UNIVERSITÀ DEGLI STUDI DI TORINO

Journal of Pharmaceutical and Biomedical Analysis, Volume 106, 2015,
doi: 10.1016/j.jpba.2014.10.036

Determination of major, minor and trace elements in Glyceric Macerates and Mother Tinctures and in the starting plant materials

Ornella Abollino[a,*], Agnese Giacomino[b], Mery Malandrino[a], Arianna Allio[b], Rosanna Toniolo[c], Maria Laura Colombo[b]

[a]Department of Chemistry, University of Torino, Via Giuria 5, Torino (Italy)

[b]Department of Drug Science and Technology, University of Torino, Via Giuria 5, Torino (Italy)

[c]Department of Food Science, University of Udine Via Cotonificio 108, Udine (Italy).

* Corresponding author. Tel.: +390116705243; fax: +390116705243.

E-mail address: ornella.abollino@unito.it

ABSTRACT

Glyceric Macerates (GMs) and Mother Tinctures (MTs) are liquid preparations obtained from plant buds (for GMs) and flowers, leaves or roots (for MT) by extraction with a mixture of solvents. Their quality depends on the quality of the plant materials and on the preparation procedures. In this work we determined the concentrations of major, minor and trace elements in buds, flowers and other plant components and in the GMs and MTs obtained from them by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) after microwave mineralization. To our knowledge, this procedure has been applied for the first time here to the analysis of buds. We have taken into account spectral interferences and other causes of errors. Analogies and differences with regard to the method reported by European Pharmacopoeia for heavy metal determination in herbal

drugs have been highlighted. The experimental results have been interpreted with chemometric techniques. No significant contamination was detected during the manufacturing step. Element concentrations in GMs and MTs, taking into account their daily dosages, are lower than acceptable intake levels.

Keywords: plants; buds; Glyceric Macerates; Mother Tinctures; metals; chemometrics.

1. Introduction

In the last decades the demand of natural, plant-based product has been increasing. In the past, only raw dry herbs were commercially available, which were usually consumed for infusions and decoctions, commonly referred to as tisanes. Nowadays many plant-derived products are marketed in several forms, *e.g.* tablets, powders and liquids. Some producers do not prepare formulations starting from raw herbs, but use semi-finished products, because they are easier to deal with, enable one to save time and are more homogeneous than the raw materials. The quality of such products is extremely important for the quality of the final formulations. Glyceric Macerates(GM) and Mother Tinctures (MT) are two examples of semi-finished stuffs; they are obtained by extraction of plant parts (section 2.2.). They are also directly consumed after dilution; in addition, MTs are used as bases for homeopathic and cosmetic products.

Plants are sources of both secondary metabolites (some of which represent the “active principles” of plants and are the reason why plants are used as health-promoting agents) and of the so-called mineral nutrients, i.e. most alkali, alkaline earth and transition metals, some metalloids (*e.g.* Si and Se) and nonmetals (*e.g.* P and Cl). These elements are essential for plant growth and for human health [2,3], but become harmful at high concentrations [1,4]. Other elements, like Pb, Hg and Cd, do not have known physiological roles and are simply tolerated by the organism at low concentrations. The “low” and “high” concentration levels depend on the effect of each element: even common metals like Na or Ca are detrimental if present in excess in the human body.

Plants assimilate mineral nutrients primarily from the soil and partly from the atmosphere and from the water used for irrigation. Such nutrients are transferred, in part or completely, to plant-derived products [5]. Also improper manufacturing of semi-finished and end products, such as the use of contaminated solvents, unclean vessels or working places can be a source of essential and non-essential elements. Therefore it is important to determine their concentrations both in plant raw materials and in plant-derived products. Many papers report element contents in plants [*e.g.* 4,6-8], but less attention was devoted to plant-derived products [*e.g.* 9]. Furthermore, to our knowledge no papers deal with the concentrations in buds or bud-derived products, and the information on MTs is scarce [10,11]. For these reasons we focused our attention on GMs obtained from buds and MTs prepared from selected plant parts, and on their starting materials; since the manufacturing steps may cause contamination, we also analyzed the extracting reagents and the filters used for the preparation.

The analytical techniques commonly adopted for element determination in plants are atomic absorption (AAS), inductively coupled plasma optical emission (ICP-OES) or mass spectrometry (ICP-MS), preceded by mineralization of the samples [*e.g.* 3,8,9]. The European Pharmacopoeia [12] reports a method for heavy metal determination in herbal drugs and fatty oils, based on mineralization with a mixture of nitric and hydrochloric acid and analyte determination by AAS. This combination of acids is quite aggressive, probably because the method is designed also for fatty oils. We used nitric acid alone, because it is extensively adopted for plant digestion [*e.g.* 13], and chose ICP-OES instead of other instrumental techniques because it is more rapid than graphite furnace AAS (GF-AAS) and less expensive than ICP-MS. We took into account the ~~possible~~ instrumental interferences and other sources of error and treated the results with chemometric techniques. The limits of quantification (LoQs) are higher than those of GF-AAS and ICP-MS: anyway we were able to evaluate the hazards associated to the presence of potentially toxic elements in GMs and MTs using worst-scenario conditions, assuming that their concentrations were

equal to the LoQs (section 3.3). For such evaluation we compared element concentrations with reference acceptable intake values.

The outcomes of our study can have different applications. First of all, we report a protocol for the analysis of buds and highlight the sources of errors and interferences. Secondly, the concentrations found in buds and MGs, presumably being the first published data on these matrices, can be used as a basis of comparison in future studies. Finally, the results reported can be of interest to both producers and consumers of plant-derived products.

2. Experimental

2.1. Sample collection

Buds, flowers and other plant parts were provided by GEALpharma (Bricherasio, Torino, Italy), a small company manufacturing GMs and MTs. Most samples had been harvested from plants spontaneously growing in areas unaffected by local sources of vehicular traffic or industrial activities in Val Pellice, Val Chisone and Val Germanasca, Torino province, Piedmont, Italy. *Echinacea angustifolia* DC, *Passiflora incarnata* L. and *Rheum officinale* Baill., which are not spontaneous in Piedmont, had been purchased from vendors growing plants in open fields in areas with the same characteristics. Table 1 reports the list of the investigated species, the identification code used in this paper, the common name, family, order, the balsamic time, the used parts and the obtained product. For the nomenclature and taxonomy of the plants the projects "The Plant List" and "Angiosperm Phylogeny Website v.13" were taken as reference [14,15].

2.2. Extraction procedure

According to the European Pharmacopoeia [12], Glyceric Macerates are liquid preparations obtained from raw materials of botanical, zoological or human origin by using glycerol or a mixture of glycerol and either alcohol of a suitable concentration or a solution of sodium chloride of a suitable concentration. Tinctures are liquid preparations usually obtained using either one part of

herbal drug or animal matter and ten parts of extraction solvent, or one part of herbal drug or animal matter and five parts of solvent.

GMs and MTs were prepared by GEALpharma according to the European Pharmacopoeia 8th edition [14], following the procedure deriving from the French Pharmacopoeia. Briefly, buds or other plant materials were transferred to glass jars and the following solvents were added: 50/20/30 (by weight) water/ethanol/glycerol for GMs; 60/40 (by weight) water/ethanol for MTs. Fresh plants were used, and their humidity was calculated on an aliquot of the material. About 1 Kg of stuff was treated, and the amount of solvent was adjusted so as to obtain a weight ratio of 1/20 between (calculated) dry plant and final product for GM and 1/10 for MT. After 21 days of maceration, the suspension was filtered, and the residue was pressed. The percolate was added to the filtrate, and the GM or MT so obtained was stored in stainless steel containers, from which it was transferred in glass vessel for commercialization.

2.3. Reagents and apparatus

High purity water (HPW) produced with Millipore Milli-Q system was used throughout. The reagents adopted were of analytical grade.

Standard and sample solutions were prepared and stored in high density polyethylene (HDPE) vessels or in polypropylene Falcon tubes. All vessels were previously washed in 1 M HNO_3, rinsed with HPW and stored in 0,01 M HCl. Standard analyte solutions were prepared by dilution of concentrated stock solutions (Merck Titrisol or Sigma Aldrich).

Sample mineralization was carried out with a Milestone MLS-1200 Mega (Milestone, Sorisole, Italy) microwave laboratory unit equipped with polytetrafluoroethylene (PTFE) bombs.

The analytes were determined with a Perkin Elmer Optima 7000 (Perkin Elmer, Norwalk, Connecticut, USA) ICP-OES.

2.4. Mineralization and analysis procedures

Buds and other plant materials were dried and smashed with a ceramic knife. Small portions of new (i.e. not used for extract preparation) filters were cut and analysed without further pretreatments. Aliquots of 0.5 g of solid or liquid sample were transferred into PTFE bombs and added with 5 ml of concentrated HNO_3. The bombs were heated in the microwave oven according to the scheme: 250 W (2 min), 0 W (2 min), 250 W (6 min), 400 W (5 min), 600 W(5 min), ventilation (5 min). The resulting solutions were filtered on Whatman 5 filters and diluted to 50 ml with HPW or to 25 ml for filter and pure solvent samples. Analyte concentrations were determined by ICP-OES using an external calibration performed with standard solutions prepared in aliquots of sample blanks. The emission wavelengths are shown in Table 1S (Supplementary data).

The accuracy of the procedure was evaluated with a Certified Reference Material (CRM), namely Tomato Leaves SRM 1573a, supplied by the National Institute of Standards and Technology (NIST); analyte recoveries are reported in Table 2S (Supplementary data). Analyses were performed in duplicate and blanks were simultaneously run. The limits of quantification (LoQ) were estimated as ten times the standard deviation of the blank ($10s_b$).

2.5. Data processing

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) [16] were carried out with the aid of XLSTAT 4.4 software package, used as a Microsoft Excel plug-in, whereas Unscrambler X 10:2 was used for Linear Discriminant Analysis (LDA). Unscrambler X 10:2 was also employed for data standardization, obtained by mean-centering (for each variable) and dividing by the corresponding standard deviation, and for substituting values below LoQs with estimated values. Analytes with most values below the LoQ were not included; in the case of Na (for MTs) and of Si (for GMs and MTs), values below the LoQ but higher than the limit of detection (LoD, estimated as $3s_b$) were also considered for PCA and HCA: of course these data have a higher uncertainty than the other ones. The Scree plot was examined in order to decide the number of

factors to be taken into account for PCA. The Euclidean distance and Ward's agglomeration method were used for HCA.

3. Results and discussion

3.1. Analytical approach

Although the analytical procedure we adopted is well established, it is important to avoid errors and take instrumental interferences into account. The main risks of errors are associated to contaminations and positive interferences, which would lead to an overestimation of the concentrations and consequently of the risks for health associated to the consumption of plant-derived products. The following aspects should be taken into account:

- sample pretreatment should be carried out with suitable tools, to avoid release of analytes into the samples. In this study, we used a ceramic knife;
- the digestion vessels should be cleaned after each sample mineralization, to avoid memory effects. We add 5 ml of HNO_3 and heat the bombs for 5 min at 250 W, then we rinse them with HPW;
- the usual precautions necessary for trace element determination should be adopted, in terms of clean laboratory environment and vessels, careful manipulation by the analyst and so on;
- we prepare the calibration standards in aliquots of sample blank, according to the matrix matching method. This procedure enables one to take into account the influence of sample density, mainly dictated by the concentration of acids, on nebulization efficiency. Alternatively, it is possible to adopt the standard addition method, which anyway is time-consuming in the presence of a large number of samples; moreover this method is excellent to overcome the effect of the sample matrix on sensitivity, i.e. on the slope of the calibration curve, but it cannot take account of background signals.

Furthermore, it is important to visually inspect the emission spectrum of each analyte, instead of just relying on the output of the instrument software in terms of the final concentration. Five main errors may occur:

- if the background, to be subtracted from the analyte signal, is measured in correspondence to a local maximum of the spectrum, the analyte signal will be underestimated; the background signal should be measured in a position of the spectrum with a baseline similar to the one present under the analyte itself;
- in our case, the software subtracts the signal of the calibration blank from the signal of the samples; if the former is negative, due to a fluctuation of the baseline, an apparent increase of the analyte emission intensity results. This happened with Sn, which was below the LoQ in the starting materials, but seemed to be present in GMs and MTs. If we had uncritically taken the concentrations provided by the software, we would have wrongly concluded that the two products had been contaminated by Sn during preparation;
- sometimes the peak height is not correctly measured, especially in the case of sloping baseline. We encountered this source of error with Al, as shown in Fig. 1a; fortunately, the software allowed us to choose the proper baseline and re-calculate all peak heights; as to the background, in the presence of sloping baselines the software measures the intensity at two points on the sides of the peak, then interpolates an intensity at the peak wavelength and subtracts it from the signal recorded at the peak itself;
- spectral interferences must be checked, with the aid of a list of emission lines. If serious overlaps between analyte and interferent peaks are present, another wavelength should be chosen, or a mathematical correction of the signal should be applied [17]. If the peaks are well separated, the analysis can be carried out without problems; this is the case of the determination of Fe in buds: as shown in Fig. 1b, a minor emission line of Fe itself at 259.837 nm does not interfere with the signal of interest;
- fluctuations of the background can be misinterpreted as signals from the analyte. We found this situation with Se (Fig. 1c); a proper estimation of the standard deviation of the background, coupled to the visualization of the emission spectrum, allowed us to avoid this error.

Fig. 1.

Emission spectra affected by interferences. X-axis: wavelength (nm). Y-axis: intensity (arbitrary units). (a) Spectra of Al: _ _ _ _ 0,05 mg/l standard solution; ____ sample JN_C; _ .. _ .. _ baseline before correction; _._._baseline after correction. (b) Spectra of Fe: …….blank; _ _ _ _ 0,05 mg/l standard solution; ____ sample JN_C; the arrow indicates a secondary emission line of Fe. (c) Spectra of Se: …….blank; _ _ _ _ 0,05 mg/l standard solution; ____ sample JN_C; the arrow indicates the emission line of Se; the peak on the right of the spectra was not identified.

In conclusion, even in the presence of a relatively simple and well known procedure, the good quality of the experimental results must not be taken for granted, but depends on proper operation and on the check of the instrument output.

3.2. Element concentrations in buds, flowers and other part materials

We analysed 17 samples of buds, 6 samples of flowers and 7 samples of other plant materials, from which GMs or MTs were obtained. Unfortunately, raw plants were not available, so we analysed the samples after maceration and pressing: therefore the concentrations determined, and reported in Tables 2 and 3, represent the residues after extraction. The total element concentrations in the starting materials were calculated from the sum of concentrations in treated samples and in GMs or MTs, taking into account the plant-solvent ratios and assuming that the contributions from the solvent and from the product preparation process is negligible with respect to that from the plant (section 3.4).

We determined the concentrations of 18 elements: Al, As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, P, Pb, Se, Si, Sn, Zn, chosen for their roles as i) nutrients in the human body (macro-nutrients: Na, K, Ca, Mg and P; micro-nutrients: Cr, Cu, Fe, Se, Si, Zn) and/or as ii) potentially toxic agents (As, Cd, Hg, Pb, Cr, Cu, Se, Sn and Zn): as expected, some analytes have both roles, depending on their concentrations, so they belong to both categories. The results are reported in Table 2 for buds and

Table 3 for flowers and other plant materials. The data are subdivided according to the kind of plant (conifers, other trees, shrubs) or to the used part (flowers, roots, leaves, berry-like fruits, whole plant). The concentrations of As, Cd, Cr, Hg, Pb, Se and Sn were lower than the LoQ in all samples. The following remarks can be made on the results obtained from buds:

- as expected, the analytes with the highest concentrations are Ca, K, Mg and P, which are macro-nutrients for plants as well as for humans; Ca has the highest concentration in most samples;
- among conifers, *Larix decidua* Mill. has the highest concentrations of most elements. Most analytes have lower concentrations in conifer buds than in the other investigated buds. The former were obviously collected at greater altitudes in comparison to the other samples;
- as to the buds from other trees, there is no specimen with outstandingly higher or lower concentrations. The concentrations of Si are higher than in conifers, which suggests that these trees assimilate it in a different way from conifers, or that they are more influenced by the resuspension of soil dust arising from agricultural activities; the influence of vehicular traffic on soil resuspension can be deemed neglectable, because the buds were collected far from congested roads;
- shrub buds were collected at lower altitudes: the concentrations of Si remarkably increased with respect to trees, corroborating the hypothesis that this element partly derives for soil resuspension, which strongly influences shrubs owing to their lower height;
- in general, the interaction of metals with plants is complex and depends on many environmental and genetic factors. Jansen et al. [18] developed a scheme that correlates the plant taxonomic Order to their ability to accumulate Al. Their data are in agreement with our results: nearly all plants belonging to the Orders Cornales, Fagales, Sapindales and Saxifragales (see Table 1), classified by Jansen et al. as Al accumulators, have high level of this metal.

The outcome of the chemometric treatment for bud data is shown in Fig. 2a and 2b for PCA loading and score plots respectively and in Fig. 3 for the HCA dendrogram. We will discuss the chemometric results for the four data sets (sections 3.2-3.3) using the approach outlined hereafter. We will mainly refer to the dendrograms for discussing similarities and differences among samples,

because they retain 100% of the variance, i.e. the information originally present in the data; on the other hand, the reported score plots only show the first two principal components, also named "factors" or "latent variables", i.e. the first two linear combinations of the original variables (element concentrations), which retain only a part of the variance: the exact percentage is shown in each plot. We will consider the score and the loading plot to identify the elements with the highest or lowest concentrations in the samples. In addition, the loading plot shows correlations among variables but, being a two-dimensional projection of a multidimensional data set, does not always allow one to correctly visualize them: for this reason we will mainly base our discussion on Pearson's correlation matrix and on the numerical values of the loadings on the first PCs. For bud samples, these data are reported in Table 3S and 4S (Supplementary data) respectively.

Fig. 2.

Loading (a) and score (b) plots obtained by PCA for bud samples.

Fig. 3.

Dendrogram obtained by HCA for bud samples.

The most interesting correlations observed for buds are among: i) Al, Fe and Si, deriving from the soil matrix; ii) Na, K, Mg, Ca, probably because of their chemical properties, which are quite different from those of transition metals and p-block elements. As to transition metals, only Cu and Mn are correlated, but they have no significant relationships with Fe and Zn. P and Zn do not show significant correlations with any analyte. The numerical values of the loadings show that: i) Ca, Mg, Na and K mainly load on F1; ii) Al, Fe and Si have high loadings on F2; iii) Cu and Mn primarily influence F3. We hypothesize that F1 is related to the nutrient content in the buds, and F2 to the input from soil silicate matrix.

The results of HCA show that conifer buds are differentiated from the other samples. Buds from plants belonging to the Betulaceae family (BE_T, CR_T and CY_S) have a certain degree of similarity. Other clusters based on plant taxonomy are not visible. Considering loadings and scores simultaneously, it can be observed that AB_C, JN_C and PI_C are characterized by low concentrations of analytes, whereas LA_C, which is far from all other samples (i.e. it is much different from them), has high levels of Al, Fe and Si. No differentiation among buds from other trees and from shrubs is apparent.

Considering the residues of flowers and other plant materials, the following considerations can be made:

- as with buds, the elements with the highest concentrations are Ca, K, Mg and P; Ca is the predominant component;
- sample EC_R has the highest levels of several analytes, whereas no sample has the lowest concentrations of most or all elements;
- for *Echinacea angustifolia* DC and *Crataegus laevigata* (Poir.) DC. a comparison can be made between two plant parts. The roots of *Echinacea angustifolia* DC have higher concentrations of most analytes that the leaves: this trend is typical for plants, which assimilate nutrients mainly from soil through their roots, that also act as reservoirs. Flowers of *Crataegus laevigata* (Poir.) DC. have higher concentrations than buds of the same plant, probably because the latter are a meristematic state of flowers or leaves, so they are at an earlier stage of development.

Pearson's correlation matrix for flowers and other plant materials, reported in Table ~~4~~5S (Supplementary data), shows less correlations in comparison to those present for buds, probably because of the heterogeneity of the samples, which comprise flowers, leaves, roots and fruits. The main feature is the strong correlation among Al and Fe, that may be indicative of their geogenic origin; the lack of correlation of these elements with Si is difficult to explain. On the other hand, neither the investigated transition metals, nor alkali and alkaline earth metals, are correlated, suggesting that they derive from different sources or they have different roles in the plant. The

correlation between Al and Fe is visible in the loading plot shown in Fig. 4a. Table 6S (Supplementary data) also shows that: i) Al, Fe and, at a lesser extent, Na mainly load on F1; the influence of Al and Fe suggests that this PC reflect the influence of soil; ii) Cu, Mg and Si have high loadings on F2: a sound interpretation of the meaning of this factor was not found; iii) the other variables load on F3, F4 and F5: no apparent relationship as a function of their chemical properties or source could be identified.

Fig. 4.

Loading (a) and score (b) plots obtained by PCA for plant component samples.

Fig. 5.

Dendrogram obtained by HCA for plant component samples.

Both the score plot in Fig. 4b and the dendrogram in Fig. 5 show that flower samples are not differentiated from the other plant parts; the score plot shows a certain degree of similarity between the two roots, i.e. EC_R and RH_R. The joint observation of scores and loadings reveals that they are characterized by the high concentrations of elements typical from soils. Samples CT_Fl, HL_Fl and especially TL_Fl are characterized by a high concentration of Mn.

3.3. Element concentrations in GMs and MTs

Tables 4 and 5 collect element concentrations in GMs and MTs respectively. The data are expressed as mg/Kg, but can be easily converted to mg/L taking into account that the densities are 1.03 Kg/L for GMs and 0.932 Kg/L for MTs. The percentages of extraction from the starting materials are reported in Tables 6 and 7.The following remarks can be made:

- overall, the extraction percentages vary in the order Ca < Mn < P < Mg < K < Na for GMs and Ca < Mn < Mg < P < K < Na for MTs. In both cases Na and K are the most extensively extractable elements, in agreement with the high solubility of their compounds. The behaviour of Ca suggests its presence as sparingly soluble species, such as oxalates. Extractability in GMs is higher than in MTs: the presence of glycerol possibly favours the release of a fraction of elements bound to organic components of the samples. Fairly similar concentrations are present in the two formulations, even if the extraction percentages are different, because analyte levels in the raw plant materials for MTs are generally higher than in those for GMs;
- the concentrations in GMs and MTs are much lower than in the starting materials even for elements with high extraction percentages, owing to the large excess of solvent with respect to the solute. Several analytes present in the plants (Al, Cu, Fe, Si and Zn) are below the LoQs in most of the extracts; the same is valid for As, Cd, Cr and Pb, which had not been found in the starting materials as well. The elements with the highest concentrations are K and P, followed by Mg and Ca;
- no substantial differences are present as a function of the kind of bud for GMs or between flowers and other plant materials for MTs. This suggests that concentrations mainly depend on the solubility of the analytes in the extracting solvents, rather than on the composition of the plant;

Elements with concentrations below the LoQs in most or all samples were not included in the chemometric treatment. Pearson's correlation matrix for GM samples is reported in Table 7S (Supplementary data). The main correlation observed is among Mg, Ca, K, Si and Mn; K is also strongly correlated with P. Such correlations are visible in the loading plot (Fig. 6a).

Fig. 6.

Loading (a) and score (b) plots obtained by PCA for GM samples.

Fig. 7.

Dendrogram obtained by HCA for GM samples.

Table 8S (Supplementary data) reports the numerical values of the loadings: K, Mg and Si mainly load on F1, whereas Ca and P have higher loadings on F2; Mn primarily influences F3. The meanings of the factors were not identified; no relationship with the solubility of the salts of these elements in the solvent is apparent.

Both the score plot (Fig. 6b) and the dendrogram (Fig. 7) show that there is no grouping of GMs according to the kind of bud present in each sample, and that conifers are no more differentiated from other trees and shrubs. The plot of F1 vs F3 (not shown) does not indicate further distinction of the samples. These findings confirm our previous hypothesis that the type of bud has a low influence on the solubility of elements, which is dictated by the solvent. The joint observation of scores and loadings shows that samples CN_S and FI_T are characterized by high concentrations of Ca and of K and P respectively.

As to MTs, Pearson's correlation matrix, reported in Table 9S (Supplementary data) shows the presence of fewer correlations, as already remarked for the starting materials. Table 9S, collecting the loading values, shows that most variables load on F1, with the exception of Mn (F2) and P (F3). The loading plot (Fig. 8a) does not add any additional information.

Fig. 8.

Loading (a) and score (b) plots obtained by PCA for MT samples.

Fig. 9.

Dendrogram obtained by HCA for MT samples.

Neither the score plot (Fig. 8b) nor the dendrogram (Fig. 9) reveal the presence of clusters of samples. Sample EC_LR is differentiated from all the others, due to high concentrations of Si and Mg.

We also processed the data for GMs and MTs together: neither PCA nor HCA enabled us to distinguish between the two types of formulations. The same data were treated with LDA: again, GMs and MTs could not be classified in two separate categories. Therefore, the content of inorganic components is not a feature that characterizes GMs and MTs.

3.4. Analysis of solvents and materials used to prepare GMs and MTs

The possible contribution of the preparation steps to the element contents in GMs and MTs was investigated. Element concentrations in the two extracting solvents are reported in Table 8; the elements not listed in the table are below the-LoQs. The samples contain only small amounts of Ca, Si and Zn. The concentration levels in the two solvents are comparable for all analytes with the exception of Si, which is present at higher concentrations in the solvent for GM. The concentrations of Si and Zn are higher in the pure solvents than in the final GMs and MTs, which suggests that these elements might have been trapped in the solid plant residue during maceration.

Table 8 shows that the filters used to separate the extract from the solid mass-contain (before use) a high concentration of Na and lower amounts of Al, Ca, K, Fe, Mg, P, Si and Zn. According to our experience, Na and K can be released from some brands of paper filters, and we usually pre-clean them with aliquots of water before use. We analyzed aliquots of the solvents used for GMs and MTs before and after filtration, and we did not find any significant difference between them: so we can conclude that elements present in the filters are not released when the solvent flows through them.

3.5. Comparison with admissible intake levels

The levels of the elements with the highest potential toxicity, namely Al, As, Cd, Cr, Cu, Mn, Pb and Zn, in GMs and MTs were compared with admissible intake levels under the following

assumptions:

- concentrations equal or lower than the LoQs in GMs and MTs were assumed to be equal to the latter, in order to consider the worst-scenario;
- the dose ingested by end-users consuming the formulations as such, was calculated according to the standard dosages of 100 drops/day of GMs and 60 drops/day of MTs (20 drops = 1 mL).

When GMs and MTs are used as semi-finished products, they are mixed with other components: in this case we do not have enough information to perform the calculation.

Table 9 reports the admissible levels issued by three international organisms: the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [19]; the European Food Safety Authority (EFSA) [20]; the Agency for Toxic Substances and Disease Registry (ATSDR) of the U.S. Department of Health and Human Services [21]. We report each value both expressed in the original units, and converted to mg/day. These last values were compared with the calculated intake of each element from GMs and MTs for a body weight of 60 Kg. As Table 9 shows. the reference values were never exceeded: we can hypothesize that no risks are present for human health, from the point of view of the contents of the considered elements, upon the consumption of these products at the dosages indicated. For this reason we did not re-analyse the samples with more sensitive analytical techniques, such as GFAAS or ICP-MS, to exactly quantify the concentrations of potentially toxic elements. Of course in order to evaluate the risk of harmful effects of such elements for an individual, all the sources to which he/she is exposed must be taken into account.

4. Conclusions

Several potentially toxic elements, namely As, Cd, Cr, Hg and Pb, are below the LoQs in the investigated GMs and MTs and in the starting plant components. Also the contents of Al and Cu in the two formulations are below the LoQ, even if they are present in the starting materials. The intake of all these elements upon consumption of GMs and MTs, was found to be lower than the admissible levels issued by JECFA, EFSA and ATSDR. Therefore we can hypothesize that the

consumption of these products does not pose risk to human health, at least from the point of view of the presence of trace elements. Of course this conclusion is not valid for all GMs and MTs present on the market, but it is applicable only to the investigated samples. Similar studies should be carried out on other commercially available products.

It can be presumed that the content of each element in the products depends on the combination of three factors: its concentration in the starting materials, the nature of the latter and its solubility in the extracting solvent. Our results suggest that the type of plant has a limited influence on the solubility of elements, which is dictated by the solvent.

The chemometric treatment of the data allowed us to inspect similitudes ad differences among the samples and to identify correlations among variables. Buds from conifers were found to be different from buds from other plants. On the other hand, neither GMs nor MTs were grouped according to the macroscopic characteristics of the species of origin. GMs and MTs could not be classified in two groups by LDA, so the content of inorganic components is not a feature that characterizes these two kinds of product.

Future development of the research can be a comparative analysis of plants and of the soils underneath, in order to obtain transfer factors between soil and plant. Moreover, it would be interesting to know if a metal-contaminated plant would give rise to contaminated MG or MT, or whether the metals would not be extracted.

Acknowledgements

The authors are grateful to GEALpharma (Bricherasio, Torino, Italy) for providing the samples of plant materials, GMs and MTs.

The financial support of the University of Torino is gratefully acknowledged.

References

[1] A. Kabata-Pendias, H. Pendias, Trace elements in soils and plants, third ed., CRC Press, Boca Raton, 2001.

[2] P.J. White, M.R. Broadley, Biofortifying crops with essential mineral elements, Trends Plant Sci. 10 (2005) 586-593.

[3] A. Arzani, H. Zeinali, K. Razmjo, Iron and magnesium concentrations of mint accessions (Mentha spp.), Plant Physiol. Biochem. 45 (2007) 323-329.

[4] H. Sarma, S. Deka, H. Deka, R.R. Saikia, Accumulation of heavy metals in selected medicinal plants, in: D.M.Whitacre (Ed.), Reviews of environmental contamination and toxicology, vol. 214, Springer, Berlin, 2001, pp. 63-86.

[5] M. Vaculik, L. Jurkovic, P. Matejkovic, M. Molnarova, A. Lux, Potential risk of arsenic and antimony accumulation by medicinal plants naturally growing on old mining sites, Water Air Soil Pollut. 224 (2013) 1-16.

[6] J. Barthwal, S. Nair, P. Kakkar, Heavy metal accumulation in medicinal plants collected from environmentally different sites, Biomed. Environ. Sci. 21 (2008) 319-324.

[7] S. Shavakh, S. Kavkani, N. Ghalandari, J. Nouri, Accumulation of Copper and Lead in Selected Medicinal Plants, Asian J. Chem. 25 (2013) 2977-2980 .

[8] J.Kováčik, J. Grúz, B. Klejdus, F. Štork, J. Hedbavny, Accumulation of metals and selected nutritional parameters in the field-grown chamomile anthodia, Food Chem.131 (2012) 55-62.

[9] T. Karak, R.M. Bhagat, Trace elements in tea leaves, made tea and tea infusion: A review, Food Res. Int. 43 (2010) 2234-2252.

[10] J. Busch, W. Werner, A. Huwer, Study of the risk of heavy metal transfer to homoeopathic mother tinctures, Pharmeuropa Bio & Scientific Notes 2012 (2012) 55-71.

[11] C.M. Witt, R. Ludtke, T.E.R. Weisshuhn, P. Quint, S.N. Willich, The role of trace elements in homeopathic preparations and the influence of container material, storage duration, and potentisation, Forsch. Komplementarmedizin Klass. Naturheilkunde 13 (2006) 15-21.

[12] Council of Europe, European Pharmacopoeia, 8th ed., Council of Europe, Strasbourg, 2013.

[13] M. Malandrino, O. Abollino, S. Buoso, A. Giacomino, C. La Gioia, E. Mentasti, Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite, Chemosphere 82 (2011) 169-178.

[14] www.theplantlist.org

[15] www.mobot.org/MOBOT/research/APweb/

[16] D.L. Massart, B.G.M. Vandenginste, L.M.C. Buydens, S. De Jono, P.J. Leqy, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Parts A and B, Elsevier, Amsterdam, 1997.

[17] O. Abollino, E. Mentasti, C. Sarzanini, E. Modone, M. Braglia, Determination of zirconium, barium, lanthanum, aluminium, sodium, hafnium and fluorine in fluorozirconate glasses, Fresenius' J. Anal.Chem., 343 (1992) 482-487.

[18] S. Jansen, T. Watanabe, S. Dessein, E. Robbrecht, E. Smets, The evolution of aluminium accumulation in angiosperms, in A.R Hemsley, I. Poole (Eds.), The evolution of plant physiology, Elsevier Academic Press, London, 2004, pp. 467-479.

[19] www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/

[20] www.efsa.europa.eu/en/topics/topic/metals.htm

[21] www.atsdr.cdc.gov/

Captions to the figures

Fig. 1. Emission spectra affected by interferences. X-axis: wavelength (nm). Y-axis: intensity (arbitrary units). (a) Spectra of Al: _ _ _ _ 0,05 mg/l standard solution; ____ sample JN_C; _ .. _ .. _ baseline before correction; _._._baseline after correction. (b) Spectra of Fe: …….blank; _ _ _ _ 0,05 mg/l standard solution; ____ sample JN_C; the arrow indicates a secondary emission line of Fe. (c) Spectra of Se: …….blank; _ _ _ _ 0,05 mg/l standard solution; ____ sample JN_C; the arrow indicates the emission line of Se; the peak on the right of the spectra was not identified.

Fig. 2. Loading (a) and score (b) plots obtained by PCA for bud samples.

Fig. 3. Dendrogram obtained by HCA for bud samples.

Fig. 4. Loading (a) and score (b) plots obtained by PCA for plant component samples.

Fig. 5. Dendrogram obtained by HCA for plant component samples.

Fig. 6. Loading (a) and score (b) plots obtained by PCA for GM samples.

Fig. 7. Dendrogram obtained by HCA for GM samples.

Fig. 8. Loading (a) and score (b) plots obtained by PCA for MT samples.

Fig. 9. Dendrogram obtained by HCA for MT samples.

Table 1
Species investigated, identification code, common name, family, order, balsamic time, part used and product obtained (Fl = flowers; L = leaves; R = Roots & Rhizome; W = Whole plant; Fr = berry-like Fruit).

Code	Species	Common name	Family	Order	Time	Part	Product
AB_C	*Abies alba* Mill. syn. *Abies pectinata* (Lam.) Lam. & DC	Fir	Pinaceae	Pinales	Late Spring	Buds	GM
AC_T	*Acer pseudoplatanus* L.	Sycamore maple	Sapindaceae	Sapindales	Late Spring	Buds	GM
AE_T	*Aesculus hippocastanum* L.	Horse chestnut	Sapindaceae	Sapindales	Late Spring	Buds	GM
BE_T	*Betula pubescens* Ehrh.	Downy birch	Betulaceae	Fagales	Early Spring	Buds	GM
CR_T	*Carpinus betulus* L.	Common hornbeam	Betulaceae	Fagales	Early Spring	Buds	GM
CS_T	*Castanea sativa* Mill. syn. *Castanea vesca* Gaertn.	Chestnut	Fagaceae	Fagales	Late Spring	Buds	GM
CN_S	*Cornus mas* L.	Cornelian cherry, European corne	Cornaceae	Cornales	Early Spring	Buds	GM
CY_S	*Corylus avellana* L.	Hazelnut	Betulaceae	Fagales	Early Spring	Buds	GM
CT_S	*Crataegus laevigata* (Poir.) DC. (syn. *Crataegus oxyacantha* auct.)	Hawthorn	Rosaceae	Rosales	Spring	Buds	GM
FI_T	*Ficus carica* L.	Fig	Moraceae	Rosales	Spring	Buds	GM
JG_T	*Juglans regia* L.	Walnut	Juglandaceae	Fagales	Spring	Buds	GM
JN_C	*Juniperus communis* L.	Juniper	Cupressaceae	Pinales	Spring	Buds	GM
LA_C	*Larix decidua* Mill.	Larch	Pinaceae	Pinales	Spring	Buds	GM

Table 1 (continued)

Code	Species	Common name	Family	Order	Time	Part	Product
PI_C	*Pinus mugo* Turra (syn. *Pinus montana* Mill.)	Mountain pine	Pinaceae	Pinales	Spring	Buds	GM
RI_S	*Ribes nigrum* L.	Blackcurrant	Grossulariaceae	Saxifragales	Spring	Buds	GM
RC_S	*Rosa canina* L.	Dog rose	Rosaceae	Rosales	Early spring	Buds	GM
VI_S	*Vitis vinifera* L.	Common grape vine	Vitaceae	Vitales	Spring	Buds	GM
CL_Fl	*Calendula arvensis* M.Bieb.	Marigold	Compositae	Asterales	Summer	Fl	MT
CM_Fl	*Matricaria chamomilla* L.	Chamomile	Compositae	Asterales	Late spring - Summer	Fl	MT
CT_Fl	*Crataegus laevigata* (Poir.) DC. (syn. *Crataegus oxyacantha* auct.)	Hawthorn	Rosaceae	Fagales	Spring	Fl	MT
EC_RL	*Echinacea angustifolia* DC	Narrow-leaved purple coneflower	Compositae		Summer (L) Autumn (R)	R + L	MT
HL_Fl	*Humulus lupulus* L.	Hop	Cannabaceae	Urticales	Early autumn	Fl	MT
PA_Fl	*Papaver rhoeas* L.	Red poppy	Papaveraceae	Papaverales	Late spring	Fl	MT
PF_FlL	*Passiflora incarnata* L.	Maypops	Passifloraceae	Malpighiales	Spring	Fl+L	MT
PI_L	*Pilosella officinarum* Vaill (syn. *Hieracium pilosella* L.)	Mouse-ear hawkweed	Compositae	Asterales	Late spring	L	MT
RH_R	*Rheum officinale* Baill.	Rhubarb	Polygonaceae	Polygonales	Autumn	R	MT
TA_W	*Taraxacum campylodes* G.E.Haglund (syn. *Taraxacum officinale* (L.) Weber ex F.H.Wigg.	Common dandelion	Compositae	Asterales	Late spring	W	MT

Table 1 (continued)

Code	Species	Common name	Family	Order	Time	Part	Product
TI_Fl	*Tilia tomentosa* Moench.	Silver lime (UK) Silver linden (USA)	Malvaceae	Malvales	Late spring	Fl	MT
VM_Fr	*Vaccinium myrtillus* L.	European blueberry	Ericaceae	Ericales	Summer	Fr	MT

Table 2.
Element average concentrations and estimates of standard deviation (in brackets) in residues of plant buds, minimum and maximum values (mg/kg dw).

Plant	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	Zn	Min	Max
Conifers													
AB_C	13.0 (0.1)	600 (50)	2.0 (0.1)	10.7 (0.8)	490 (15)	180 (5)	12.6 (0.3)	≤2	1530 (20)	7 (1)	13.2 (0.3)	Na	P
JN_C	4.5 (0.5)	1800 (200)	2.6 (0.1)	17 (1)	280 (50)	263 (9)	103 (4)	≤2	1140 (40)	5.8 (0.8)	11.3 (0.3)	Na	Ca
LA_C	190 (10)	1800 (400)	4.0 (0.2)	183 (8)	580 (70)	380 (58)	62 (9)	≤2	1300 (100)	260 (10)	17 (2)	Na	Ca
PI_C	80 (10)	720 (10)	3.2 (0.2)	22 (3)	570 (70)	270 (10)	51.4 (0.1)	≤2	800 (100)	27 (8)	15.9 (0.3)	Na	P
Min	4.5 JN_C	600 AB_C	2.0 AB_C	10.7 AB_C	280 JN_C	180 AB_C	12.6 AB_C	-	800 PI_C	5.8 JN_C	11.3 JN_C		
Max	190 LA_C	1800 LA_C	4.0 LA_C	183 LA_C	580 LA_C	380 LA_C	103 JN_C	-	1530 AB_C	260 LA_C	17 LA_C		
Other trees													
AC_T	3.3 (0.9)	2000 (400)	7.6 (0.5)	20 (1)	700 (100)	640 (90)	62 (8)	≤2	1160 (30)	60 (10)	29 (2)	Na	Ca
AE_T	21 (2)	2530 (90)	11 (1)	30 (10)	1480 (20)	670 (11)	7.9 (0.7)	2.2 (0)	1100 (100)	50 (15)	17 (3)	Na	Ca
BE_T	31 (4)	2310 (30)	6.2 (0.1)	39 (1)	800 (100)	570 (10)	218 (9)	2.0 (0.1)	1420 (20)	210 (40)	62 (2)	Na	Ca
CR_T	21 (1)	3300 (100)	15 (1)	27.9 (0.1)	700 (100)	610 (50)	231 (7)	≤2	1330 (80)	200 (100)	28.8 (0.4)	Na	Ca
CS_T	49 (6)	2400 (200)	15 (1)	43 (3)	900 (200)	610 (30)	490 (20)	≤2	1700 (100)	50 (5)	33 (1)	Na	Ca
FI_T	17.3 (0.5)	5200 (600)	7.2 (0.6)	44 (1)	1350 (60)	980 (50)	9.1 (0.3)	2.7 (0.1)	543 (1)	56 (8)	12.0 (0.1)	Na	Ca

Table 2 (continued)

Plant	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	Zn	Min	Max
JG_T	19 (1)	5600 (400)	6.0 (0.3)	41 (2)	1800 (100)	700 (50)	26 (1)	3.0 (0.2)	1240 (60)	57 (5)	24 (2)	Na	Ca
Min	3.3 AC_T	2000 AC_T	6.0 JG_T	20 AC_T	700 CR_T	570 BE_T	7.9 AE_T	≤2	543 FI_T	50 CS_T	12.0 FI_T		
Max	49 CS_T	5600 JG_T	15 CS_T	44 FI_T	1800 JG_T	980 FI_T	490 CS_T	3.0 JG_T	1700 CS_T	210 BE_T	62 BE_T		
Shrubs													
CN_S	31 (3)	9700 (400)	6.8 (0.3)	40 (4)	820 (30)	590 (20)	2.6 (0.2)	2.9 (0.6)	900 (30)	112 (9)	37.2 (0.4)	Na	Ca
CY_S	11.1 (0.1)	5500 (500)	10.9 (0.5)	26 (2)	670 (60)	580 (40)	200 (10)	2.5 (0.7)	1050 (90)	86 (10)	10 (1)	Na	Ca
CT_S	8.4 (0.9)	4000 (500)	5.7 (0.4)	17 (1)	770 (77)	730 (30)	12.2 (0.3)	2.2 (0.3)	343 (8)	20 (10)	23 (1)	Na	Ca
VI_T	16 (1)	2430 (40)	12.2 (0.6)	28 (1)	1050 (40)	430 (10)	37 (1)	2.0 (0.1)	1370 (20)	31 (5)	35 (2)	Na	Ca
RI_S	22 (1)	3500 (200)	15 (1)	36 (2)	1000 (200)	730 (50)	12.6 (0.5)	2.7 (0.3)	2300 (200)	34 (2)	24 (1)	Na	Ca
RC_S	7.0 (1)	3790 (40)	6.9 (0.5)	32 (2)	770 (90)	820 (40)	20.5 (0.9)	2.4 (0.2)	1700 (200)	140 (20)	19 (1)	Na	Ca
Min	7.0 RC_S	2430 VI_S	5.7 CT_S	17 CT_S	670 CY_S	430 VI_S	2.6 CN_S	2.0 VI_T	343 CT_S	20 CT_S	19 RC_S		
Max	31 CN_S	9700 CN_S	15 RI_S	40 CN_S	1050 VI_S	820 RC_S	200 CY_S	2.9 CN_S	2300 RI_S	140 RC_S	37.2 CN_S		
Elements below LoQ	As	Cd	Cr	Hg	Pb	Se	Sn						
LoQ/mg/Kg	1.3	0.4	0.7	1.2	3	1.2	n.d.						

n.d.: not determined

Table 3.
Element average concentrations and estimates of standard deviation (in brackets) in residues of flowers and other plant materials, minimum and maximum values (mg/kg dw).

Plant	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	Zn	Min	Max
Flowers													
CL_Fl	230 (20)	13000 (400)	31.9 (0.2)	250 (30)	2720 (40)	1770 (60)	17 (1)	22.8 (0.7)	1360 (70)	240 (13)	112 (5)	Mn	Ca
CM_Fl	110 (3)	9940 (40)	29.5 (0.5)	172 (1)	7100 (100)	2840 (10)	53.9 (0.1)	12.9 (0.3)	3440 (100)	187 (9)	217 (3)	Na	Ca
CT_Fl	51.6 (0.8)	15200 (100)	10.6 (0.2)	70.6 (0.7)	1240 (10)	2357 (4)	57.6 (0.1)	11.0 (0.1)	1279 (9)	172 (8)	50.9 (0.2)	Na	Ca
HL_Fl	46.2 (0.4)	11000 (1000)	12.7 (0.1)	120 (4)	3680 (40)	2240 (30)	83 (1)	5.2 (0.1)	3000 (100)	70 (5)	93 (2)	Na	Ca
PA_Fl	57 (8)	8800 (200)	12 (1)	151 (6)	6100 (400)	2130 (10)	26 (1)	16 (1)	1600 (100)	123 (4)	90 (10)	Cu	Ca
TI_Fl	45 (4)	11000 (30)	13.4 (0.2)	78.5 (0.8)	3600 (200)	2500 (100)	540 (20)	6.0 (0.1)	1200 (40)	95 (5)	45.7 (0.9)	Na	Ca
Min	45 TI_Fl	8800 CMFl	10.6 CT_Fl	70.6 CT_Fl	1240 CT_Fl	1770 CL_Fl	17 CL_Fl	5.2 HL_Fl	1200 TI_Fl	70 HL_Fl	45.7 TI_Fl		
Max	230 CL_Fl	15200 CL_Fl	31.9 CL_Fl	250 CL_Fl	7100 CMFl	2840 CMFl	540 TI_Fl	22.8 CL_Fl	3440 CMFl	240 CL_Fl	217 CMFl		
Other-parts													
EC_L	88 (11)	24000 (1000)	9.6 (0.3)	121 (9)	3500 (400)	7950 (88)	64 (3)	7.7 (0.4)	1940 (60)	48 (2)	34 (2)	Na	Ca
EC_R	930 (70)	6400 (500)	33.5 (0.3)	1300 (100)	3500 (200)	4800 (100)	71 (3)	18 (1)	751 (7)	58 (3)	50 (3)	Na	Ca
PF_FL	21 (3)	19000 (400)	21.7 (0.1)	85 (6)	1900 (60)	1270 (50)	31.2 (0.4)	9.9 (0.3)	1100 (30)	44 (1)	370 (12)	Na	Ca

Table 3 (continued)

Plant	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	Zn	Min	Max
PI_L	143 (7)	9900 (300)	52.9 (0.6)	106 (1)	3000 (200)	1920 (50)	139 (5)	7.9 (0.1)	680 (30)	200 (30)	215 (4)	Na	Ca
RH_R	520 (30)	30800 (100)	23.0 (0.2)	640 (20)	2500 (200)	1350 (20)	17 (1)	9.3 (0.3)	263 (8)	150 (37)	13.5 (0.7)	Na	Ca
TA_W	98.9 (0.3)	10400 (300)	22 (1)	163 (5)	4800 (90)	2060 (30)	31 (1)	16 (1)	1340 (60)	146 (7)	130 (10)	Na	Ca
VM_Fr	37 (4)	2750 (80)	21 (1)	41 (1)	2200 (100)	810 (25)	18.6 (0.9)	6.0 (0.2)	1600 (100)	100 (4)	33.6 (0.9)	Na	Ca
Min	21 PFFL	2750 VMFr	9.6 EC_L	41 VMFr	1900 PFFL	810 VMFr	1 RH_R	6.0 VMFr	263 RH_R	44 PFFL	13.5 RH_R		
Max	930 EC_R	30800 EC_R	52.9 PI_L	1300 EC_R	3500 EC_R	7950 EC_R	139 PI_L	18 EC_R	1940 EC_R	200 PI_L	370 PFFL		
Elements below LoQ	As	Cd	Cr	Hg	Pb	Se	Sn						
LoQ/mg/Kg	1.3	0.4	0.7	1.2	3	1.2	n.d.						

n.d.: not determined

Table 4.
Element average concentrations and estimates of standard deviation (in brackets) in GM obtained from by extraction from gems, minimum and maximum values (mg/kg).

Plant	Ca	Fe	K	Mg	Mn	Na	P	Si	Min	Max
Conifers										
AB_C	≤ 5	≤ 0.5	115 (7)	8.4 (0.6)	≤ 0.1	≤ 2	28 (2)	≤ 4	-	K
JN_C	27 (4)	1.3 (0.9)	107 (6)	21 (1)	3.8 (0.1)	≤ 2	24.4 (0.1)	≤ 4	-	K
LA_C	11.8 (0.9)	≤ 0.5	120 (10)	16 (1)	1.3 (0.1)	≤ 2	35 (2)	≤ 4	-	K
PI_C	8 (1)	≤ 0.5	160 (20)	17.6 (0.5)	1.7 (0.1)	≤ 2	44 (1)	≤ 4	-	K
Min	≤ 5 AB_C	≤ 0.5	107 JN_C	8.4 AB_C	≤ 0.1 AB_C	-	24.4 JN_C	-		
Max	27 JN_C	1.3 JN_C	160 PI_C	21 JN_C	3.8 JN_C	.	44 PI_C	-		
Other trees										
AC_T	≤ 5	0.73 (0.08)	203 (1)	16.9 (0.2)	0.76 (0.01)	≤ 2	87.5 (0.6)	≤ 4	-	K
AE_T	15 (2)	≤ 0.5	110 (8)	20 (2)	0.39 (0.03)	≤ 2	19 (2)	≤ 4	-	K
BE_T	7 (2)	≤ 0.5	105 (6)	12.2 (0.3)	1.80 (0.01)	≤ 2	37 (1)	≤ 4	-	K
CR_T	21 (3)	≤ 0.5	101 (1)	20.3 (0.5)	3.1 (0.1)	≤ 2	30.3 (0.9)	≤ 4	-	K
CS_T	6.2 (0.2)	0.8 (0.2)	136 (2)	24.2 (0.2)	8.90 (0.06)	≤ 2	46.0 (0.3)	≤ 4	-	K

Table 4 (continued)

Plant	Ca	Fe	K	Mg	Mn	Na	P	Si	Min	Max
FI_T	36 (2)	≤ 0.5	330 (13)	38 (1)	≤ 0.1	≤ 2	85 (1)	7 (2)	-	K
JG_T	≤ 5	≤ 0.5	260 (20)	30 (2)	0.34 (0.07)	2.6 (0.1)	68 (4)	≤ 4	-	K
Min	≤ 5	-≤ 0.5	101 CR_T	12.2 BE_T	≤ 0.1 FI_T	≤ 2	19 AE_T	≤ 4		
Max	36 FI_T	0.8 FI_T	330 FI_T	38 FI_T	8.90 CS_T	2.6 JG_T	87.5 AC_T	7 FI_T		
Shrubs										
CN_S	144 (7)	≤ 0.5	128 (2)	30.9 (0.6)	≤ 0.1	≤ 2	23.3 (0.9)	≤ 4		K
CY_S	28 (7)	≤ 0.5	100 (10)	23 (3)	3.3 (0.4)	≤ 2	27 (4)	≤ 4		K
CT_S	14.4 (0.3)	≤ 0.5	100 (10)	17.6 (0.3)	≤ 0.1	≤ 2	14.6 (0.9)	≤ 4		K
VI_S	16.2 (0.1)	≤ 0.5	189 (8)	11.7 (0.8)	0.41 (0.03)	≤ 2	49 (2)	≤ 4		K
RI_S	8 (1)	≤ 0.5	169 (9)	13.8 (0.7)	≤ 0.1	≤ 2	48 (2)	≤ 4		K
RC_S	25.0 (0.3)	1.1 (0.5)	180 (10)	25 (2)	0.26 (0.01)	2.3 (0.7)	72 (5)	5 (3)		K

Table 4 (continued)

Plant	Ca	Fe	K	Mg	Mn	Na	P	Si	Min	Max
Min	8 RI_S	≤ 0.5	100	11.7 VI_S	≤ 0.1	≤ 2	14.6 CT_S	≤ 4		
Max	144 CN_S	1.1 RC_S	189 VI_S	30.9 CN_S	3.3 CY_S	2.3 RC_S	72 RC_S	5 RC_S		
Elements below LoQ	Al	As	Cd	Cr	Cu	Hg	Pb	Se	Sn	Zn
LoQ/mg/Kg	0.5	1.3	0.4	0.7	0.5	1.2	3	1.2	n.d.	0.8

n.d.: not determined

Table 5.
Element average concentrations and estimates of standard deviation (in brackets) in MTs obtained by extraction from flowers and other plant materials minimum and maximum values (mg/kg).

Plant	Al	Ca	Fe	K	Mg	Mn	Na	P	Si	Min	Max
Flowers											
CL_Fl	≤ 0.5	9.0 (3)	≤ 0.5	80.9 (0.5)	8.6 (0.3)	≤0.1	4.1 (0.6)	17.2 (0.1)	≤4	-	K
CM_Fl	≤ 0.5	7.1 (0.3)	≤ 0.5	177 (8)	19.5 (0.7)	≤0.1	2.6 (0.1)	50 (4)	≤4	-	K
CT_Fl	1.4 (0.6)	13 (1)	≤ 0.5	110 (1)	19.6 (0.1)	0.36 (0.01)	2.0 (0.3)	18 (1)	≤4	-	K
HL_Fl	≤ 0.5	16.6 (0.8)	≤ 0.5	174 (2)	22.4 (0.5)	0.18 (0.01)	2.0 (0.1)	28.1 (0.6)	7.4 (0.3)	-	K
PA_Fl	1.0 (0.5)	≤ 5	≤ 0.5	152 (1)	13.1 (0.1)	≤0.1	2.9 (0.1)	27 (1)	≤4	-	K
TI_Fl	≤ 0.5	16.3 (4)	≤ 0.5	111 (3)	19.8 (0.9)	2.2 (0.1)	3 (0.4)	19.0 (0.2)	≤4	-	K
Min	≤ 0.5	≤ 5 PA_Fl	-	80.9 CL_Fl	8.6 CL_Fl	≤0.1	2.0	17.2 CL_Fl	≤4		
Max	1.4 CT_Fl	16.6 HL_Fl		177 CM_Fl	22.4 HL_Fl	2.2 TI_Fl	4.1 CL_Fl	50 CM_Fl	7.4 HL_Fl		
Other parts											
EC_LR	0.51 (0.30)	28 (4)	≤ 0.5	176 (7)	177 (7)	0.21 (0.06)	5.3 (0.5)	35.1 (0.3)	7 (1)	-	K
PF_FL	≤ 0.5	18 (6)	≤ 0.5	100 (40)	7 (2)	0.2 (0.1)	2.8 (0.4)	16 (4)	≤4	-	K
PI_L	≤ 0.5	20 (8)	≤ 0.5	100 (30)	12 (2)	0.23 (0.09)	2.9 (0.3)	17 (3)	≤4	-	K

Table 5 (continued)

Plant	Al	Ca	Fe	K	Mg	Mn	Na	P	Si	Min	Max
RH_R	≤ 0.5	18 (2)	0.58 (0.02)	112 (4)	32.4 (0.8)	0.23 (0.07)	2.0 (0.1)	10.2 (0.2)	≤4	-	K
TA_W	≤ 0.5	32 (2)	≤ 0.5	163 (1)	15.2 (0.3)	≤0.1	6.0 (1)	20.1 (0.9)	≤4	-	K
VM_Fr	≤ 0.5	7.5 (0.6)	≤ 0.5	65 (3)	6.0 (0.1)	≤0.1	2.3 (0.7)	9.3 (0.2)	≤4	-	K
Min	≤ 0.5	7.5	≤ 0.5	65 VM_Fr	6.0 VM_Fr	≤0.1	2.0 RH_R	9.3 TA_W	≤4		
Max	0.51 EC_LR	32 TA_W	0.58 RH_R	176 EC_LR	177 EC_LR	0.23	6.0 TA_W	35.1 EC_LR	7 EC_LR		
Elements below LoQ	As	Cd	Cr	Cu	Hg	Pb	Se	Sn	Zn		
LoQ/mg/Kg	1.3	0.4	0.7	0.5	1.2	3	1.2	n.d.	0.8		

n.d.: not determined

Table 6.
Percentages of extraction of elements from buds.

Plant	Ca	K	Mg	Mn	P
Conifers					
AB_C	n.d.	82.4	48.3	n.d.	26.8
JN_C	23.1	88.4	61.5	42.5	30.0
LA_C	11.6	80.5	45.7	29.5	35.0
PI_C	18.2	84.9	56.6	39.8	52.4
Other trees					
AC_T		85.3	34.6	19.7	60.1
AE_T	10.6	59.8	37.4	49.7	25.7
BE_T	5.7	72.4	30.0	14.2	34.3
CR_T	11.3	77.1	40.0	21.2	31.3
CS_T	4.9	75.1	44.2	26.6	35.1
FI_T	12.2	83	43.9	n.d.	75.8
JG_T	n.d.	75.4	46.2	n.d.	52.3
Shrubs					
CN_S	22.9	75.7	51.2	n.d.	34.1
CY_S	9.2	74.9	44.2	24.8	34.0
CT_S	6.7	72.2	32.5	n.d.	46.0
VI_S	11.8	78.3	35.2	18.1	41.7
RI_S	4.4	77.2	27.4	n.d.	29.4
RC_S	11.7	82.4	37.9	20.2	45.9

n.d.: not determined

Table 7.
Percentages of extraction of elements from flowers and other plant materials.

Plant	Ca	K	Mg	Mn	Na	P
Flowers						
CL_Fl	0.7	22.9	4.6	n.d.	64.3	11.2
CM_Fl	0.7	20	6.4	n.d.	66.8	12.7
CT_Fl	0.8	47	7.7	5.9	64.5	12.3
HL_Fl	1.5	32.1	9.1	2.1	79.4	8.6
PA_Fl	n.d.	19.9	5.8	n.d.	64.4	14.4
TI_Fl	1.5	23.6	7.3	3.9	83.3	13.7
Other parts						
EC_LR	1.2	34.1	18.2	3.2	87.3	15.3
PF_FL	2.7	22.2	1.4	2.7	60.9	17.6
PI_L	1.0	34.5	8.6	6.9	74.6	13.4
RH_R	1.8	27.2	14.4	1.6	71.7	13
TA_W	1.0	39.5	10.1	n.d.	86.6	43.3
VM_Fr	0.7	11.9	2.8	n.d.	59	6.5

n.d.: not determined

Table 8.

Element average concentrations and estimates of standard deviation (in brackets) in solvents and filters used for the preparation of GMs and MTs (mg/Kg). The elements not reported in the table are below the LoQ.

	Al	Ca	Fe	K	Mg	Na	P	Si	Zn
Solvent for GM	≤0.5	2.6 (0.1)	≤0.5	≤20	2.06 (0.04)	≤2	≤3	22 (2)	3.2 (0.1)
Solvents for MT	≤0.5	4.3 (0.2)	≤0.5	≤20	2.35 (0.04)	≤2	≤3	0.18 (0.01)	1.4 (0.1)
Filters	6.6 (0.1)	25.3 (0.1)	5.8 (0.2)	24.5 (0.1)	19.1 (0.1)	1468 (1)	8.3 (0.1)	16.9 (0.1)	18.9 (0.7)

Table 9.

Comparison between concentrations in GMs and MTs and admissible intake levels. Dosages: 100 drops/day for GMs; 60 drops/day for MT. 20 drops = 1 ml. bw = body weight (60 Kg). d = day; w = week; m = month. Highest: the dosage for the GM and MT sample with the highest concentration is reported

Element	JECFA limit	Limit name[a]	EFSA limit	Limit name[a]	ATDSR	Limit name[a]	GM Highest	MT Highest
Al	1 mg/kg bw/w 8.6 mg/d	PWTI	1 mg/kg bw/w 8.6 mg/d	TWI	1 mg/kg bw/d 60 mg/d	MRL	2.58×10^{-3} mg/d	1.58×10^{-3} mg/d
As	2.1 μg/kg bw/d[b] 0.126 mg/d	PTMDI	0,3-8 μg/kg bw/d 0.018-0.48 mg/d	BMDL	0.003/0.005 mg/kg bw/d [c] 0.18-0.30 mg/d	MRL	6.70×10^{-3} mg/d	3.64×10^{-3} mg/d
Cd	25 μg/kg bw/m 0.05 mg/d	PTMI	2,5 μg/kg bw/w 0.021 mg/d	TWI	$1\times10^{-4}/5\times10^{-4}$ mg/kg bw/d [c] $6\times10^{-3}/3\times10^{-2}$ mg/d	MRL	2.06×10^{-3} mg/d	1.12×10^{-3} mg/d
Cr	-		0,3 mg/kg bw/d 18 mg/d	TDI	$9\times10^{-4}/5\times10^{-3}$ mg/kg bw/d $5.4\times10^{-2}/3\times10^{-1}$ mg/d	MRL	3.60×10^{-3} mg/d	1.96×10^{-3} mg/d
Cu	0.5 mg/kg bw/d 30 mg/d	PMTDI	5 mg/d	UL	0.01 mg/kg bw/d 0.6 mg/d	MRL	2.58×10^{-3} mg/d	1.40×10^{-3} mg/d
Fe	0.8 mg/kg bw/d 48 mg/d	PMTDI	10 mg/d	UL	-		2.58×10^{-3} mg/d	1.40×10^{-3} mg/d
Hg	0.004 mg/kg bw/w 0.034 mg/d	PTWI	0,004 mg/kg bw/w 0.034 mg/d	PTWI	0.007/0.002/0.0003 mg/kg bw/d 0.42/0.12/0.018	MRL	6.18×10^{-3} mg/d	3.36×10^{-3} mg/d
Mn	-		0,05 mg/kg bw/d 3 mg/d	TDI			1.03×10^{-3} mg/d	5.60×10^{-4} mg/d

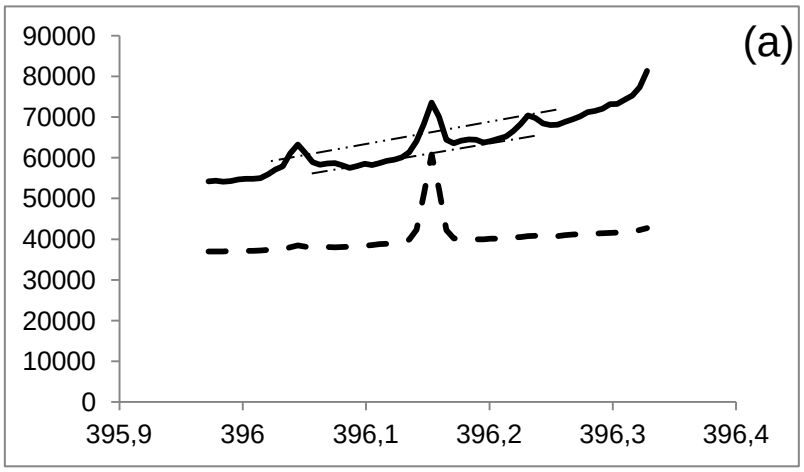
Table 9 (continued)

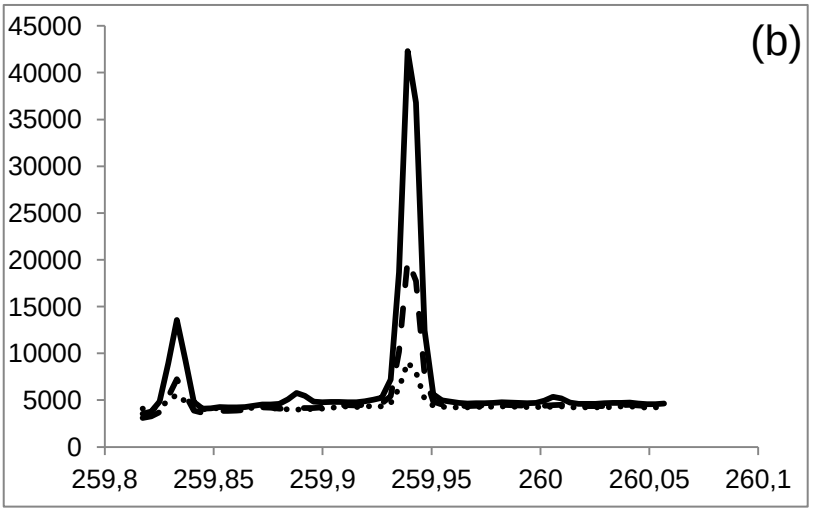
Element	JECFA limit	Limit name[a]	EFSA limit	Limit name[a]	ATDSR	Limit name[a]	GM Highest	MT Highest
Pb	0.025 mg/kg bw/w[b] 0.21 mg/d	PWTI	0,025 mg/kg bw/w[b] 0.21 mg/d	PWTI	-		1.54×10^{-2} mg/d	8.39×10^{-3} mg/d
Zn	0.3-1 mg/kg bw/d 18-60 mg/d	PMTDI	25 mg/d	UL	0.3 mg/kg bw/d 18 mg/d	MRL	4.12×10^{-3} mg/d	2.24×10^{-3} mg/d

[a] ADI: Admissible Daily Intake; $BMDL_{01}$: Benchmark Dose Lower Confidence Limit; MRL: Maximum Residue Limit; PMTDI: Provisional Maximum Tolerable Daily Intake; PMTI; Provisional Tolerable Monthly Intake; PTWI: Provisional Tolerable Weekly Intake; TDI: Tolerable Daily Intake, TWI: Tolerable Weekly Intake; UL: Upper Level

[b]this value has been now withdrawn

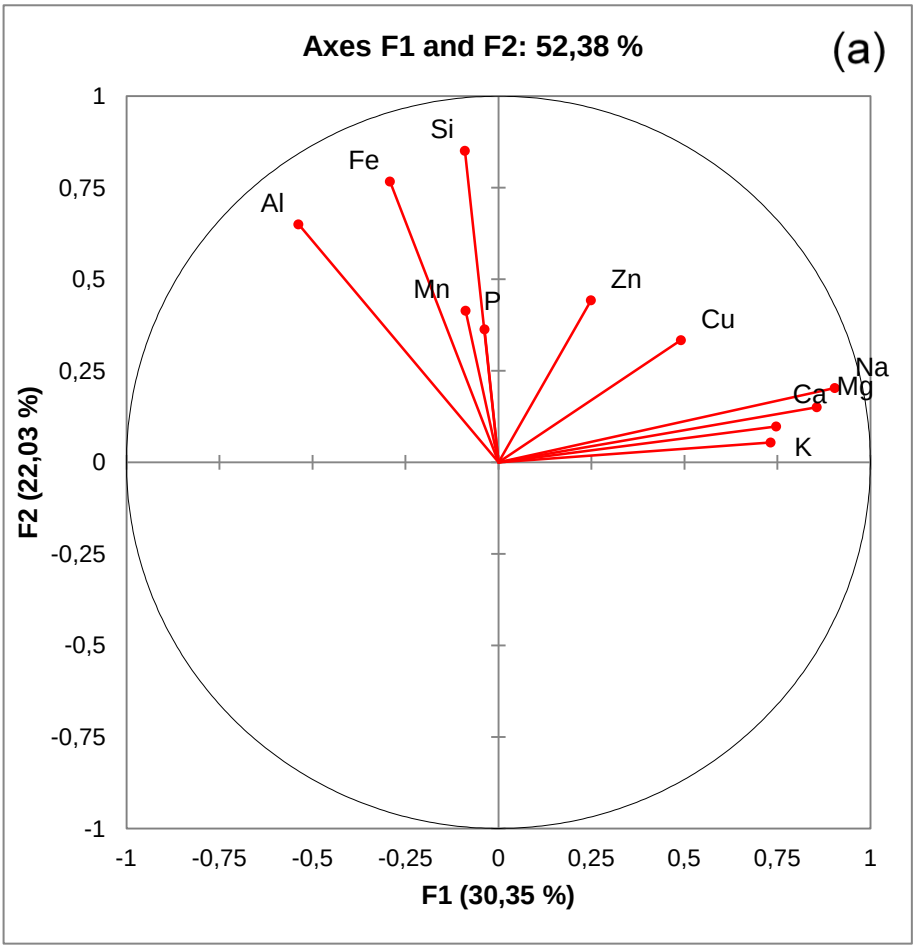
[c]For As, 0.003: limit for chronic exposition; 0.005: limit for acute exposition. For Cd, 1×10^{-4}: limit for chronic exposition; 5×10^{-4}: limit for intermediate exposition. For Cr, 9×10^{-4}: limit for chronic exposition; 5×10^{-3}: limit for intermediate exposition. For Hg: 0.007: limit for chronic exposition; 0.002: imit for intermediate exposition; 0.0003: limit for acute exposition.


(a)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
395,9
396
396,1
396,2
396,3
396,4


(b)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
259,8
259,85
259,9
259,95
260
260,05
260,1


(c)
500
450
400
350
300
250
200
150
100
50
0
195,9
195,95
196
196,05
196,1
196,15

Fig. 1


Axes F1 and F2: 52,38 %
(a)
Si
Fe
Al
Mn
P
Zn
Cu
Na
Mg
Ca
K
F2 (22,03 %)
F1 (30,35 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1
-1
-0,75
-0,5
-0,25
0
0,25
0,5
0,75
1

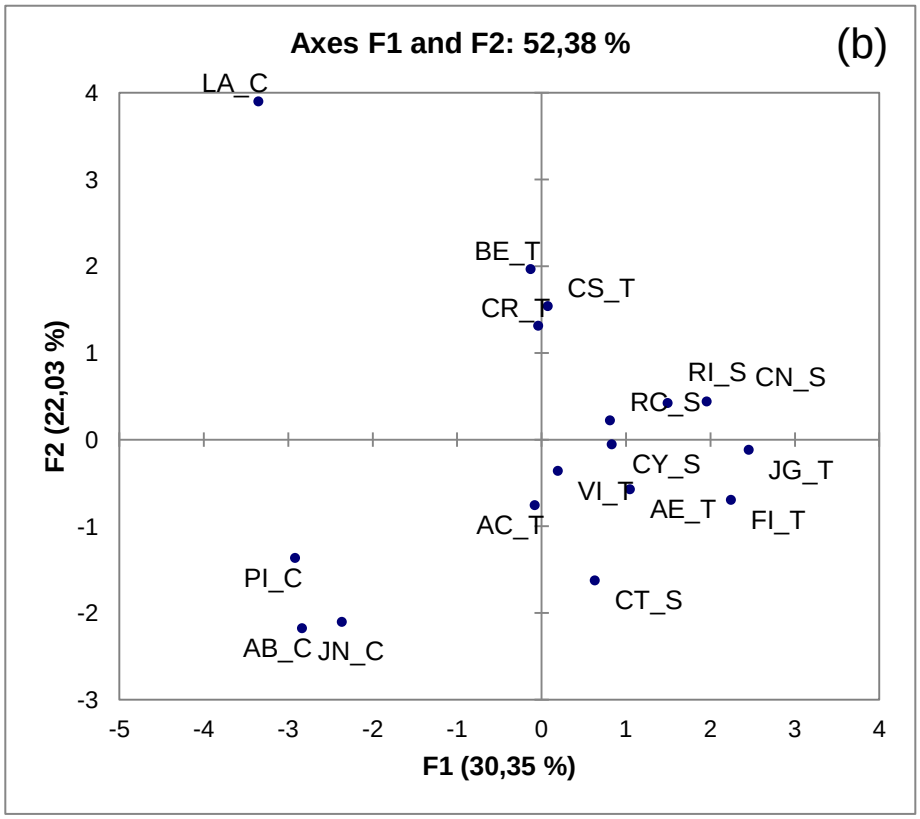

Axes F1 and F2: 52,38 %
(b)
LA_C
BE_T
CS_T
CR_T
RI_S
CN_S
RG_S
CY_S
JG_T
VI_T
AE_T
FI_T
AC_T
PI_C
CT_S
AB_C
JN_C
F2 (22,03 %)
F1 (30,35 %)
4
3
2
1
0
-1
-2
-3
-5
-4
-3
-2
-1
0
1
2
3
4

Fig. 2

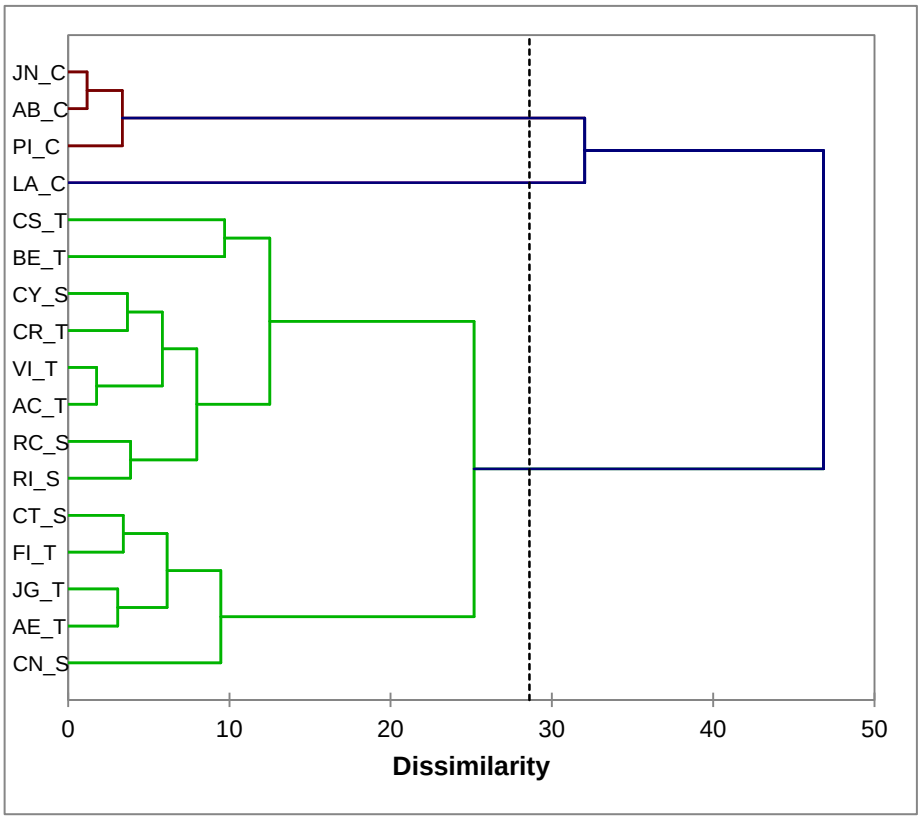
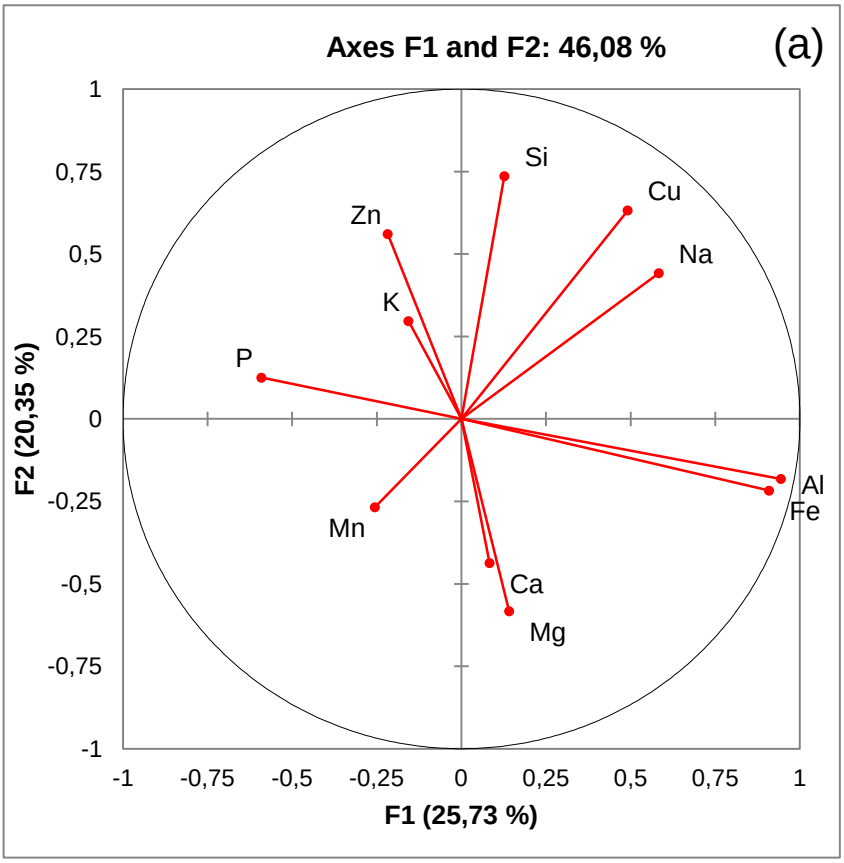

JN_C
AB_C
PI_C
LA_C
CS_T
BE_T
CY_S
CR_T
VI_T
AC_T
RC_S
RI_S
CT_S
FI_T
JG_T
AE_T
CN_S
0
10
20
30
40
50
Dissimilarity

Fig. 3


Axes F1 and F2: 46,08 %
(a)
Si
Cu
Zn
Na
K
P
Al
Fe
Mn
Ca
Mg
F2 (20,35 %)
F1 (25,73 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1
-1
-0,75
-0,5
-0,25
0
0,25
0,5
0,75
1

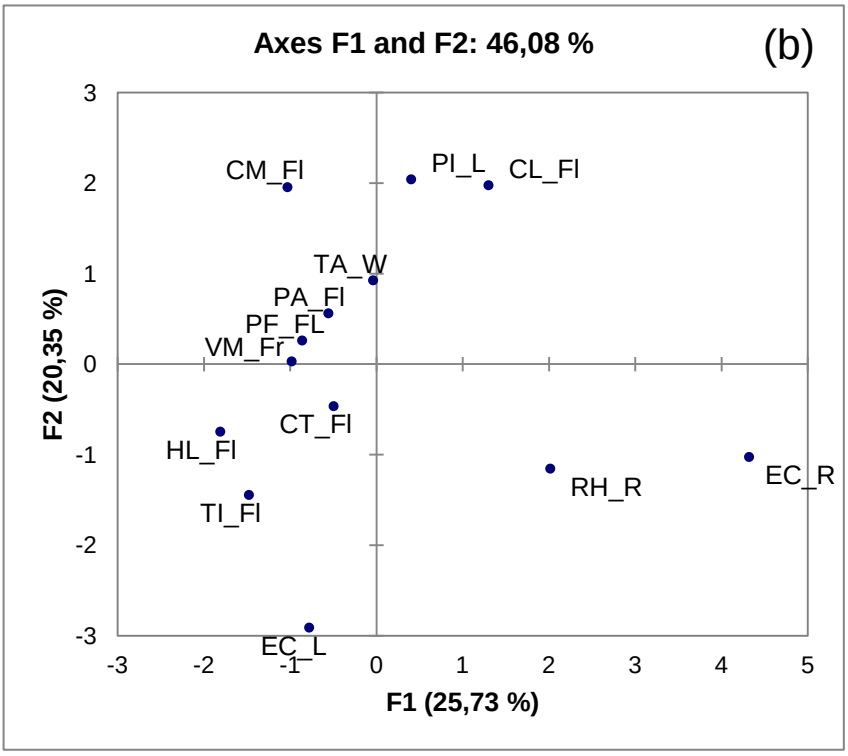

Axes F1 and F2: 46,08 %
(b)
CM_Fl
Pl_L
CL_Fl
TA_W
PA_Fl
PF_FL
VM_Fr
CT_Fl
HL_Fl
RH_R
EC_R
TI_Fl
EC_L
F2 (20,35 %)
F1 (25,73 %)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
5

Fig. 4

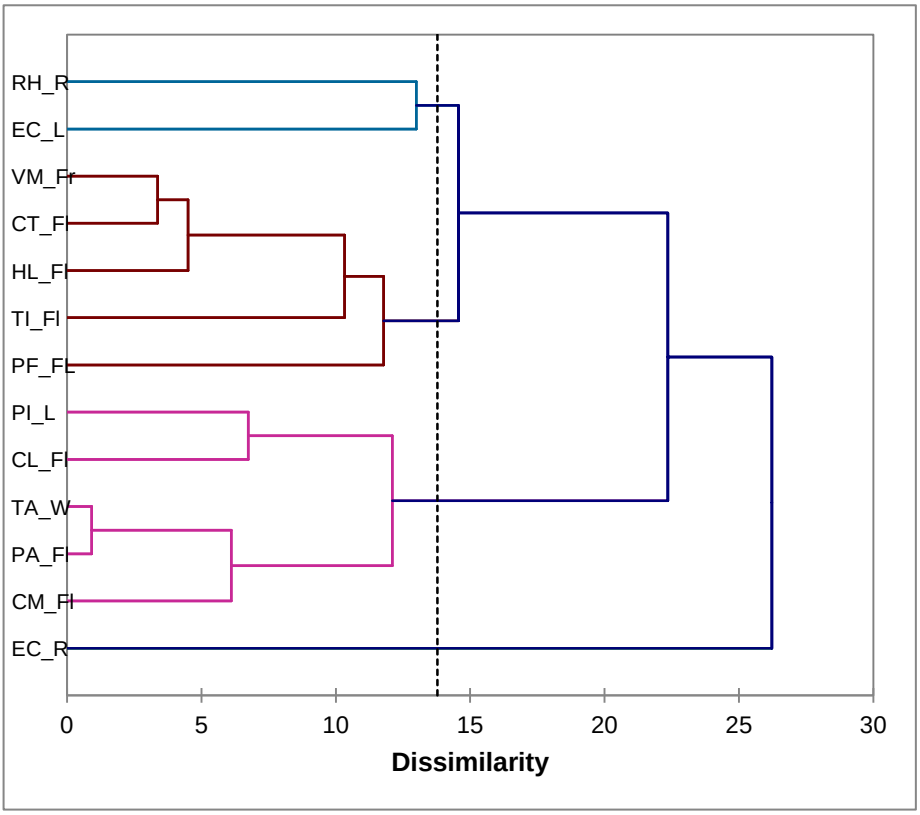
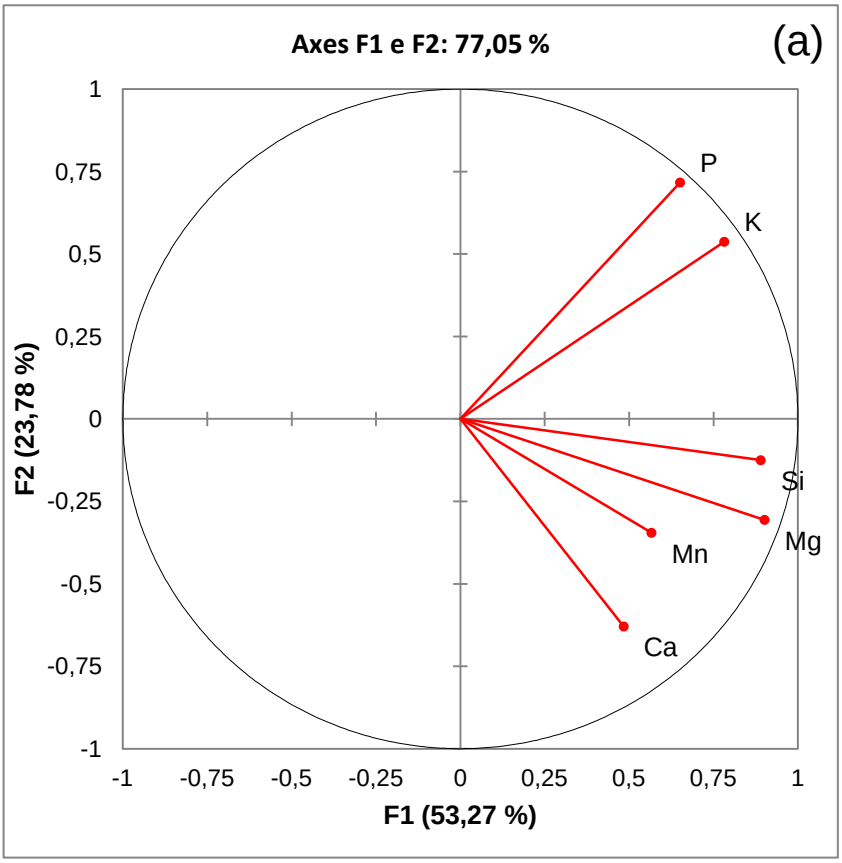

RH_R
EC_L
VM_Fr
CT_Fl
HL_Fl
TI_Fl
PF_FL
PI_L
CL_Fl
TA_W
PA_Fl
CM_Fl
EC_R
0
5
10
15
20
25
30
Dissimilarity

Fig. 5


Axes F1 e F2: 77,05 %
(a)
P
K
Si
Mg
Mn
Ca
F2 (23,78 %)
F1 (53,27 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1
-1
-0,75
-0,5
-0,25
0
0,25
0,5
0,75
1

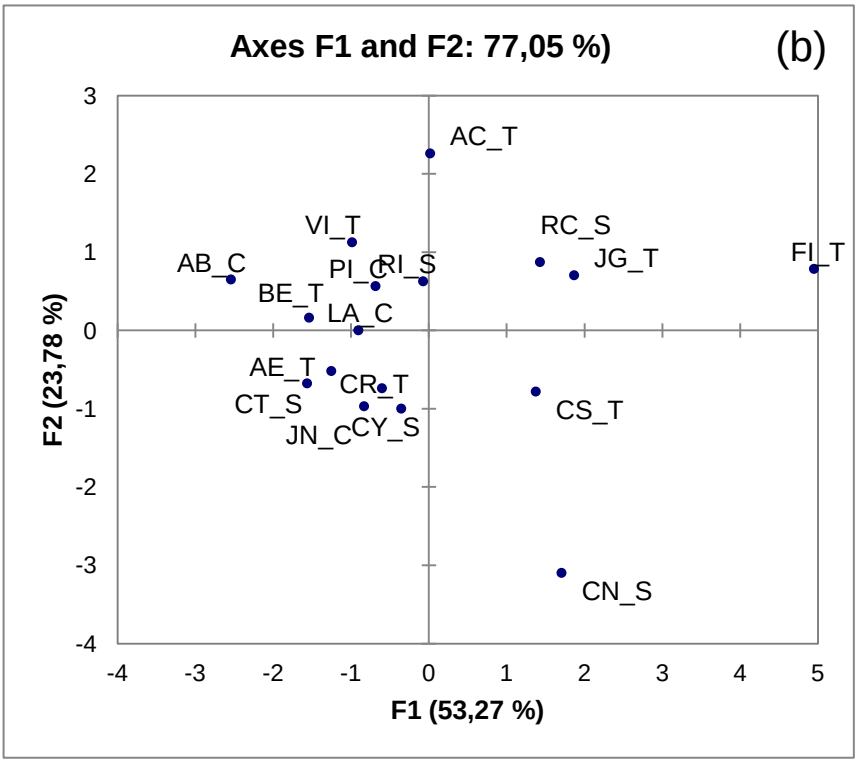

Axes F1 and F2: 77,05 %)
(b)
AC_T
VI_T
RC_S
AB_C
PI_C
RI_S
JG_T
FI_T
BE_T
LA_C
AE_T
CR_T
CT_S
CS_T
JN_C
CY_S
CN_S
F2 (23,78 %)
F1 (53,27 %)
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4
5

Fig. 6

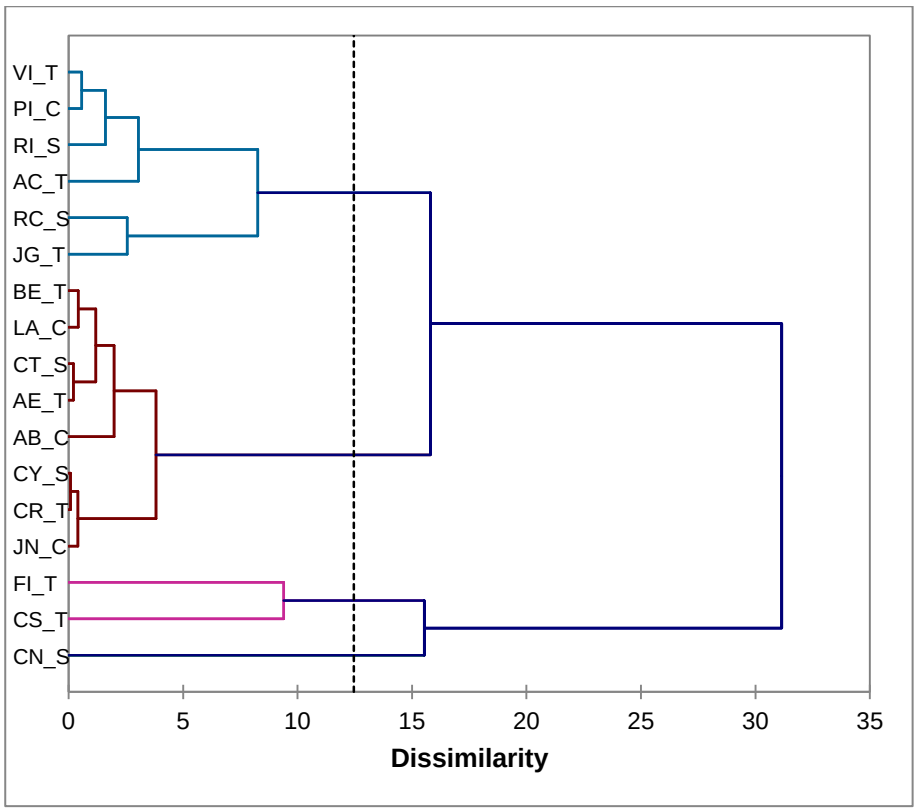
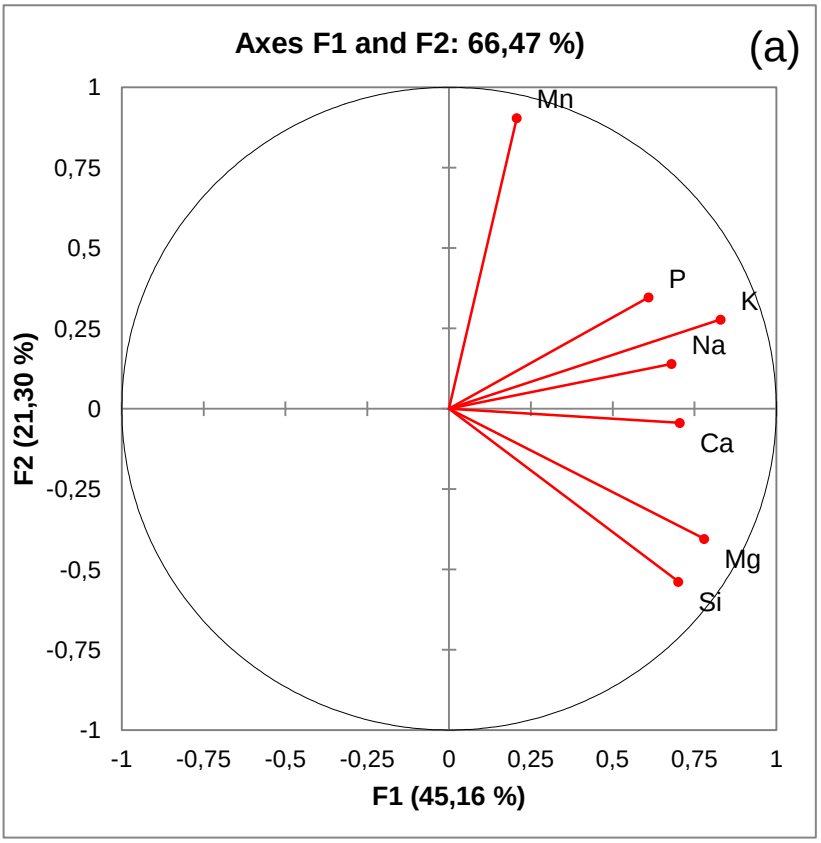

VI_T
PI_C
RI_S
AC_T
RC_S
JG_T
BE_T
LA_C
CT_S
AE_T
AB_C
CY_S
CR_T
JN_C
FI_T
CS_T
CN_S
0
5
10
15
20
25
30
35
Dissimilarity

Fig. 7


Axes F1 and F2: 66,47 %)
(a)
Mn
P
K
Na
Ca
Mg
Si
F2 (21,30 %)
F1 (45,16 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1
-1
-0,75
-0,5
-0,25
0
0,25
0,5
0,75
1

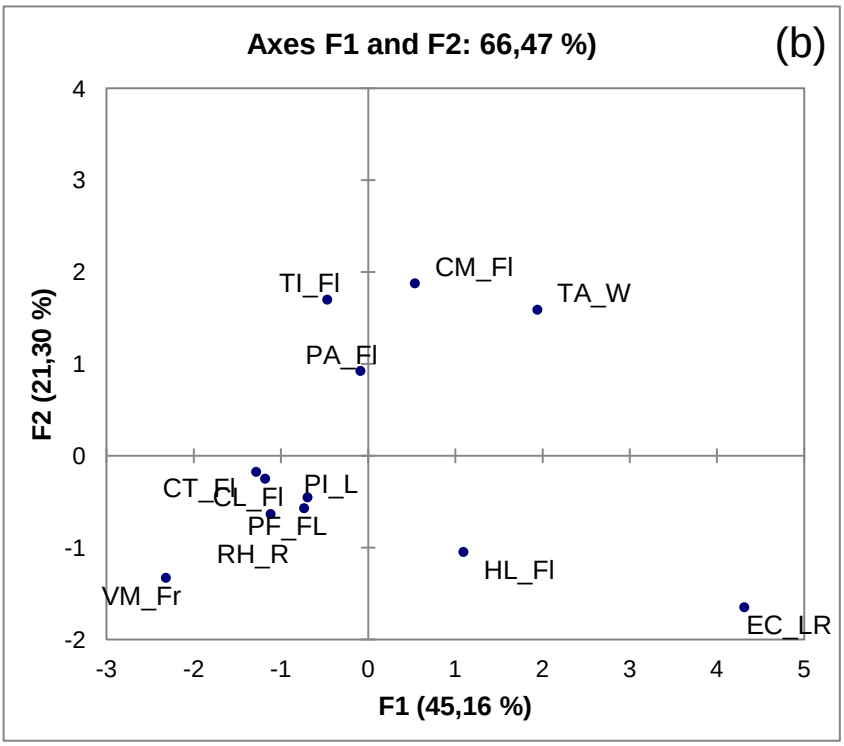

Axes F1 and F2: 66,47 %)
(b)
TI_FI
CM_FI
TA_W
PA_FI
CT_FI
CL_FI
PI_L
PF_FL
RH_R
HL_FI
VM_Fr
EC_LR
F2 (21,30 %)
F1 (45,16 %)
4
3
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
4
5

Fig. 8