. arvensis* may contribute to an adequate supply of Ca, while *P. oleracea* may be a good source of Mg. Nevertheless, considering the EC regulation 1881/2006, Cd content resulted over the legal threshold in *A. retroflexus* harvested from IPC area. Moreover, Pb content was over the maximum level defined by the EC regulation 1881/2006 in *P. lagopus* and *A. retroflexus* harvested from the IPC areas. Finally, Ni content in *D. tenuifolia* harvested from IPC areas could result in an intake higher than the toxicity level for nickel-sensitized persons. However, the higher concentrations of these element in some plants were not caused only by environmental reasons, but also by species-dependent factors.

Nevertheless, more information about the retention of elements in WEP after cooking will be required for a better evaluation of elements intake by the consumption of these vegetables. Moreover, the assessment of bioaccessibility and bioavailability of elements after in vitro gastro-digestion process could be carried out in order to evaluate the real availability of these elements for human beings.

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Table 1 – Nomenclature, edible portion and food use of wild edible plants selected in the study.

Family	**Scientific name**	**Common name**	**Edible portion**	**Food use**
Amaranthaceae	*Amaranthus retroflexus* L.	Redroot pigweed or common amaranth	Leaves and young stems	Raw and/or cooked
Apiaceae	*Foeniculum vulgare* Mill.	Fennel	Leaves, young stems and flower buds	Raw and/or cooked
Asteraceae	*Cichorium intybus L.* s.l.	Common chicory or blu daisy	Leaves	Cooked
Asteraceae	*Glebionis coronaria* (L.) Spach	Crown daisy or Chop-Suey Greens	Leaves and young stems	Cooked
Asteraceae	*Sonchus* spp.	Sow thistles	Leaves and flower buds	Cooked
Boraginaceae	*Borago officinalis* L.	Borage	Leaves and flowers	Raw and/or cooked
Brassicaceae	*Diplotaxis tenuifolia* (L.) DC.	Rocket	Leaves	Raw and/or cooked
Brassicaceae	*Sinapis arvensis* L. subsp. *arvensis*	Wild mustard or charlock	Leaves and flower buds	Raw and/or cooked
Papaveraceae	*Papaver rhoeas* L. subsp. *rhoeas*	Red poppy	Leaves	Raw and/or cooked
Plantaginaceae	*Plantago lagopus* L.	Plantain hare's foot	Leaves	Cooked
Portulacaceae	*Portulaca oleracea* L. s.l.	Common purslane	Leaves and young stems	Raw and/or cooked

Table 2 – Detection limits of the ICP spectrometer.

Element	**Detection limit** ($\mu g\ L^{-1}$)
Na	0.0595
K	0.1557
Ca	0.0558
Mg	0.0145
Fe	0.2385
Mn	0.0794
Cu	0.0785
Zn	0.0760
Cr	0.2858
Co	0.1646
Cd	0.0739
Ni	0.3065
Pb	0.8211

Table 3 – Elements content ± SD of the selected wild edible plants harvested from different areas (n = 6) .

Species	**Area**	**Value**	**Na**	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**	**Cr**	**Co**	**Cd**	**Ni**	**Pb**
			mg g^{-1} DW				**mg kg^{-1} DW**								
Amaranthus retroflexus	**IPC**	**Mean**	**0.19**	**23.80**	**22.53**	**6.48**	**786.8**	**41.36**	**8.41**	**54.64**	**1.47**	**0.20**	**0.40**	**6.31**	**1.94**
		SD	0.14	3.26	3.09	0.30	279.7	10.69	1.35	11.59	0.38	0.11	0.56	3.40	0.65
	NR	**Mean**	**0.15**	**26.37**	**24.35**	**7.05**	**747.3**	**35.60**	**7.57**	**51.50**	**1.16**	**0.15**	**0.23**	**8.39**	**1.33**
		SD	0.03	2.44	2.82	0.63	160.3	5.60	2.36	8.39	0.42	0.11	0.05	2.68	0.44
Foeniculum vulgare	**IPC**	**Mean**	**2.35**	**19.21**	**12.70**	**1.97**	**132.7**	**53.41**	**6.00**	**21.11**	**0.73**	**ND**	**0.06**	**1.56**	**0.13**
		SD	0.80	1.87	1.99	0.35	49.1	21.29	1.42	2.38	0.10	-	0.03	0.92	0.06
	NR	**Mean**	**3.69**	**19.60**	**10.14**	**1.68**	**155.8**	**44.59**	**7.33**	**37.46**	**2.56**	**ND**	**0.16**	**22.14**	**0.24**
		SD	0.55	3.07	0.64	0.33	23.0	3.07	2.27	12.12	2.11	-	0.15	20.01	0.09
Cichorium intybus	**IPC**	**Mean**	**4.98**	**18.25**	**13.22**	**2.19**	**716.2**	**66.90**	**7.53**	**29.31**	**1.56**	**0.15**	**0.25**	**9.66**	**0.74**
		SD	1.17	1.96	1.37	0.21	286.8	13.02	2.67	3.76	0.29	0.19	0.07	7.02	0.43
	NR	**Mean**	**7.58**	**18.80**	**11.69**	**2.34**	**875.5**	**65.75**	**10.47**	**33.27**	**1.90**	**0.32**	**0.27**	**6.28**	**1.08**
		SD	3.15	2.54	2.17	0.38	318.4	29.20	4.03	14.40	0.56	0.18	0.09	3.84	0.35
Glebionis coronaria	**IPC**	**Mean**	**6.58**	**16.16**	**9.42**	**2.24**	**155.1**	**100.03**	**17.10**	**31.95**	**0.59**	**0.12**	**0.05**	**ND**	**1.08**
		SD	1.54	1.45	0.72	0.19	41.7	15.05	11.30	10.22	0.51	0.12	0.01	-	1.91
	NR	**Mean**	**5.40**	**13.15**	**9.74**	**2.20**	**193.4**	**67.23**	**10.60**	**29.99**	**1.40**	**0.01**	**0.03**	**0.19**	**0.38**
		SD	1.59	2.18	1.20	0.37	57.3	13.92	5.40	4.47	1.00	0.03	0.03	0.43	0.42
Sonchus spp.	**IPC**	**Mean**	**7.32**	**16.32**	**10.98**	**2.25**	**152.5**	**89.88**	**8.40**	**51.28**	**0.95**	**ND**	**0.18**	**ND**	**0.37**
		SD	2.27	3.19	1.14	0.20	111.9	26.20	1.33	11.22	0.62	-	0.02	-	0.20
	NR	**Mean**	**5.94**	**14.28**	**12.58**	**2.27**	**177.7**	**102.08**	**8.42**	**61.63**	**0.78**	**ND**	**0.19**	**ND**	**0.38**
		SD	2.67	1.23	1.33	0.21	50.8	22.98	2.93	33.79	0.33	-	0.11	-	0.24
Borago officinalis	**IPC**	**Mean**	**8.88**	**16.14**	**17.80**	**1.86**	**173.8**	**113.55**	**4.24**	**28.51**	**0.83**	**0.36**	**0.02**	**4.91**	**0.01**
		SD	1.44	1.07	2.67	0.28	60.4	13.81	2.90	4.89	0.64	0.25	0.02	7.82	0.01
	NR	**Mean**	**5.61**	**19.51**	**20.73**	**1.45**	**292.1**	**155.92**	**3.70**	**30.30**	**1.50**	**0.40**	**0.02**	**0.48**	**1.36**
		SD	0.87	1.47	2.48	0.17	98.5	29.26	2.14	5.65	0.38	0.17	0.03	0.76	1.71
Diplotaxis tenuifolia	**IPC**	**Mean**	**0.40**	**16.67**	**20.43**	**1.81**	**372.0**	**44.81**	**5.16**	**47.90**	**2.08**	**ND**	**0.40**	**34.94**	**1.83**
		SD	0.15	1.30	1.09	0.29	74.2	5.83	0.49	12.10	0.90	-	0.09	13.71	2.50
	NR	**Mean**	**2.63**	**15.00**	**21.26**	**1.79**	**566.9**	**47.06**	**5.38**	**35.00**	**1.64**	**ND**	**0.38**	**17.60**	**0.38**
		SD	2.60	3.07	2.0	0.33	178.4	7.24	1.07	17.40	0.54	-	0.12	21.83	0.28
Sinapis arvensis	**IPC**	**Mean**	**0.99**	**12.96**	**27.44**	**1.40**	**383.3**	**41.41**	**5.70**	**34.55**	**2.40**	**ND**	**0.47**	**13.48**	**0.62**
		SD	0.88	1.45	3.04	0.18	44.7	4.28	0.50	6.03	0.93	-	0.12	8.08	1.13
	NR	**Mean**	**0.87**	**13.61**	**27.16**	**1.36**	**680.0**	**58.56**	**5.28**	**26.28**	**1.60**	**ND**	**0.41**	**8.27**	**0.31**
		SD	0.45	3.24	3.22	0.34	319.2	27.13	0.88	3.96	0.39	-	0.09	4.10	0.24
Papaver rhoeas	**IPC**	**Mean**	**0.36**	**27.03**	**14.51**	**2.84**	**1732.2**	**97.21**	**10.07**	**28.21**	**2.25**	**0.64**	**0.16**	**3.97**	**1.18**
		SD	0.11	3.40	1.91	0.22	446.6	18.31	1.25	2.94	0.52	0.21	0.04	1.03	0.22
	NR	**Mean**	**2.06**	**23.41**	**13.97**	**2.70**	**904.9**	**64.44**	**12.51**	**121.50**	**2.03**	**0.30**	**0.08**	**37.90**	**1.26**
		SD	2.01	2.23	3.54	0.48	603.5	21.24	2.76	53.30	0.84	0.23	0.05	20.11	0.72
Plantago lagopus	**IPC**	**Mean**	**1.97**	**15.48**	**12.79**	**1.93**	**184.2**	**41.35**	**27.19**	**55.92**	**0.78**	**ND**	**0.02**	**0.21**	**9.76**
		SD	0.27	0.78	0.90	0.19	55.3	5.48	13.66	4.90	0.15	-	0.02	0.30	6.28
	NR	**Mean**	**2.92**	**12.20**	**14.79**	**1.76**	**350.7**	**35.80**	**6.35**	**50.32**	**0.93**	**ND**	**0.01**	**0.35**	**1.15**
		SD	0.52	3.02	2.78	0.53	113.1	12.19	1.27	16.74	0.13	-	0.01	0.55	0.48
Portulaca oleracea	**IPC**	**Mean**	**0.60**	**34.07**	**8.62**	**12.28**	**546.1**	**50.43**	**20.02**	**68.65**	**0.82**	**0.14**	**0.08**	**6.20**	**1.22**
		SD	0.16	3.19	1.16	1.27	127.8	6.32	1.52	8.90	0.30	0.05	0.01	2.09	0.19
	NR	**Mean**	**3.20**	**38.26**	**11.94**	**12.20**	**423.7**	**37.82**	**20.01**	**89.51**	**1.80**	**0.16**	**0.07**	**36.26**	**1.91**
		SD	1.64	4.58	0.90	2.06	305.9	10.93	0.82	13.91	1.29	0.19	0.02	11.07	0.65

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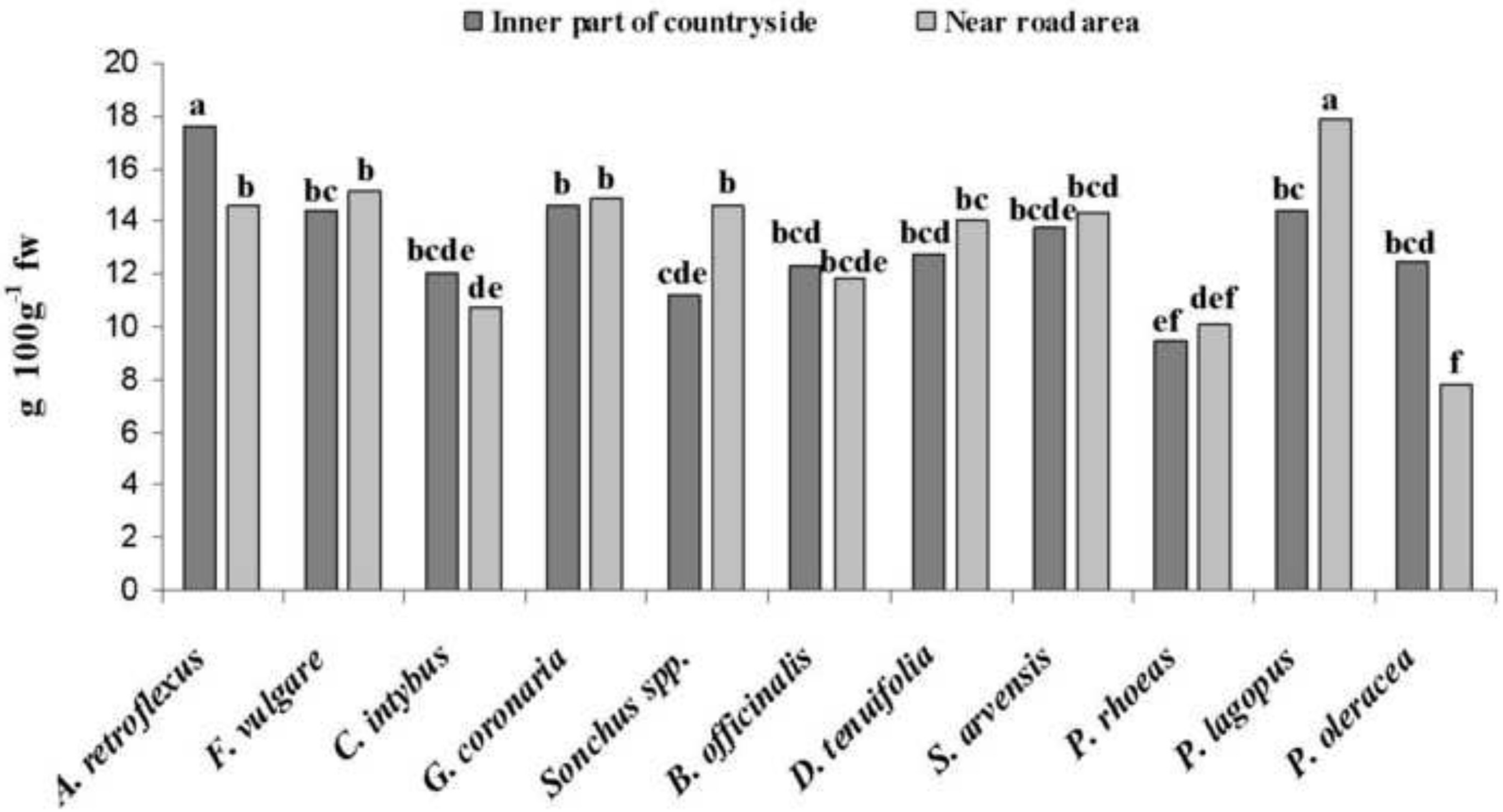

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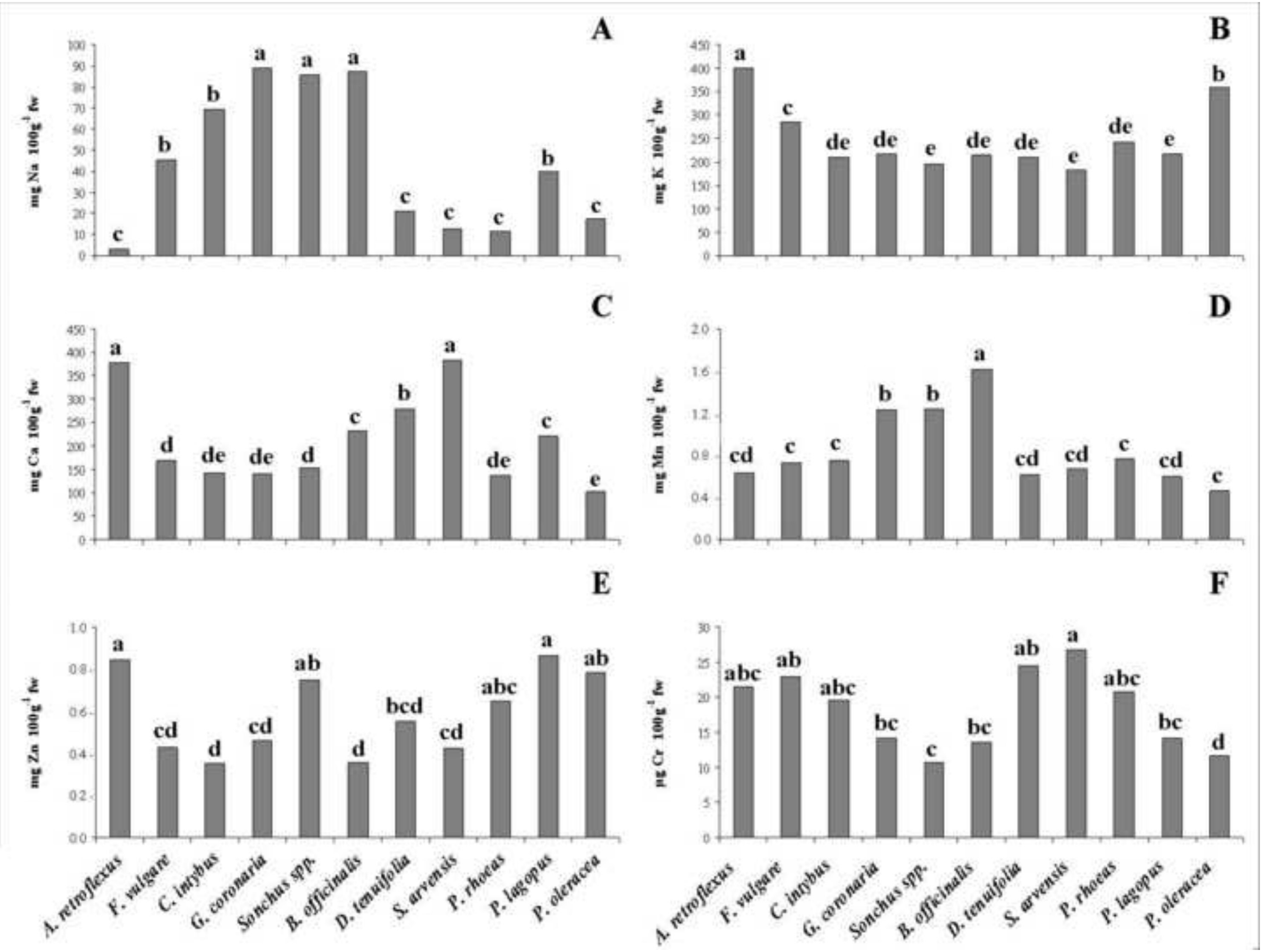

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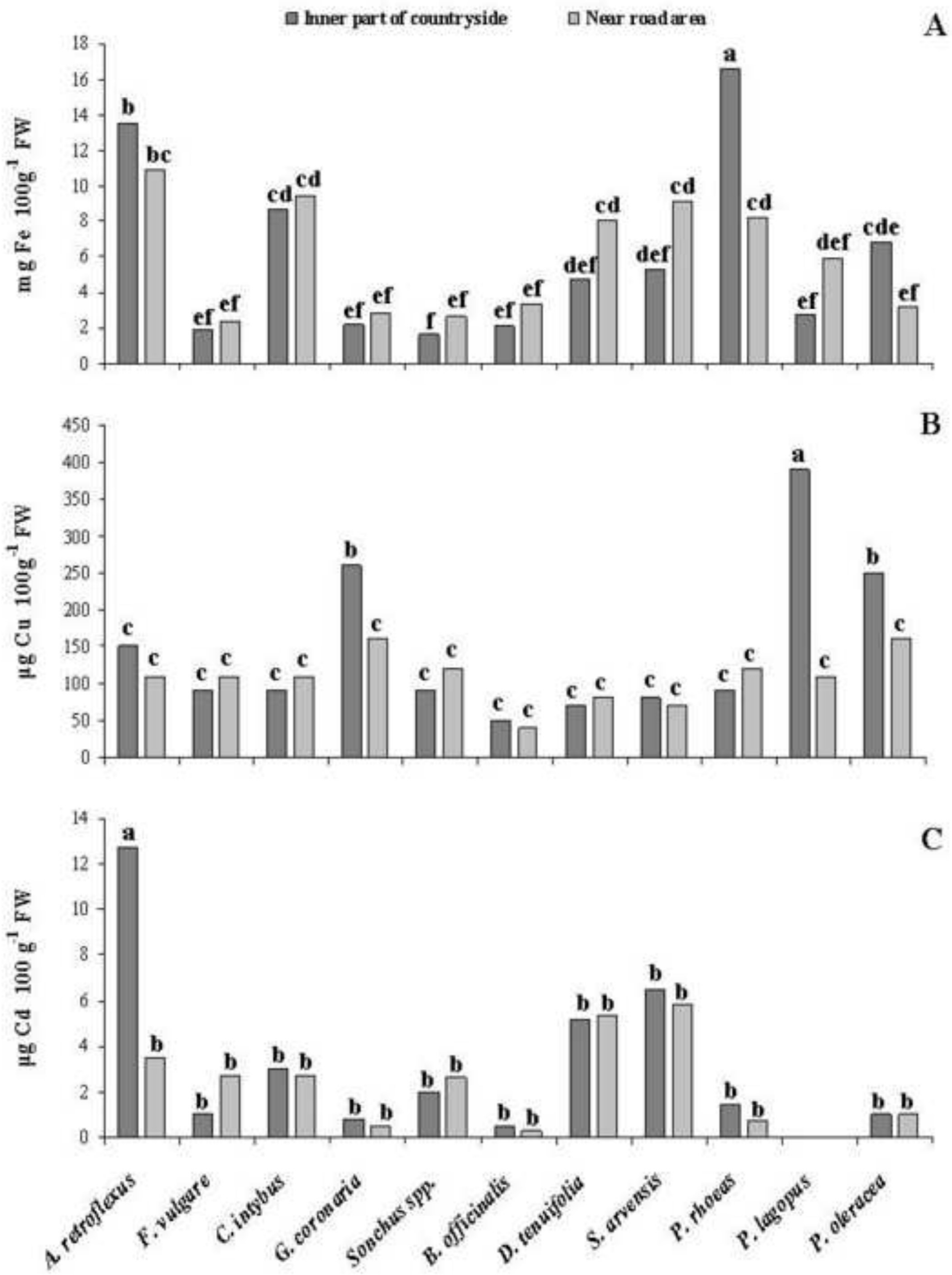

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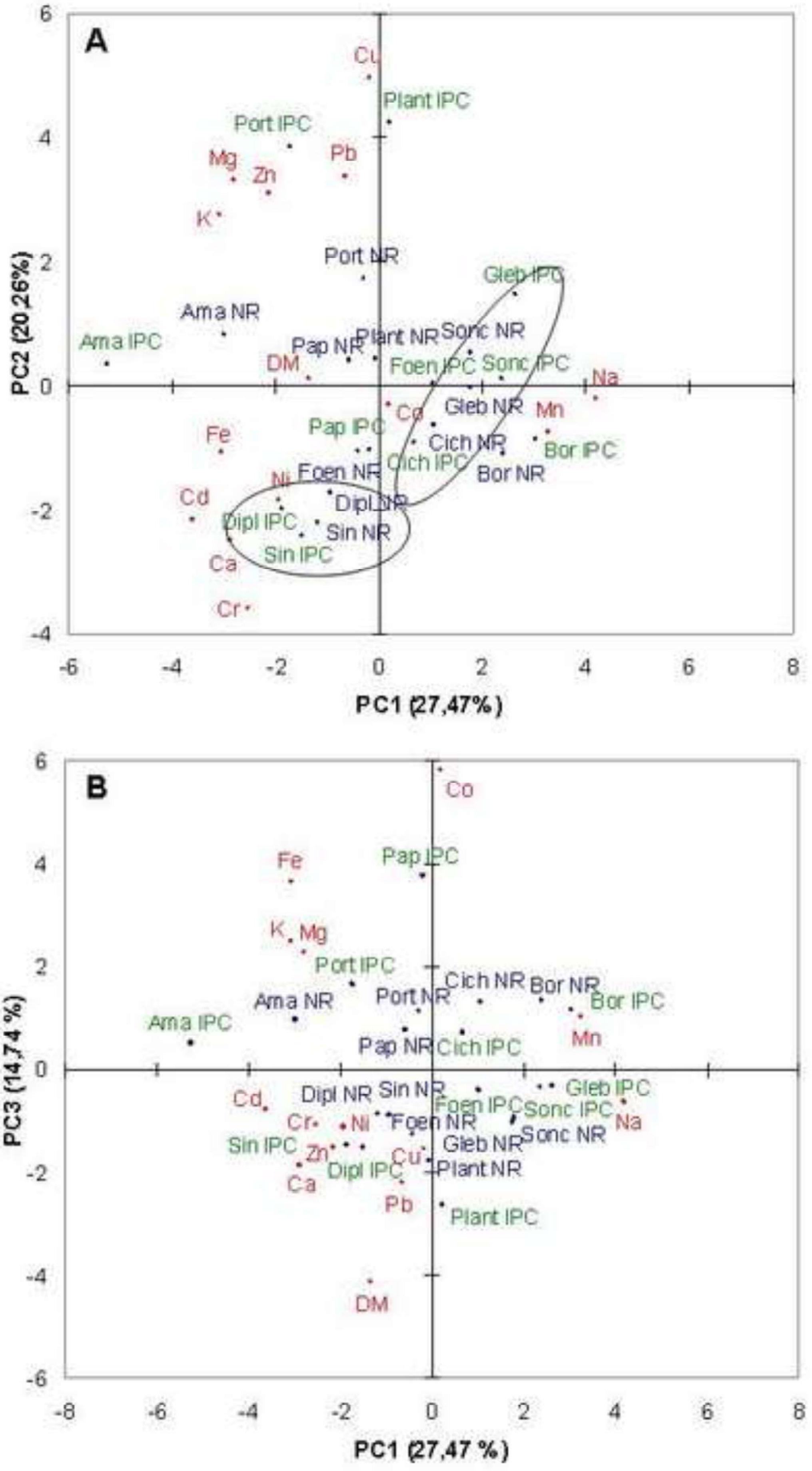

Figure(s)

Figure 1 – Map of the study area with the harvesting sites: near road of urban area (X); inner part of countryside(Δ).

Figure 2 – Dry matter content of the selected wild edible plants harvested from different areas ($P \leq 0.001$). The same letters indicate that mean values are not significantly different ($P = 0.05$).

Figure 3 – Average elements content in wild edible plants grouped for species ($n = 6$). The interaction between species and harvesting site was not significant. The same letters indicate that mean values are not significantly different ($P = 0.05$).

Figure 4 – Elements content in the fresh matter of the selected wild edible plants harvested from different areas ($n = 6$). The same letters indicate that mean values are not significantly different ($P = 0.05$).

Figure 5 – PCA biplots (**A**: PC1 vs PC2; **B**: PC1 vs PC3) describing the spatial distribution of DM and the IPC-OES chemical properties of eleven wild edible plants harvested from two different areas; **IPC** (Inner Part of Countryside) area and **NR** (Near Road) area. **Ama** (*A. retroflexus*), **Bor** (*B. officinalis*), **Cich** (*C. intybus*), **Dipl** (*D. tenuifolia*), **Foen** (*F. vulgare*), **Gleb** (*G. coronaria*), **Pap** (*P. rhoeas*), **Plant** (*P. lagopus*), **Port** (*P. oleracea*), **Sin** (*S. arvensis*), **Sonc** (*Sonchus* spp.). **DM** (dry matter). PCA was performed on fresh basis data.