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Determination and assessment of the contents of essential and potentially toxic elements in Ayurvedic medicine formulations by inductively coupled plasma-optical emission spectrometry

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Abstract

Traditional Ayurvedic remedies are easily available nowadays not only in India, their country of origin, but also in Western countries. Some of these products contain high concentrations of potentially toxic elements as main or secondary ingredients, in addition to elements essential for human health; for these reasons, it is interesting to determine their elemental composition. In this study we assessed the concentrations of fifteen elements (Al, As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Si and Zn) in five products of the Parpati family, a group of Ayurvedic medicines containing high concentrations of mercury, manufactured in various places of India. Concentrations were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) or (for Pb and Cd) by graphite furnace atomic absorption spectrometry (GF-AAS) after sample mineralization. We compared calculated daily intake of each element with reference values, considering maximum tolerable intake levels or recommended nutrient amounts. The experimental results were treated with chemometric pattern recognition techniques. We found differences in the composition of products of the same denomination manufactured by different companies and strong correlations among

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groups of variables. As expected, the daily intake of mercury upon consumption of Parpati medicines largely exceeded the tolerable intake level of this element.

Keywords: Ayurvedic medicines; Parpati; Potentially toxic elements; Metals; Chemometrics

1. Introduction

Ayurvedic medicine originated in India several thousand years ago [1-3]; it is extensively used nowadays in this country and is becoming increasingly popular in Western nations: Ayurvedic formulation are easily available from ethnic markets, medical practitioners, health food stores, and the Internet [4,5]. Generally, Ayurvedic practice involves the use of medications that typically contain herbs, metals, minerals, or other materials [2,3,6]. Ayurvedic practitioners usually make up their own medicines, but several companies manufacture and sell such formulations for the Indian market and/or other countries. Ayurvedic remedies can be classified into two groups, namely Kasthausadhi, which are mainly based on plants, and Rasausadhi, which primarily contain metals (and/or arsenic) and minerals.

Although such remedies can have health benefits, there are numerous reports of significant adverse effects due to some of their components. For example, Dargan et al [7] have recently reported the risks of heavy metals poisoning associated with the use of Ayurvedic medicines. Several literature reports have also demonstrated lead poisoning from these formulations [8-12] or the presence of significantly high levels of lead, mercury and arsenic [13-14]. The effects of elements like Cd, Hg and Pb on humans is well known; these elements have no known biological function in human body and are simply tolerated at low levels, but become toxic above certain concentrations. Other elements, such as Cr, Cu, Fe, Mn, Zn and surprisingly As, are essential to human life at adequate levels, but they can have

negative effects if their concentrations exceed certain threshold limits [15]. Hence it is very interesting to determine the element content in traditional Ayurvedic medicines, taking into account their role as nutrient and/or toxins. In this study we assessed the levels of fifteen elements (Al, As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Si and Zn) in a family of commercial Ayurvedic medicines commonly used in India, namely the Parpati group, belonging to the Rasausadhi class. The term Parpati means "flake". Parpati formulations are obtained by pouring molten metals over leaves of plants. Concentrations were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) or (for Pb and Cd) by graphite furnace atomic absorption spectrometry (GF-AAS) after sample mineralization in a microwave oven. We examined two Rasa Parpati and three Panchamrit Parpati products manufactured and purchased in various places in India and compared the estimated daily intake of each element with reference values, considering maximum tolerable intake levels or recommended nutrient amounts issued by internationally recognized organizations. Finally, we treated the data with chemometric pattern recognition techniques, in order to obtain a visual representation of the composition of the different products and find out similarities and differences among samples and correlations among variables, i.e. the investigated elements, which might be more difficult to detect just from the dataset.

2. Material and methods

2.1. General method of preparation and uses of Parpati family remedies

Rasa Parpati formulations are based on mercury and sulphur, previously "purified", i.e. treated according to a traditional procedure. First a black powder (Kajjali) is obtained by grinding the two elements, then other grinded drugs are added. The powder is then heated in iron vessels and melted. This melted material is purified as per Ayurvedic method, cooled

and again flakes of the product are powdered. Rasa Parpati formulations are mainly used to treat gastro-intestinal diseases, anemia and alopecia.

Panchamrit Parpati is made of five components (Pancha means “five” and Amrita represents a nectar which gives immortality, like the Greek ambrosia); in addition to mercury, it contains iron (lauth), mica (abhrak), copper (amra) and gold (svarna). It is used as an intestinal antiseptic.

2.2. Pre-treatment of the samples

The Ayurvedic medicinal products analyzed were purchased from big Ayurvedic manufacturing companies or small local firms in India. The names of the products, their manufacturers and types of formulation are summarized in Table 1. The purchased samples originally appeared as a black power or solid. Thus, they were grinded in a mortar in order to obtain a powdered sample with a uniform colour.

2.3. Apparatus and reagents

Element concentrations were determined with: i) a Varian Liberty 100 inductively coupled plasma-optical emission spectrometer (ICP-OES) equipped with a Czerny-Turner monochromator, a Sturman-Masters spray chamber, a V-groove nebuliser and a radio frequency (RF) generator or with ii) a Perkin Elmer 5100 graphite furnace absorption spectrometer (GF-AAS) equipped with a Zeeman-effect background correction and an autosampler. A Milestone MLS-1200 Mega microwave laboratory unit was used for the dissolution of the samples. High purity water (HPW) produced with a Millipore Milli-Q system was used throughout. All the reagents used in this study were of analytical grade. The standard metal solutions were prepared from concentrated stock solutions (Merck Titrisol).

2.4. Procedures

2.4.1. Dissolution and analysis

Sample treatment and analysis were carried out in triplicate. Acid digestion in a microwave oven was adopted as sample dissolution procedure. Aliquots of 200 mg were treated with 5 ml of concentrated nitric acid in polytetrafluoroethylene (PTFE) bombs. Four heating steps of 5 min each (at a power of 250, 400, 600 and 250 W respectively), followed by a ventilation step of 25 min, were applied. Finally, the resulting solutions were diluted to 25 or 50 ml with HPW. The solutions were directly employed for ICP-OES (Al, Ca, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Si and Zn) or GF-AAS (As, Cd and Pb) analysis, depending on the analyte concentration level. In both cases calibrations were performed with standard solutions prepared in aliquots of sample blanks. The accuracy of the procedure was verified by analysis of a standard reference material (NIST SRM 1573a, tomato leaves).

2.4.2. Chemometric data processing

A chemometrics analysis of the experimental results was performed by principal component analysis (PCA) and hierarchical cluster analysis (HCA), with the aid of XLStat 4.4 software package, used as a Microsoft Excel plug-in. For the principles of the two techniques, the reader is referred to existing textbooks on chemometrics [e.g. 16,17]**.** The data were preprocessed by column standardization, i.e. by subtracting the mean (for a variable) from each value and dividing by the corresponding standard deviation. For HCA, the Euclidean distance and Ward's agglomeration method were used.

3. Results and discussion

3.1. Comparison of element contents in Parpati samples

Element concentrations in the investigated samples are reported in Table 2. Most analytes, including mercury, were determined by ICP-OES. Trace levels of Hg are commonly

determined by cold vapour atomic absorption spectrometry (CV-AAS), but the high concentrations present in Parpati samples enabled us to perform the analysis by ICP-OES: this technique had the advantage of being multi-elementary, hence it provides the concentrations of the analytes of interest in a single run. ICP-OES was used by other researchers for the analysis of Ayurvedic medicines or their components [18-20] as well as for the analysis of other Asian drugs [21,22]. The concentrations of lead and cadmium were close to or lower than the detection limits for emission spectrometry, so we determined them by GF-AAS: this technique is slower, but more sensitive than ICP-OES. Atomic absorption spectrometry was used for the analysis of Ayurvedic products or their components also in other studies [23-24]. Such products were also analyzed by voltammetry [25], instrumental neutron activation analysis (INAA) [26], X-Ray fluorescence (XRF) spectroscopy [5,13,27] or inductively coupled plasma mass spectrometry (ICP-MS) [28]. In particular, XRF has the advantage of being non-destructive, but it cannot be applied to elements present at trace levels; on the other hand, ICP-MS has an extremely high sensitivity, but it requires a very expensive instrumentation.

The most outstanding characteristic is obviously the high concentration of mercury, which is higher than 10 g/kg in all samples. The two Rasa Parpati samples have different compositions. Sample 1, manufactured by a big company, has higher concentrations of As, Ca, Cd, Fe, Mg and Si than sample 2, which was produced by a small local company and has higher concentrations of the other elements. In particular, the content of Hg, the main constituent of the product, in sample 2 is more than twice as much as in sample 1. Large differences are also present among the three Panchamrit Parpati products. In this case, the lowest concentration of mercury is found in sample 5, manufactured by a small local company. There is not a common trend in element contents, but in general sample 5 has higher concentrations of several analytes than the other two samples. Sample 3 has a

remarkably high concentration of arsenic, whereas the levels of Cd, Cr and Pb are distinctly lower than those present in samples 4 and 5.

Clear differences are observable between Rasa and Panchamrit Parpati products. The former have distinctly lower concentrations of most elements. The high levels of Cu, Fe, Al, K and Na in Panchamrit Parpati remedies are not unexpected and derive from its main constituents (see section 2.1).

3.2. Daily intake and reference values

The daily intake of each investigated element upon consumption of Ayurvedic medicines was calculated taking into account the posology reported in the product packages, when present, or indications from the literature. Minimum and maximum amounts ingested daily are reported in Table 3. As expected from the observations reported in section 3.1, the intake is different for each sample, even for samples with the same denomination. The elements ingested at highest levels following product consumption are Hg, for all the five products, and Al, Cu, Fe, K and Na for Panchamrit Parpati formulations.

Table 4 reports reference values of Tolerable Weekly Intake (TWI) for Al, Provisional Tolerable Weekly Intake (PTWI) for As, Cd, Hg and Pb, Safe Upper Level (SUL) for Si and Recommended Levels of Nutrient Intake (Italian LARN) for Ca, Cr, Fe, K, Mg, Mn, Na and Zn; the organizations who issued the reference values are also indicated [29-31]. As expected, the intake of mercury with all investigated medicines largely exceeds the PTWI for this element. The LARN for Cu and Fe are exceeded for Panchamrit Parpati products, and the amount of As ingested with sample 3 is higher than the corresponding PTWI. Of course these reference levels have different meanings, since LARN are recommended levels, and not limits, so that exceeding LARN values does not necessarily imply a risk for health.

3.3. Chemometric data processing

PCA and HCA are multivariate chemometric techniques, i.e. they enable one to take into account the behaviour of more variables (in this case, element concentrations) simultaneously. Even if the number of samples examined is relatively small in this case, a chemometric treatment can be of use to gain insight into the characteristics of the samples.

Figure 1 shows the plot of scores and loadings for PC1 vs. PC2, together with the variance explained by each PC (79.2 % in all). Rasa Parpati samples are separated from Panchamrit Parpati samples, which are more scattered in the plot, i.e. more differentiated from one another. The position of sample 3 is due to its high concentrations of As and Si, whereas samples 4 and 5 have generally higher concentrations of most elements. The correlations between variables are reported in Table 5. Figure 1 shows that the investigated elements can be divided into four groups: i) Hg, which is not correlated to other variables, possibly due to the "purification" treatment to which it is subjected in the samples; ii) Cd, Cr, Mn, Na and Pb, which are strongly correlated; iii) Al, Ca, Fe, K, Mg, Zn, which again have a strong correlation; Cd is also correlated with these variables, i.e. it has an intermediate behaviour between groups ii) and iii), as clearly shown in the figure; iv) As and Si. The causes of such variable associations are not clear: they are not apparently due to similarities in the chemical properties of the elements, and they might be related to their sources.

Figure 2 shows the dendrogram obtained by HCA. Despite the differences in their composition, the two Rasa Parpati products are closely clustered, i.e. they are more similar to each other than to the other samples. Surprisingly, sample 3 is more tightly clustered to these samples than to the other two Panchamrit Parpati products, which form another cluster, with a lower degree of similarity in comparison to the one formed by samples 1 and 2.

4. Conclusions

The analysis of Ayurvedic medicines of Parpati family showed that they have a remarkably high content of mercury. Some samples have also high concentrations of other potentially toxic elements, such as arsenic and lead. Products with the same denomination, i.e. Rasa Parpati or Panchamrit Parpati, manufactured by different companies showed differences in composition, probably arising from different procedures of preparation. An interesting prosecution of this research study would be to verify the degree of similarity within a lot and among different lots of a product manufactured by the same company. The chemometric data processing showed the similarities and differences within Rasa and Panchamrit Parpati products and between these two groups of medicines.

The comparison between the calculated daily intake of each element upon use of the investigated products and reference values showed that the PWTI for mercury are by far exceeded for all products. The PWTI for arsenic is exceeded in one Panchamrit Parpati sample. These results show that the consumption of Parpati remedies might cause risks associated to metal toxicity and confirm the need of further studies and monitoring of these kinds of products.

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References

[1] B. Patwardhan, A.D.B. Vaidhya, M. Chorchade, Ayurveda and natural products drug discovery, Curr. Sci. 86 (2004) 789-799.

[2] P. Balachandran, R. Govindarajan, Ayurvedic drug discovery, Expert Opin. Drug Discov. 2 (2007) 1631-1652.

[3] A. Chopra, V.V. Doiphode, Ayurvedic medicine: core concept, therapeutic principles, and current relevance, Med. Clin. North Am. 86 (2002) 75-89.

[4] M.D. McElvaine, E.M. Harder, L. Johnson, R.D. Baer, R.D. Satzger, Lead poisoning from the use of Indian folk medicines, J. Am. Med. Ass. 264 (1990) 2212–2213.

[5] R. B. Saper, R.S. Phillips, A. Sehgal, N. Khouri, R.B. Davis, J. Paquin, V. Thuppil, S.N. Kales, Lead, Mercury, and Arsenic in US- and Indian-Manufactured Ayurvedic Medicines Sold via the Internet, J. Am. Med. Ass. 300 (2008) 915-923.

[6] U.M. Thatte, N.N. Rege, S.D. Phatak, S.A. Dahanukar, The flip side of Ayurveda, J. Postgrad. Med. 39 (1993) 179–182.

[7] P.I. Dargan, I.B. Gawarammana, J.R.H. Archer, I.M. House, D. Shaw, D.M. Wood, Heavy metal poisoning from Ayurvedic traditional medicines: an emerging problem?, Intern. J. Environ. Health 2 (2008) 463-474.

[8] D.W. Dunbabin, G.A. Tallis, P.Y. Popplewell, R.A. Lee, Lead poisoning from Indian herbal medicine (Ayurveda). Med J Aust. 157 (1992) 835–836.

[9] R.W. Keen, A.C. Deacon, H.T. Delves, J.A. Moreton, P.G. Frost, Indian herbal remedies for diabetes as a cause of lead poisoning, J. Postgrad. Med. 70 (1994) 113–114.

[10] G.R. Bayly, R.A. Braithwaite, T.M. Sheehan, N.H. Dyer, C. Grimley, R.E. Ferner, Lead poisoning from Asian traditional remedies in the West Midlands – report of a series of five cases, Hum. Exp. Toxicol. 14 (1995) 24–28.

[11] D. Prpic-Majic, A. Pizent, J. Jurasovic, J. Pongracic, N. Restek- Samarzija, Lead poisoning associated with the use of Ayurvedic metal-mineral tonics, J. Toxicol. Clin. Toxicol. 34 (1996) 417–423.

[12] P.A. Tait, A. Vora, S. James, D.J. Fitzgerald, B.A. Pester, Severe congenital lead poisoning in a preterm infant due to a herbal remedy, Med J Aust. 177 (2002) 193–195.

[13] R.B. Saper, S.N. Kales, J. Paquin, M.J. Burns, D.M. Eisenberg, R.B. Davis, R.S. Phillips, Heavy metal content of Ayurvedic herbal medicine products, J. Am. Med. Ass. 292 (2004) 2868–2873.

[14] M. Aslam, S.S. Davis, M.A. Healy, Heavy metals in some Asian medicines and cosmetics', Public Health 93 (1979) 274–284.

[15] E. Merian, Metals and their compounds in the environment: occurrence, analysis and biological relevance, Wiley-VCH, Weinheim, 1990.

[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] M. Otto, Chemometrics: Statistics and Computer Application in Analytical Chemistry, Wiley-VCH, Weinheim, 1999.

[18] V. Rai, P. Kakkar, C. Misra, S.K. Ojha, N. Srivastava, S. Mehrotra, Metals and organochlorine pesticide residues in some herbal ayurvedic formulations, Bull. Environ. Contam. Toxicol. 79 (2007) 269-272.

[19] V. Rai, P. Kakkar, J. Singh, C. Misra, S. Kumar, S. Mehrotra, Toxic metals and organochlorine pesticides residue in single herbal drugs used in important ayurvedic formulation - 'Dashmoola', Environ. Monit. Assess. 143 (2008) 273-277.

[20] S. Shailajan, K.K. Jaripatke, S. Smruti, Evaluation of Some Heavy Metals in Swertia Species Collected from Different Geographical Regions, Asian J. Chem. 21 (2009) 4485-4488.

[21] G.J. Garvey, G. Hahn. R.V. Lee, R.D. Harbison, Heavy metal hazards of Asian traditional remedies, Int. J. Environ. Health Res.11 (2001) 63–71.

[22] J.L. Qin, Z.F. Chen, Y.C. Liu, H. Liang, Determination of Trace Metal Elements in *Zanthoxylum Nitidum* by ICP-AES, Spectrosc. Spectral Anal. (2009) 2851-2854.

[23] S.A. Gaikwad, A.A. Kale, K.S. Mundhe, N.R. Deshpande, J.P. Salvekar, Detection of Elements Present in Leaves of Tanner's cassia, Asian J. Chem. 21 (2009) 6607-6609.

[24] S.K. Singh, A. Chaudhary, D.K. Rai, S.B. Rai, Preparation and characterization of a mercury based Indian traditional drug-Ras-Sindoor, Indian J. Traditional Knowl. 8 (2009) 346-351.

[25] S. Sharma, P. Dhingra, R.S. Pandey, Trace determination of Pb, Cu, Cd and Zn in Ayurvedic, drug, "Mahayograj Guggulu" via polarographic technique, J. Indian Chem. Soc. 85 (2008) 962-965.

[26] A. Kumar, A.G.C. Nair, A.V.R. Reddy, A.N. Garg, Availability of essential elements in bhasmas: analysis of Ayurvedic metallic preparations by INAA, J. Radioanal. Nucl. Chem. 270 (2006) 173-180.

[27] P. Mahawatte, K.R. Dissanayaka, R. Hewamanna, Elemental concentrations of some Ayurvedic drugs using energy dispersive XRF, J. Radioanal. Nucl. Chem. 270 (2006) 657-660.

[28] M.J. Martena, J.C.A. Van Der Wielen, I.M.C.M. Rietjens, W.N.M. Klerx, H.N. De Groot, E.J.M. Konings, Monitoring of mercury, arsenic, and lead in traditional Asian herbal preparations on the Dutch market and estimation of associated risks, Food Additives and Contaminants 27 (2010) 190–205.

[29] European Food Safety Authority (EFSA), www.efsa.europa.eu.

[30] Joint FAO/WHO Expert Committee on Food Additive (JEFCA), www.codexalimentarius.net/web/jecfa.jsp.

[31] Italian Society for Human Nutrition (SINU), http://www.sinu.it/larn/tab_rias.asp.

Captions to figures

Figure 1. Combined plot of scores and loadings obtained by PCA for Ayurvedic medicine samples

Figure 2. Dendrogram obtained by HCA for Ayurvedic medicine sample

Table 1. List of commercial Ayurvedic medicines of Parpati family examined in this study

S. No	Denomination	Manufacturing company	Formulation
1	Rasa Parpati	Dindayal Aushadhi, India	Black Powder
2	Rasa Parpati	Local company, India	Black Solid
3	Panchamrit Parpati	Dabur, India	Black Solid
4	Panchamrit Parpati	Baidyanath, Kolkata, India	Black Solid
5	Panchamrit Parpati	Local company, India	Black Solid

Table 2. Total element concentrations (mg/kg) in Ayurvedic medicine samples of Parpati family

S. No	Al	As	Ca	Cd	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	Pb	Si	Zn
1	36.1	0.15	1260	0.02	n.d.	n.d.	517	13483	44.5	279	n.d.	28.5	2.92	59.0	18.2
2	44.1	0.04	738	0.01	n.d.	40.4	436	28258	121	201	n.d.	40.8	5.27	27.8	36.5
3	4557	771	1764	0.07	n.d.	18655	21890	25260	5243	1271	175	1729	6.41	258	141
4	3681	8.07	3526	0.23	65.9	11723	19337	25711	10656	2555	916	3392	75.9	40.0	95.8
5	6926	12.9	8439	0.26	31.5	39985	21913	13266	21440	4236	413	1317	31.4	47.9	565

n.d. not detected

Table 3. Estimation of element daily intake upon consumption of Ayurvedic medicines of Parpati family (mg/day, min-max)

Element	1. Rasa Parpati	2. Rasa Parpati	3. Panchamrit Parpati	4. Panchamrit Parpati	5. Panchamrit Parpati
Al	$4.5\cdot10^{-3}$ - $4.5\cdot10^{-2}$	$5.5\cdot10^{-3}$- $5.5\cdot10^{-2}$	1.71 – 5.13	1.38 – 4.14	2.60 – 7.79
As	$1.9\cdot10^{-5}$ - $1.9\cdot10^{-4}$	$4.6\cdot10^{-6}$- $4.6\cdot10^{-5}$	$2.9\cdot10^{-1}$ – $8.7\cdot10^{-1}$	$3.0\cdot10^{-3}$ – $9.1\cdot10^{-3}$	$4.8\cdot10^{-3}$ – $1.4\cdot10^{-2}$
Ca	$1.6\cdot10^{-1}$- 1.57	$9.2\cdot10^{-2}$- $9.2\cdot10^{-1}$	$6.6\cdot10^{-1}$ – 1.98	1.32 – 3.96	3.16 – 9.49
Cd	$2.9\cdot10^{-7}$- $2.9\cdot10^{-6}$	$1.1\cdot10^{-6}$- $1.1\cdot10^{-5}$	$2.8\cdot10^{-5}$ – $8.2\cdot10^{-5}$	$8.5\cdot10^{-5}$ – $2.5\cdot10^{-4}$	$9.9\cdot10^{-5}$ – $3.0\cdot10^{-4}$
Cr	n.d.	n.d.	n.d.	$2.5\cdot10^{-2}$ – $7.4\cdot10^{-2}$	$1.2\cdot10^{-2}$ – $3.5\cdot10^{-2}$
Cu	n.d.	$5.1\cdot10^{-3}$- $5.1\cdot10^{-2}$	6.99 – 20.9	4.40 – 13.2	14.9 – 44.9
Fe	$6.5\cdot10^{-2}$- $6.5\cdot10^{-1}$	$5.5\cdot10^{-2}$- $5.5\cdot10^{-1}$	8.21 - 24.6	7.25 – 21.7	8.22 – 24.6
Hg	1.88 – 18.8	3.53 - 35.3	9.47 – 28.4	9.64 – 28.9	4.97 – 14.9
K	$5.6\cdot10^{-3}$ – $5.6\cdot10^{-2}$	$1.5\cdot10^{-2}$- $1.5\cdot10^{-1}$	1.96 – 5.90	4.00 – 12.0	8.04 – 24.1
Mg	$3.5\cdot10^{-2}$– $3.5\cdot10^{-1}$	$2.5\cdot10^{-2}$- $2.5\cdot10^{-1}$	$4.8\cdot10^{-1}$ – 1.43	$9.6\cdot10^{-1}$ – 2.87	1.59 – 4.76
Mn	n.d.	n.d.	$6.6\cdot10^{-2}$ – $2.0\cdot10^{-1}$	$3.4\cdot10^{-1}$ – 1.03	$1.6\cdot10^{-1}$ – $4.6\cdot10^{-1}$
Na	$3.6\cdot10^{-3}$ -$3.6\cdot10^{-2}$	$5.1\cdot10^{-3}$- $5.1\cdot10^{-2}$	$6.5\cdot10^{-1}$ - 1.94	1.27 – 3.81	$4.9\cdot10^{-1}$ – 1.48
Pb	$3.6\cdot10^{-4}$- $3.6\cdot10^{-3}$	$6.6\cdot10^{-4}$- $6.6\cdot10^{-3}$	$2.4\cdot10^{-3}$ – $7.2\cdot10^{-3}$	$2.8\cdot10^{-2}$ – $8.5\cdot10^{-2}$	$1.2\cdot10^{-2}$ – $3.5\cdot10^{-2}$
Si	$7.4\cdot10^{-3}$ – $7.4\cdot10^{-2}$	$3.5\cdot10^{-3}$- $3.5\cdot10^{-2}$	$9.7\cdot10^{-2}$ – $2.9\cdot10^{-1}$	$1.5\cdot10^{-2}$ – $4.5\cdot10^{-2}$	$1.8\cdot10^{-2}$ – $5.4\cdot10^{-2}$
Zn	$2.3\cdot10^{-3}$ – $2.3\cdot10^{-2}$	$4.5\cdot10^{-3}$- $4.5\cdot10^{-2}$	$5.3\cdot10^{-2}$ – $1.6\cdot10^{-1}$	$3.6\cdot10^{-2}$ – $1.1\cdot10^{-1}$	$2.1\cdot10^{-1}$ – $6.4\cdot10^{-1}$

n.d.: not detected in the sample

Table 4. Reference values for element intake (values refer to an individual of 60 kg)

Element	Dosage	Reference
Al	60 mg/week	TWI (EFSA)
As	0.9 mg/week	PTWI (JECFA)
Ca	1000 mg/day	LARN (SINU)
Cd	0.42 mg/week	PTWI (JECFA)
Cr	50-200 μg/day	LARN (SINU)
Cu	1,2 mg/day	LARN (SINU)
Fe	10 mg/day	LARN (SINU)
Hg	0.3 mg/week	PTWI (JECFA)
K	3100 mg/day	LARN (SINU)
Mg	150-500 mg/day	LARN (SINU)
Mn	1-10 mg/day	LARN (SINU)
Na	0,575-3,5 g/day	LARN (SINU)
Pb	1,5 mg/week	PTWI (JECFA)
Si	700 mg/day	SUL (EVM)
Zn	10 mg/day	LARN (SINU)

TWI: Tolerable Weekly Intake
PTWI: Provisional Tolerable Weekly Intake
LARN: Recommended level of nutrient intake
SUL: Safe Upper Level
EFSA: European Food Safety Authority [18]
JEFCA: Joint FAO/WHO Expert Committee on Food Additive [19]
SINU: Italian Society for Human Nutrition [20]
EVM: Expert group on Vitamins and Minerals [18]

Table 5. Pearson's correlation coefficients for the investigated variables

Variable	Al	As	Ca	Cd	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	Pb	Si	Zn
Al	1	0,297	0,841	0,823	0,350	0,963	0,940	-0,228	0,909	0,908	0,531	0,579	0,417	0,293	0,852
As	0,297	1	-0,231	-0,211	-0,443	0,170	0,462	0,310	-0,126	-0,128	-0,174	0,182	-0,314	0,993	-0,061
Ca	0,841	-0,231	1	0,876	0,437	0,917	0,641	-0,532	0,980	0,964	0,475	0,327	0,431	-0,219	0,958
Cd	0,823	-0,211	0,876	1	0,782	0,777	0,769	-0,240	0,936	0,962	0,840	0,721	0,801	-0,216	0,734
Cr	0,350	-0,443	0,437	0,782	1	0,230	0,426	0,155	0,531	0,591	0,952	0,795	0,986	-0,473	0,193
Cu	0,963	0,170	0,917	0,777	0,230	1	0,814	-0,393	0,934	0,912	0,367	0,359	0,267	0,173	0,959
Fe	0,940	0,462	0,641	0,769	0,426	0,814	1	0,005	0,766	0,788	0,650	0,776	0,525	0,450	0,624
Hg	-0,228	0,310	-0,532	-0,240	0,155	-0,393	0,005	1	-0,387	-0,358	0,144	0,297	0,150	0,212	-0,515
K	0,909	-0,126	0,980	0,936	0,531	0,934	0,766	-0,387	1	0,996	0,601	0,495	0,543	-0,126	0,925
Mg	0,908	-0,128	0,964	0,962	0,591	0,912	0,788	-0,358	0,996	1	0,666	0,563	0,609	-0,128	0,890
Mn	0,531	-0,174	0,475	0,840	0,952	0,367	0,650	0,144	0,601	0,666	1	0,934	0,987	-0,195	0,254
Na	0,579	0,182	0,327	0,721	0,795	0,359	0,776	0,297	0,495	0,563	0,934	1	0,876	0,157	0,159
Pb	0,417	-0,314	0,431	0,801	0,986	0,267	0,525	0,150	0,543	0,609	0,987	0,876	1	-0,337	0,189
Si	0,293	0,993	-0,219	-0,216	-0,473	0,173	0,450	0,212	-0,126	-0,128	-0,195	0,157	-0,337	1	-0,055
Zn	0,852	-0,061	0,958	0,734	0,193	0,959	0,624	-0,515	0,925	0,890	0,254	0,159	0,189	-0,055	1

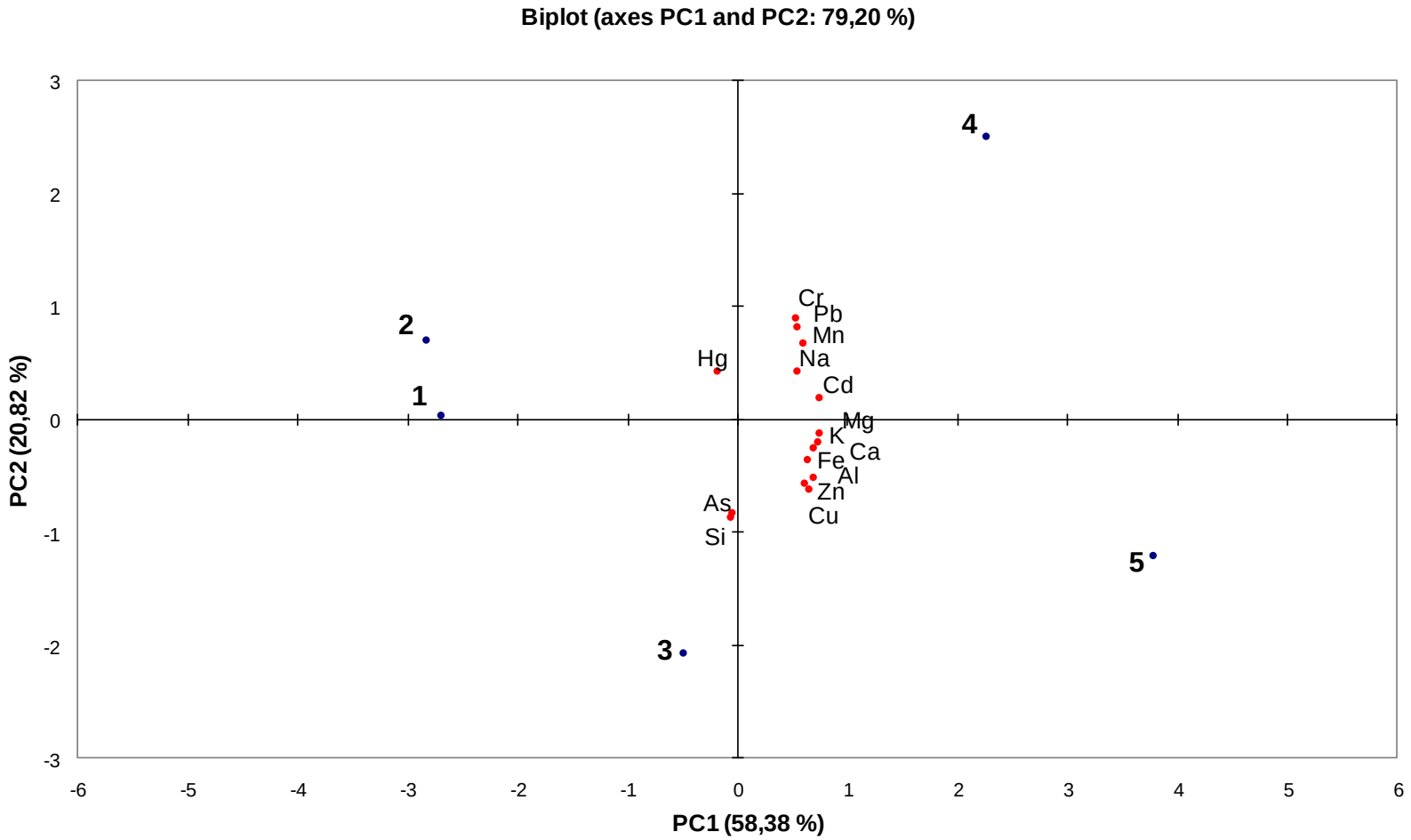
Biplot (axes PC1 and PC2: 79,20 %)
PC2 (20,82 %)
PC1 (58,38 %)
3
2
1
0
-1
-2
-3
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
4
2
1
3
5
Cr
Pb
Mn
Na
Cd
Mg
K
Ca
Fe
Al
Zn
Cu
Hg
As
Si

Figure 1

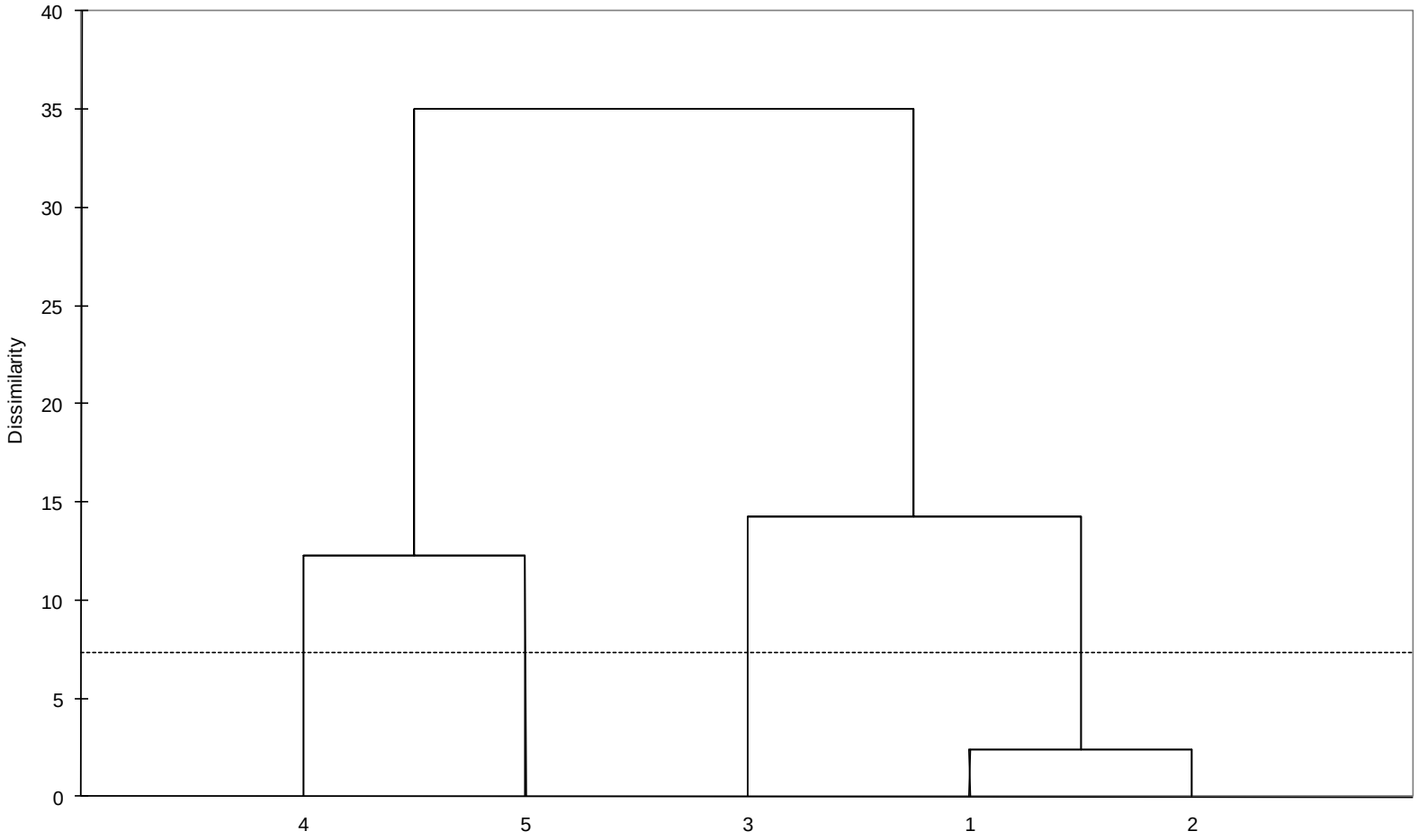

Figure 2