**Potentially toxic elements in ayurvedic formulations: Total and bioaccessible content**

Ornella Abollino, Agnese Giacomino, Gaia Paparella, Emanuele Magi, Eleonora Conca, Mery Malandrino

Potentially toxic elements in ayurvedic formulations:
total and bioaccessible content

Microchemical Journal 136 (2018) 236-243
DOI: 10.1016/j.microc.2017.01.005

## Highlights

- Some ayurvedic medicines contain deliberately added metals or arsenic
- Bioaccessibility of Potentially Toxic Elements can be estimated by extraction with synthetic gastric and intestinal media
- Bioaccessibility strongly depends on the operating conditions adopted
- Element bioaccessibility decreases when ayurvedic formulations are mixed with butter

**Potentially toxic elements in ayurvedic formulations: total and bioaccessible content**

Ornella Abollino[a]*, Agnese Giacomino[b], Gaia Paparella[a], Emanuele Magi[c], Eleonora Conca[a], Mery Malandrino[a]

[a] Department of Chemistry, University of Torino, Via Giuria 5, 10125 Torino, Italy

[b] Department of Drug Science and Technology, University of Torino, Via Giuria 9, 10125 Torino, Italy

[c] Department of Chemistry and Industrial Chemistry, University of Genova, Via Dodecaneso 31, 16146 Genova, Italy

**Abstract**

Some formulations used in ayurvedic medicine are based on herbs deliberately combined with arsenic, metals, and minerals. Some of these preparations have been suspected to be harmful to health, due to the content of potentially toxic elements (PTE), hence it is very important to value the possible risks associated to their consumption. Such risks depend not only on the total PTE concentrations, but also on their bioaccessibility, which influences their assimilation. In this work, the total concentrations of As, Cr, Cu, Hg, Mn and Pb in nine ayurvedic medicines purchased in India were measured. After sample mineralization, concentrations were determined by atomic emission or absorption spectroscopy. The results showed the presence of high amounts of As (19-479,000 mg/Kg), Cu (27-675,000 mg/Kg), Hg (100-15,600 mg/Kg) or Pb (3-248 mg/Kg) in five of these products, whereas much smaller amounts (As, ≤ 1.3-19 mg/Kg; Cu, 0.6-3.2 mg/Kg; Hg, ≤ 1.0-5.3 mg/Kg; Pb, 0.5-1.8 mg/Kg) were present in the other ones. Subsequently, the bioaccessibility of PTE was estimated *in vitro* by extraction into synthetic gastric and intestinal fluids. The effect of different operating conditions was assessed. The results obtained show as elements are mainly extracted into gastric juices, and the extent of extraction is strongly influenced by the adopted conditions. The data were treated with chemometric techniques that helped to visualize the differences and similarities among samples. We calculated the daily intake of each PTE from its concentration and from the posology of each medicine, and compared it with the maximum tolerable intake levels: the intake of As, Cr, Cu and Hg from some products exceeded such limits (whose values, expressed in mg/day for a 60 kg individual, are: As, 0.018; Cr, 0.05; Cu, 30; Hg, 0.034), mainly when total concentrations were considered, but also for some bioaccessible values. Our study shows the importance of adopting homogeneous conditions to evaluate bioaccessibility.

The availabilty of standard reference materials for ayurvedic medicines on the market would also be highly desirable.

Keywords: Ayurvedic formulations, Arsenic, Metals, Bioaccessibility, *In vitro* test

**1. Introduction**

Ayurveda is a major traditional system of Indian medicine that is still being widely used in many countries [1]. It advocates the use of both herbal preparations, similar to other ancient medicines in the World, and metallic preparations, that are unique in Ayurveda and, to our knowledge, are not known elsewhere [2]. In some ayurvedic products, herbs are deliberately combined with metals (e.g., mercury, lead, iron, zinc), minerals (e.g. mica) and gems (e.g., pearl) according to traditional procedures known as *Rasa shastra* [3, 4]. Ayurvedic experts have estimated that 35–40% of the approximately 6000 medicines in the ayurvedic formulary intently contain at least one potentially toxic element (PTE). Although the presence of heavy metals is reported among the pharmacologic recommendations in or on the packaging for some of these remedies, the specific active ingredient is seldom identified and there is little information available to the practitioner or patient about the toxicity and safety of the so-called 'natural' organic or inorganic ingredients. Some metal-based preparations used in Indian system of medicine are suspected to be harmful, causing hepatic, renal and neurotoxicity and many other side effects [5], because over recent years, an increasing number of published cases deals with patients that have been poisoned by heavy metals after the ingestion of traditional remedies. [6-10]. It is relatively easy to determine total metal content in ayurvedic formulations either directly, using analytical techniques suitable for solid samples (e.g., X-ray fluorescence spectroscopy), or after acid digestion and measurement by atomic spectroscopy or mass spectrometry with plasma source. However, the determination of the total inorganic content in ayurvedic formulation is not sufficient for understanding the chemical risk to humans upon consumption of these preparations: it is important to assess the amount of elements potentially available for absorption in the stomach and intestine or to be excreted. A medicine, administered orally, undergoes three steps of transformation: i) the pharmaceutic step, in which the product is disgregated and the active principle is dissolved in the gastrointestinal tract; ii) the pharmacokinetic step, during which the active principles undergo

adsorption, distribution, metabolism and excretion (ADME); iii) the pharmacodynamics step, involving the interaction between the active principle and the receptor.

Bioavailability can be defined as the amount of a substance reaching the systemic circulation, and is influenced by the chemico-physical characteristics of the substance (e.g. its solubility, lipophilicity, or ionization), its morphology (e.g. powder or tablet) and by the conditions of the individual.

In the present study we focused our attention onto the pharmaceutic step and considered bioaccessibility, which can be defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (i.e. enters the blood stream) [11]. The bioaccessible fraction of a substance includes both the actually and potentially bioavailable amounts. This parameter can be determined with *in vivo* studies on animal models, but such studies are expensive, time-consuming and ethically critical. Therefore, several *in vitro* approaches have been developed in attempts to mimic the effects of the human-digestion process [12]. Such approaches are in agreement with the well-known "Three Rs" principle, which involves: i) Replacing the use of animals with alternative techniques, or avoid the use of animals altogether; ii) Reducing the number of animals used to a minimum; iii) Refining the way experiments are carried out, to make sure animals suffer as little as possible [13]. "Three Rs" principle is the basis of Directive 2010/63/EU, revising Directive 86/609/EEC on the protection of animals used for scientific purposes [14].

The gastrointestinal digestive processes are quite complicated and difficult to simulate *in vitro*. A number of different models is based on extraction with solutions simulating the effect of gastric and intestinal fluids [11, 12]. Several studies in the area of human nutrition have reported i*n vitro* methods to assess bioavailable iron in foodstuffs [15, 16]. In addition, some approaches include the addition of food constituents to the extractant, since food constituents (e.g. milk, bread and starch) can affect the fraction of the contaminant released into the digestive fluids during transit through the gastrointestinal tract after ingestion [17, 18]. Similar tests are also used for testing the oral bioaccessibility of soil contaminants upon soil ingestion, see e.g. [19-22], and the methods have been reviewed [23, 24]. Table 1 summarizes the characteristics of some popular simulated gastrointestinal extraction methods; as it can be seen, such methods are referred to with different names: i) physiologically-based extraction test (PBET) [25]; ii) Simple Bioaccessibility Extraction Test (SBET) [24, 26, 27]; iii) I*n vitro* digestion model, by the National Institute of Public Health and the Environment (RIVM, The Netherland) [11, 20] , iv) Simulator of Human Intestinal Microbial

Ecosystem of Infants (SHIME) [11, 28]; v) TNO Gastro-Intestinal Model (TIM; TNO: The Netherlands Organization for Applied Scientific Research) [11, 29]; vi) the German standard method for absorption availability of organic and inorganic pollutants from contaminated soil material (Deutsches Institut für Normung - DIN E 19738 method) [30, 31]. These methods have demonstrated good correlation with *in vivo* tests in some studies [25, 11, 24, 32]. However, there is still a lack of information regarding the behavior of contaminants and materials, and research is in progress to identify a common and accepted test method in view of an international standardization [24, 32]. Despite the large number of studies on bioaccessibility of PTEs in soils and food, few papers deal with this topic in ayurvedic formulations [33-36] and in traditional Chinese medicines [37-42]. Attention was mainly focused on the behavior of As and Hg, owing to their high toxicity, but studies on other elements such as Cd, Cu, Fe, Mn and Zn are also available. Conversely, Yan et al. assessed the effect of traditional Chinese medicines on the bioaccessibility and speciation of As and Hg [38].

In a previous study we compared the characteristics of ayurvedic medicines purchased from different commercial channels and studied bioaccessibility with single extraction tests [36].

In this work, after the evaluation of the total PTE content in a set of ayurvedic products purchased in India, we estimated the bioaccessibility of the analytes by *in vitro* sequential extractions with synthetic gastric and intestinal fluids. We also applied modifications to the experimental procedure to assess the influence of changing of operative conditions on the final results. Finally, the concentration of each analyte was compared with established acceptable daily intake values for metal ingestion indicated by the guidelines of international organizations.

## 2. Material and methods

### *2.1. Samples and sample pretreatment*

Unfortunately, no reference materials for ayurvedic medicines are available on the market. Two Certified Reference Materials (CRMs), namely *Tomato Leaves SRM 1573a*, and *Saint Joaquin Soil 2709* supplied by the National Institute of Standards and Technology (NIST), were analysed to value the efficiency and the accuracy of analyte quantification.

Nine ayurvedic formulations were purchased in India. The name of the products, the ingredients declared on the label and the aspect of formulations are summarized in Table 2. The purchased samples appeared as powder (seven products) or gel (two specimens). All the samples were analyzed without any pretreatment.

*2.2. Apparatus and reagents*

Sample dissolution was performed in polytetrafluoroethylene (PTFE) bombs, with a Milestone ETHOS-One (Milestone, Sorisole, Italy) microwave laboratory unit. Most of the analyses were carried out with a Perkin Elmer Optima 7000 (Perkin Elmer, Norwalk, Connecticut, USA) inductively coupled plasma-optical emission spectrometer (ICP-OES). For the determination of element concentrations lower than the Limit of Quantification (LoQ) of ICP-OES, a Perkin Elmer Analyst 600 (Perkin Elmer, Norwalk, Connecticut, USA) graphite furnace equipped-atomic absorption spectrometer (GF-AAS) was used.

Standard metal solutions were prepared from concentrated stock solutions (Sigma Aldrich, Darmstadt, Germany).

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

Two of the bioaccessibility tests performed (see section 2.3) were conducted with the aid of dialysis tubes (Sigma Aldrich).

The gastric juice was prepared by dissolving 2.0 g of NaCl in 7.0 mL of HCl. The solution was diluted to 1000 mL with HPW ad the pH adjusted to the final value of 1.2.

The intestinal medium was obtained by dissolving 6.8 g of $KH_2PO_4$ in 250 mL of HPW; then 77.0 mL of 0.2 N NaOH and 500 mL of HPW were added; finally pH was adjusted to 6.8 with the aid of 0.2 N NaOH or 0.2 N HCl and the solution was diluted to 1000 mL with HPW.

*2.3. Procedures*

*2.3.1. Determination of the total element content*

Acid digestion in the microwave oven was adopted to dissolve the samples. Aliquots of 0.5 g of each ayurvedic medicine were treated with 6 mL of $HNO_3$ and 2 mL of $H_2O_2$ in PTFE bombs. After the heating and ventilation steps, the resulting solutions were filtered on Whatman 5 filters and then diluted to 30 mL with HPW. The solutions were directly employed for ICP-OES or GF-AAS analysis, depending on the analyte concentration level. In both cases, the calibrations were performed with standard solutions prepared in aliquots of sample blanks diluted in the same ratios as the sample solutions. Standard solutions were periodically analyzed and their signals were used to correct the sample signals for drift of instrumental sensitivity. The limits of detection (LoD) and

the LoQ were estimated as three and ten times the standard deviation of the blank respectively. Tables in this paper report LoQ values.

In order to check the accuracy of the experimental procedure *Tomato Leaves SRM 1573a* and *Saint Joaquin Soil 2709* were analyzed to assess the effect of vegetal and mineral matrices on element determination respectively. Among the certified elements, we considered for *Tomato Leaves SRM 1573a* Cr (1.99 ± 0.06 mg/kg), Cu (4.70 ± 0.14 mg/kg), Hg (0.034 ± 0.004 mg/kg), Mn (246 ± 8 mg/kg),-and for *Saint Joaquin Soil 2709* As (17.7 ± 0.8 mg/kg), Hg (1.4 ± 0.08 mg/kg) and Pb (18.9 ± 0-5 mg/kg). The recoveries ranged between 93 and 108% for all analytes with the exception of Cr (76%) and Pb (87%).

*2.3.2. Determination of the bioaccessible content*

Different procedures were adopted to value the bioaccessible fraction [43].

*Method A.* 0.2 g aliquots of ayurvedic product were subjected to two sequential steps: i) extraction with 25 mL of gastric medium for 2 h; ii) extraction with 25 mL of intestinal medium for 6 h. During each step the suspensions were maintained at the temperature of 37 °C, shaking periodically to mimic peristaltic motions of stomach and intestine. After the first extraction, the suspension was subjected to centrifugation for 10 min at 4,000 rpm. The solution was separated, filtered on Whatman 5 filter and analyzed, while the solid residue (from step i) underwent the second extraction step. All experiments were performed in triplicate.

*Method B*. The same procedure as described for *Method A* was followed, but the sample was mixed with 1 g of *ghee* (traditional Indian clarified butter).

*Method C.* Aliquots of 0.2 g of each samples were transferred into a piece of membrane for dialysis in the form of a tube (length: 10 cm) with 1 mL of HPW. Then the tube was closed at both ends and put in contact with 25 mL of gastric medium for 2 h at 37 °C and shaken periodically. After extraction the tube was removed from the solution and dipped in 25 mL of intestinal medium for 6 h. Then both extracting solutions were analyzed.

*Method D*. The same procedure as reported for *Method C* was followed, but the sample was mixed with 1 g of *ghee*.

*2.3.3. Chemometric treatments*

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were carried out with the aid of XLSTAT4.4 software package, used as a Microsoft Excel plug-in. Unscrambler X 10:2 was

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. The Euclidean distance and Ward's agglomeration method were used for HCA.

## 3. Results and discussion

### *3.1. Total concentrations*

Among PTEs, attention was focused onto As, Cu, Cr, Hg, Mn and Pb content taking into account the results of a preliminary semiquantitative analysis of the investigated medicines and our knowledge on their composition [44]. Table 3 shows the total concentrations of the analytes in each product. Overall, samples 5-9 have higher concentrations of the selected PTEs than samples 1-4, with sample 4 having higher analyte levels than specimens 1-3. Some products have outstandingly high concentrations of As (5 and 6), Cu (7 and 8), Hg (5-9) and Pb (9). This trend was not unexpected, considering the available information on sample composition, reported in the Introduction, and the names of some samples: *Talaka* or *Thalaka* means "arsenic" (samples 5 and 6), *Tamra* means "copper" (sample 7).

The dataset was processed with chemometric pattern recognition techniques, so as to obtain an overview of the results and visualize similarities and differences among the investigated medicines. Figure 1 shows the combined plot of scores and loadings resulting from PCA and the dendrogram obtained by HCA. As to scores (Figure 1a), samples 1-4 are grouped together, owing to the relatively low content of the analytes. The position of the other products indicates the element prevailing in their composition: As for samples 5 and 6, which are closely clustered, Cu, Pb and Hg for samples 7, 8, 9 respectively. The results of HCA (Figure 1b) show an analogue clustering of the medicines. Pearson's correlation matrix (not shown) indicates that there is only a correlation between Mn, Cr and Pb; the cause of this relationship is difficult to explain. Variable correlations usually indicate the presence of a common origin or a similar chemical behavior in a sample matrix: this is true for instance in environmental samples, but it is not valid for ayurvedic medicines, in which elements are intentionally added by manufacturers. For this reason As, Cu and Hg are not correlated to other elements. Therefore, in the present study pattern recognition techniques are useful to distinguish different groups of formulations, but provide little information on the behavior of their components.

The interpretation of these results can be aided by comparing the intake of each considered element upon consumption of ayurvedic medicines with tolerable levels issued by international

organisms (Table 4). When possible, we adopted the values issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA); as to the elements for which no JECFA values existed, we referred to the levels established by the Agency for Toxic Substances and Disease Registry (ATSDR, USA) or, for Mn, by the European Food Safety Authority (EFSA). In the case of Pb, JECFA has withdrawn a previously established tolerable intake level, stating that it is not possible to provide a new value that would be considered health-protective. Anyway, since no values from ATSDR or other institutions are available for Pb (to the best of our knowledge), we referred to the withdrawn level: of course the conclusions reached for this element must be considered just indicative and regarded with great caution. The daily intake was calculated from element concentration and the posology of each medicine reported in the leaflet or taken from the literature. To simplify the comparison, all tolerable levels were converted to the same unit, i.e. mg per day for an average body weight of 60 kg. Table 4 shows that most samples, with the exception of specimens 3 and 4, exceed one or more tolerable intake level. Obviously the extent of exceedance is particularly dramatic for samples 5-9. For this reason, we decided to gain insight into the actual possible uptake of PTE in such samples, and consequently the risks for consumers, with the aid of *in vitro* studies.

### *3.2. Bioaccessible concentrations*

#### *3.2.1. Element extractability in synthetic gastric and intestinal juices*

The bioaccessibility of the investigated elements in samples 5-9 was estimated by extraction with solutions simulating gastric and intestinal juices, following Method A (section 2.3.2). In our previous paper [36] we performed single extractions, in order to assess the extracting efficiency of each medium on fresh sample aliquots. In the present study we made two sequential extractions, as prescribed by the American Pharmacopoeia [43]. The results are reported in Table 5, in terms of both concentrations and extraction percentages.

We did not add enzymes, as we observed in previous experiments that the extraction efficiency of the reagents towards metals were not influenced by these reagents, which have other roles in our organism. The results confirm our earlier findings that extraction mainly takes place in the gastric medium, thanks to its acidic pH, which favors the dissolution of hydroxides and oxides of metals and the breakage of the bonds between metals and organic components of the medicines [36].

The bioaccessibility data do not show a definite trend, either as a function of an element or as a function of a sample. The chemical form and behavior of the elements, such as the solubility of

their salts at the working pH, is probably the main factor influencing the results. All the investigated samples are in the form of powders, i.e. they are in close contact with the extractants: so the form of the product is not a cause of the different behavior of the samples; in the next step of our study we plan to compare the behavior of the same product in the form of entire tablet and powder.

Figure 2 shows the combined plot of scores and loadings resulting from PCA and the dendrogram obtained by HCA applied to bioaccessible concentrations. As observed for total amounts samples 5 and 6 are differentiated from the other ones owing to the high concentration of As. Again sample 9 has very different characteristics from the other ones.

*3.2.2. Influence of operating conditions*

The results of bioaccessibility studies depend on the procedure adopted, not only from the point of view of the composition of the extracting fluids, but also for the effect of the operative conditions. This is a main drawback of investigations based on single and sequential extraction also in other fields, e.g. in the evaluation of element mobility in soils [24].

One of the main parameters affecting *in vitro* bioaccessibility tests is surely the extent of the contact between sample and extracting solution. For this reason we carried out the extractions in different conditions.

Many of the investigated samples must be swallowed with honey or with *ghee*, the typical Indian butter. For this reason we investigated the effect of *ghee* on element bioaccessibility according to Method B (section 2.2.3). We performed these experiments with samples 5, 6 and 7. Table 6 shows that the amount of extracted elements decrease in the presence of *ghee*. This results might be due to the fact that the hydrophobic nature of butter and the lowest surface area of the sample after mixing with *ghee* reduce its contact with the extractant.

We finally tested a different approach to expose the sample to the fluids, using specimen 6 as a probe. We inserted the powder into tubes made of membrane for dialysis. The advantage of this procedure is that the centrifugation and filtration steps after extraction are avoided, thus reducing the risk of sample losses, since the specimen is just transferred from the gastric to the intestinal medium. This experiment was performed both without and with *ghee*, according to *Method C* and *D* respectively (section 2.2.3). The results, summarized in Table 7, show that element extractability decreases when the sample is inside the membrane. This might be again due to a less extensive contact between the sample and the solution, but also to a low transfer of ions through the pores

of the membrane. So the use of this procedure requires a further optimization, to avoid an underestimation of bioaccessibility. We plan to perform further experiments and monitor the extent of extraction as a function of time, since longer times might be required for a complete extraction. Table 7 also shows that mixing with *ghee* causes a reduction of PTEs extractability, confirming the trend discussed above. This finding suggests that the traditional indication of assuming the medicines with *ghee* might be meaningful.

*3.2.3. Comparison with reference values*

As we made with total concentrations, we compared daily intake levels, computed from bioaccessible concentrations, with tolerable reference levels. Table 8 shows that the number of intake values higher than the limits is greatly decreased in comparison to Table 4. Therefore, considering the total or bioaccessible concentrations leads to different conclusions regarding the potential toxicity of a product.

**4. Conclusions**

Our study confirms that some ayurvedic medicines have very high concentrations of PTEs, intentionally added according to the tradition. Anyway, many ayurvedic formulations are herb-based and do not pose any threat to health, at least from the point of view of the metal content. Therefore it is important that consumers of these medicines consult experts in Ayurveda to choose safe products.

To be marketed in Western countries, ayurvedic medicines have to be declared “metal free”. So only herb-based ayurvedic products can be sold in Italy. In our previous study [36] we had analyzed medicines bought in an Italian farmacy, and found that they complied with the legislation.

Bioaccessibility studies showed that only a part of the total content is soluble in synthetic gastric and intestinal fluids: the bioaccessible fraction was found to be different for the same element in different products, so it is probably related to the chemical form of the element, e.g. on the solubility of its salt, and on the components of the sample matrix.

The procedure adopted for extraction has a strong influence on the release of the elements from the medicines: therefore it is important to homogenize the protocol adopted in different laboratories, so that data can be comparable.

**Acknowledgements**

The financial support of the University of Torino (Ricerca Locale) is gratefully acknowledged. We thank Dr. Federica Crea for her helpful contribution in revising the manuscript.

**References**

[1] A.D. Vaidya, T.P. Devasagayam. Current status of herbal drugs in India: an overview. Clin Biochem. Nutr. 41 (2007) 1-11.

[2] 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.

[3] 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. JAMA 300 (2008) 915–923.

[4] B. Kumar, M. Vijayakumar, R. Govindarajan. P. Pushpangadan. Ethnopharmacological approaches to wound healing—Exploring medicinal plants of India. J. Ethnopharmacology 114 (2007) 103–113.

[5] G. Kumar, A. Srivastava, S.K. Sharma, Y.K. Gupta. Safety evaluation of an Ayurvedic medicine, Arogyavardhini vati on brain, liver and kidney in rats. J. Ethnopharmacology 140 (2012) 151– 160.

[6] D. M. Wood, S. Athwal, A. Panahloo. The advantages and disadvantages of a 'herbal' medicine in a patient with diabetes mellitus: a case report. Diabet. Med. 21 (2004) 625 – 627.

[7] N. Gupta, B. Goswami, N. Singh, B.C. Koner, R. Garg. Lead poisoning associated with Ayurvedic drug presenting as intestinal obstruction: A case report. Clin. Chim. Acta 412 (2011) 213–214.

[8] L. Borgese, A. Zacco, E. Bontempi, M. Pellegatta, L. Vigna, L. Patrini, L. Riboldi, F.M. Rubino, L.E. Depero. Use of total reflection X-ray fluorescence (TXRF) for the evaluation of heavy metal poisoning due to the improper use of a traditional ayurvedic drug. J. Pharm. Biomed. Anal. 52 (2010) 787–790.

[9] N.J. Gogtay, H.A. Bhatt, S.S. Dalvi, N.A. Kshirsagar. The Use and Safety of Non-Allopathic Indian Medicines. Drug Safety 25 (2002) 1005–1019.

[10] R.C. Kapoor. Some observations on the metal-based preparations in the Indian systems of medicine. Indian J Tradit Knowl. 9 (2010) 562–75.

[11] A.G. Oomen, A. Hack, M. Minekus, E. Zeijdner, G. Schoeters, W. Verstraete, T.V.D. Wiele, J. Wragg, C.J.M. Rompelberg, A.J.A.M. Sips, J.H.Van Wijnen. Comparison of five *in vitro* ingestion

models to study the bioaccessibility of soil contaminants. Environ. Sci. Technol. 36 (2002) 3326-3334.
[12] M. Intawongse, J.R. Dean, In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples, Trend Anal. Chem. 25 (2006) 876–886.
[13] W.M.S. Russell, R.L. Burch. The Principles of Humane Experimental Technique. Methuen, London, 1959.
[14] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Official Journal of the European Union L 276/33, 20.10.2010.
[15] E.E. Miller, B. R. Schricker; R. R. Rasmussen, D. Van Campen, An in vitro method for estimation of iron availability from meals. J. Clin. Nutr. 34 (1981) 2248-2256.
[16] R.A. Schwartz, Z. Belko, E. M. Wien. An in vitro system for measuring intrinsic dietary mineral exchangeability: alternative to intrinsic isotopic labeling. J. Nutr. 112 (1982) 497-504.
[17] J.R. Malagelada, G.G. Lonstreth, W.H.J. Summerskill, V. L. W. Go. Measurement of Gastric Functions During Digestion of Ordinary Solid Meals in Man. Gastroenterology 70 (1976) 203-210.
[18] H.M. Crews, J.A. Burrel, D.J. McWeeny. Comparison of trace element solubility from food items treated separately and in combination. Z. Lebensm-Unters.- Forsch.180 (1985) 405.
[19] M.E. Kelley, S. E. Brauning, R. A. Schoof, M. V. Ruby. Assessing Oral Bioavailability of Metals in Soil. Battelle Press, Columbus, OH, USA, 2002.
[20] A.G. Oomen, C. J. M. Rompelberg, M. A. Bruil, C. J. G. Dobbe, D. P. K. H. Pereboom. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants, Arch. Environ. Contam. Toxicol. 44 (2003) 281–287.
[21] J. Drexler, The In-Vitro Method, 26-4-2005.
(http://www.colorado.edu/geolsci/legs/invitro1.html)
[22] R.R. Rodriguez, N.T. Basta, S.W. Casteel, L.W. Pace. An In Vitro Gastrointestinal Method To Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media. Environ. Sci. Technol. 33 (1999) 642-649.
[23] C. Grøn, L. Andersen, Human Bioaccessibility of Heavy Metals and PAH from Soil. Miljøprojekt Nr. 840, Miljøstyrelsen, Miljøministeriet, Copenhagen, Denmark, 2003.
[24] J. Wragg, M. R. Cave. In-Vitro Methods for the Measurement of the Oral Bioaccessibility of Selected Metals and Metalloids in Soils: A Critical Review (Ed: British Geological Survey), Environment Agency, Nottingham, UK, 2002. (www.environment-agency.gov.uk).

[25] M.V. Ruby, A. Davis, R. Schoof, S. Eberle, C. M. Sellstone. Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test. Environ. Sci. Technol. 30 (1996) 422-430.
[26] J. Kim, K. Kim, J. Lee, J. Lee, J. Cook. Assessment of As and heavy metal contamination in the vicinity of Duckum Au-Ag mine, Korea. Environ. Geochem. Health 24 (2002) 215–227.
[27] F. Madrid, M. Biasioli, F. Ajmone-Marsan, Availability and bioaccessibility of metals in fine particles of some urban soils. Arch. Environ. Contam. Toxicol. 55 (2008) 21-32.
[28] T-Van de Wiele, P. Van den Abbeele, W. Ossieur, S. Possemiers, M. Marzorati, The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) in: K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, H. Wichers (Eds.), The Impact of Food Bio-Actives on Gut Health. In Vitro and Ex Vivo Models, Springer International Publishing, Heildelberg (Germany), 2015, pp.305-317.
[29] M. Minekus, The TNO Gastro-Intestinal Model (TIM), in: K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, H. Wichers (Eds.), The Impact of Food Bio-Actives on Gut Health. In Vitro and Ex Vivo Models, Springer International Publishing, Heildelberg (Germany), 2015, pp.37-46.
[30] DIN. Soil quality — absorption availability of organic and inorganic pollutants from contaminated soil material. DIN E 19738. Berlin, Germany: Deutsches Institut für Normung; 2000.
[31] V. Hollriegl, W. B. Li, K. Leopold, U. Gerstmann, U. Oeh. Solubility of Uranium and Thorium from a Healing Earth in Synthetic Gut Fluids: A Case Study for Use in Dose Assessments. Sci. Tot. Environ. 408 (2010) 5794-5800.
[32] J. Li, K. Li, X.Y Cui, N.T. Basta, L.P. Li, H.B. Li, L.Q. Ma, In vitro bioaccessibility and in vivo relative bioavailability in 12 contaminated soils: Method comparison and method development. Sci. Tot. Environ. 532 (2015) 812–820.
[33] I. Koch, M. Moriarty, J. Sui, A. Rutter, R.B. Saper, K.J. Reimer. Bioaccessibility of mercury in selected ayurvedic medicines, Sci. Total Environ. 454-455 (2013) 9-15.
[34] I. Koch, M. Moriarty, K. House, J. Sui, W.R. Cullen, R.B. Saper, K.J. Reimer. Bioaccessibility of lead and arsenic in traditional Indian medicines. Sci. Total Environ. 409 (2011) 4545-4552.
[35] I. Jayawardene, R Saper, N. Lupoli, A. Sehgal, R.O. Wright, C. Amarasiriwardena. Determination of in vitro bioaccessibility of Pb, As, Cd and Hg in selected traditional Indian medicines. J. Anal. At. Spectrom. 25 (2010) 1275-1282.

[36] A. Giacomino, O. Abollino, C. Casanova, C. La Gioia, E.Magi, M. Malandrino. Determination of the total and bioaccessible contents of essential and potentially toxic elements in ayurvedic formulations purchased from different commercial channels. Microchem. J. 120 (2015) 6–17.
[37] L. Liu, Y. Zhang, Z. Yun, B. He, G. Jiang. Estimation of bioaccessibility and potential human health risk of mercury in Chinese patent medicines. J. Environ. Sci.39 (2015) 37-44.
[38] L.L. Yan, J.J. Chen,Y. Zhou, J. Hu. Influence of traditional Chinese medicines on bioaccessibility and speciation of arsenic in realgar and mercury in cinnabar. Asian J. Chem. 27 (2015) 2891-2895.
[39] P. Xiao, H. Huang, J. Chen, X. Li. In vitro antioxidant and anti-inflammatory activities of Radix Isatidis extract and bioaccessibility of six bioactive compounds after simulated gastro-intestinal digestion. J. Ethnopharmacology 157 (2014) 55-61.
[40] Y. Zhou, X. Gao, C. Wu, Y. Wu. Bioaccessibility and safety assessment of trace elements from decoction of "Zhebawei" herbal medicines by in vitro digestion method. J. Trace Elem.Med. Biol. 28 (2014) 173-178.
[41] D.H. Yang, Y.J. Lee. Determination of cadmium bioaccessibility in herbal medicines and safety assessment by in vitro dissolution and ICP-AES. Microchim. Acta 167 (2009) 117-122.
[42] I. Koch, S. Sylvester, V.W.M. Lai, K.J. Reimer, W.R. Cullen. Bioaccessibility and excretion of arsenic in Niu Huang Jie Du Pian pills. Toxicol. Appl. Pharmacol. 222 (2007) 357-364.
[43] The United States Pharmacopoeia – National Formulary [USP 37 NF 32], Volume 1, The United States Pharmacopoeial Convention, Rockville, MD, 2013.
[44] A. Giacomino, O. Abollino, M. Malandrino, M. Karthik, V. Murugesan, Determination and assessment of the contents of essential and potentially toxic elements in Ayurvedic medicine formulations by inductively coupled plasma-optical emission spectrometry, Microchem. J. 99 (2011) 2–6.

## Captions to figures

Figure 1. Combined plot of scores and loadings obtained by PCA (a) and dendrogram obtained by HCA (b) for total element concentrations.

Figure 2. Combined plot of scores and loadings obtained by PCA (a) and dendrogram obtained by HCA (b) for bioaccessible element concentrations.

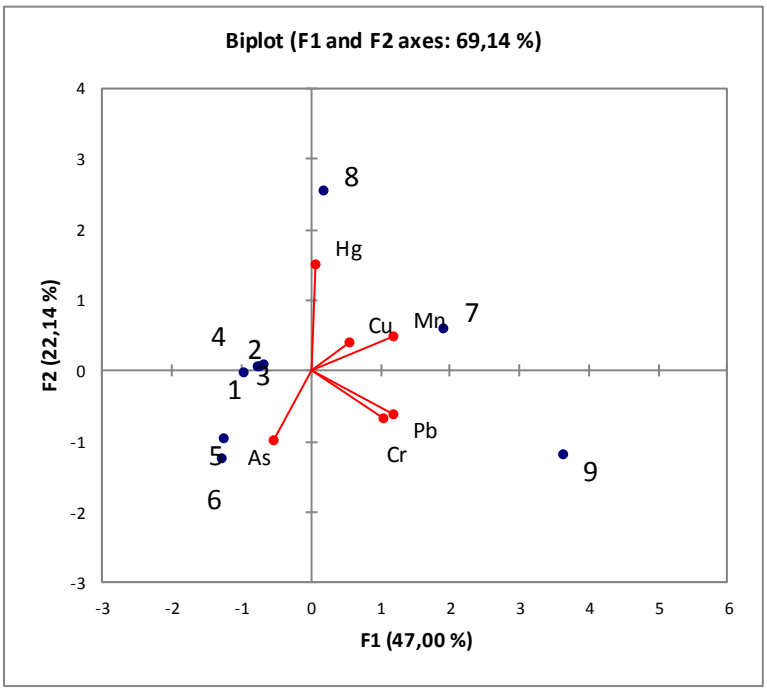


Figure 1 a

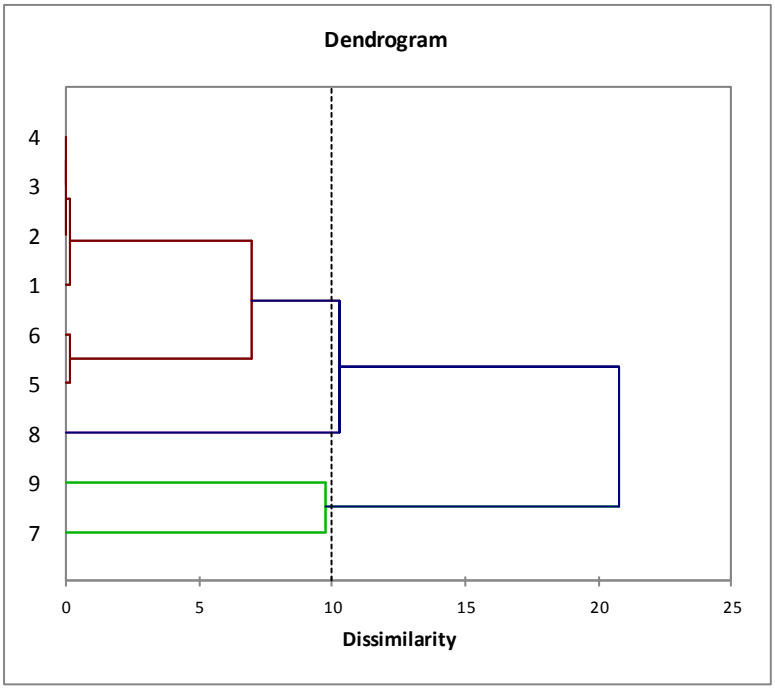


Figure 1 b

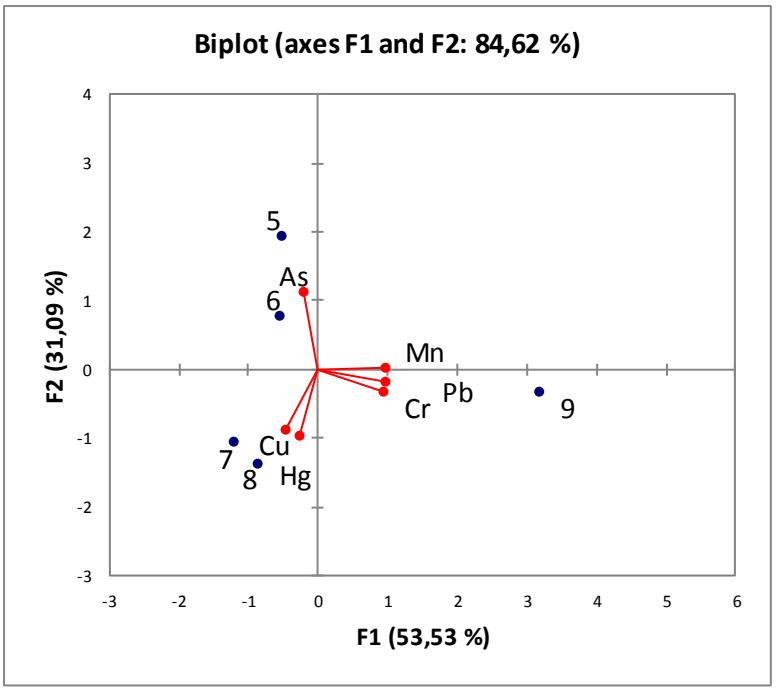


Figure 2 a

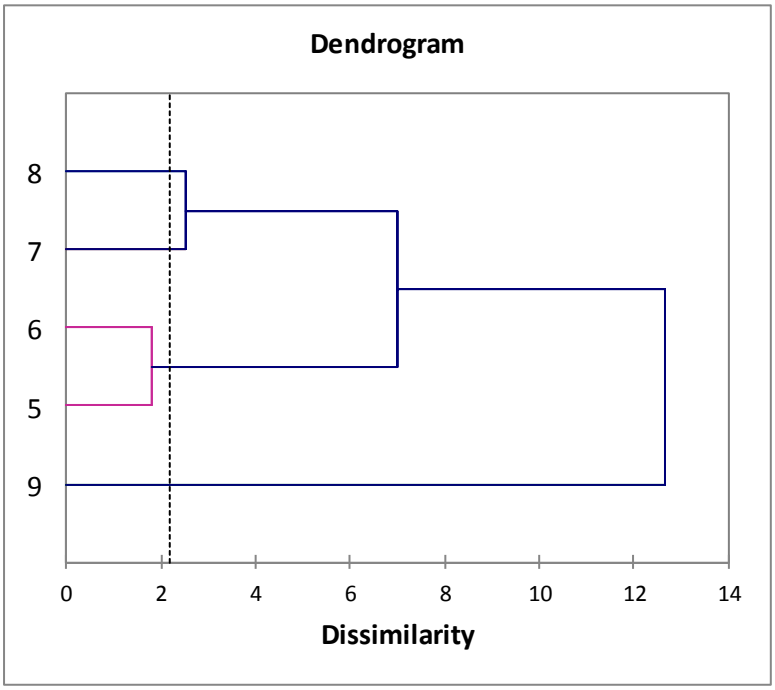


Figure 1 b

**Table 1**

Some *in vitro* methods reported for the evaluation of bioaccessibility.

| **Method**[1] | **Sample weight**[2] | **Oral cavity (saliva)** | **Stomach (gastric juice)** | **Intestine (intestinal juice)** |
|---|---|---|---|---|
| PBET [25] | 0.4 g | - | For 1 L of extracting medium: 0.50 g citrate, 0.50 g malate, 420 µg lactic acid, 500 µg acetic acid, pepsin<br>pH = 2.5 (40 mL, 60 min) | 40 ml gastric juice adjusted to pH 7.0, 70 g mg bile salt, 20 mg panchreatin<br>(40 mL, 180 min) |
| SBET [24,26,27] | 1 g | - | 0.4 M glycine<br>pH = 1.5 (100 mL, 60 min) | - |
| In vitro digestion model[3] [11,20] | 0.6 g | KCl, KSCN, $NaH_2PO_4$, $Na_3PO_4$, NaCl, urea, α-amylase, uric acid, mucin<br>pH = 6.5 (9 mL, 5 min)[3] | NaCl, $NaH_2PO_4$, KCl, $CaCl_2.2H_2O$, $NH_4Cl$, HCl, glucose, glucuronic acid, urea, glucoseamine hydrochloride, bovine serum albumin, pepsin, mucin<br>pH = 1.1(13,5 mL, 120 min) | Duodenal juice (NaCL, $NaHCO_3$, $KH_2PO_4$, KCl, $MgCl_2$, HCl, urea, , $CaCl_2.2H_2O$, bovine serum albumin, pancreatin, lipase<br>pH 7.8<br>Bile (NaCl, $NaHCO_3$, KCl, HCl, urea, $CaCl_2.2H_2O$, bovine serum albumin, bile (chicken-)<br>pH 8.0<br>(27 mL duodenal fluids, 9 ml bile, 120 min) |
| SHIME [11,28] | 10 g | - | For 1 L of extracting medium: 15 g Nutrilon (commercial baby food), 16 g pectine, 8 g mucin, 5 g starch, 1 g cellobiose, 1 g glucose, 1 g proteose peptone, 18 ml milk cream<br>pH = 4.0 (25 mL. 180 min) | Pancreatic fluid. For 1 L of extracting medium: 12 g $NaHCO_3$, 4 g bovine bile, 0.9 g pancreatine<br>pH = 6.5 (15 mL, 300 min) |
| TIM[4] [11,29] | | Electrolytes, α-amylase pH = 5.0 (50 mL, 5 min) | Electrolytes, pepsin, lipasepH decreasing from 5.0 to2.0, (250 mL, 90 min) | Electrolytes, bile and pancreatin pH increasing from 6.5 to7.2 (210 mL, 360 min) |

**Table 1 (continued)**

| Method[1] | Sample weight[2] | Oral cavity (saliva) | Stomach (gastric juice) | Intestine (intestinal juice) |
|---|---|---|---|---|
| E DIN 19738 [30,31] | | - | For 1 L of extracting medium: 2.9 g NaCl, 0.7 g KCl, 0.27 g $KH_2PO_4$, pepsin, mucin pH = 2.0 (100 mL, 120 min) | Porcine bile, trypsin, pancreatin pH = 7.5 (100 mL, 360 min) |

[1] PBET: Physiologically Based Extraction Test [25]; SBET: Simplified Bioaccessibility Extraction Test [24] or Simple bioavailability extraction test [26] or Simple Bioaccessibility Extraction Test [27]; SHIME: Simulator of Human Intestinal Microbial Ecosystem of Infants [11,28]; TIM: TNO Gastro-Intestinal Model (TNO: The Netherlands Organization for Applied Scientific Research) [11,29]; DIN: Deutsches Institut für Normung. Standard method: Soil quality — absorption availability of organic and inorganic pollutants from contaminated soil material [30,31].

[2] Sample weights are expressed as dry weight

[3] Details on the amount of each constituent are reported in ref. [20]

[4] TIM is a dynamic multi-compartmental apparatus, simulating conditions of the gastro-intestinal tract, which can be used with different extracting media. Data reported for saliva, gastric and intestinal juice are taken from ref. [29]

**Table 2**

Characteristics of the investigated samples.

| Sample Id. | Sample name | Ingredients | Aspect | Uses and posology |
|---|---|---|---|---|
| 1 | ASHWAGANDHI LEGIYAM | Ashwagandhi (*Seemai Amakkar, Withania somnifera*), Thiratchai (*Vitis vinifera*), Seeragam (*Cuminum cyminum*), Pareechu (*phonex dactylifera*), Sandanam (*Sandalum album)*, Jadhikkai (*Myristica fragrans*), Korai Kizhangu (*Cyprus rotundus*), Kadukkaithol (*Rhus succidina*), Kokkaineer (*Marntana aurundiracea*), Chukku (*Zingiber officinale*), Milagu (*Piper nigrum*), Thippili (*Piper longum*), Vilamichanver (*Cymbopogan jwarangusa*), Kirambu (*Syzigium aromaticum*), Sevviyam (*Piper nigrum*, radice), Chithiramoolam (*Plumbago zeylanica*), Lavanga Pattai (*Cinnamon zeylanicum*), Sirunagapoo (*Cynnammon verum*), Sarkkai (*Saccharum officinuram*). Milk, Pasunei (ghee) | Brown gel | Anemia, lack of appetite, icterus: improvement of well-being in general.<br>3 -6 g with milk, twice a day |
| 2 | THIPPILI RASAYANAM | Thippili (*Piper longum*), Chukku (*Zingiber officinale*), Milagu (*Piper nigrum*), Seeragam (*Cumium cyminum*), Karum Seeragam (*Nigella sativa*), Omum (*Trachyspermum ammi*), Sitraraththai (*Alpina calcarata*), Peraraththai (*Alpina galanga*), Kaddukai (*Terminalia chebula*), Nellikkai (*Emblica officinalis*), Thaanrikkai (*Terminalia belerica*), Lavangam (*Syzygium aromaticum*), Lavanga paththiri (*Cinnammomum tamala*), Thaaleesa paththiri (*Taxus baccata*), Kodiveli. Ver Pattai (*Plumbago indica*), Elakkai (*Eletteria cardamomum*), Lavanga Pattai (*Cinnamomum cassia*), Sarkkarai (*Saccharum officinarum*). Thein (honey) | Green gel | Cough, cold, hemoptysis, bronchitis, lung disease, dyspnea, diarrhea.<br>5 - 10 g with milk or ghee, twice a day |
| 3 | THAALEESRADHI CHOORANAM | Thaaleespatra (*Abies webbiana*), Milagu (*Piper nigrum*), Chukku (*Zingiber officinale*), Thippili (*Piper nigrum*), Lavanga Pattai (*Cinnamomum zeylanicum*), Elakkai (*Elettaria cardammomum*), Sarkkarai (*Saccharum officinuram*) | Pale brown powder | Cough, cold, congestion.<br>1 - 5 g with honey or hot water |
| 4 | PAVAZHA PARPAM | Corals, rabbit blood | Fine white powder | Anemia, anorexia, emesis, burning sensation.<br>100 - 200 mg with milk or ghee, , twice a day |
| 5 | THALAKA CHENDURAM | Appala karam, Padikaram, Paththira thalakam (Suththi seithathu) | Fine yellow powder | Urinary tract infection.<br>50 - 100 mg with milk or ghee, twice a day |

**Table 2. Continued**

| Sample Id. | Sample name | Ingredients | Aspect | Uses and posology |
|---|---|---|---|---|
| 6 | TALAKA BHASMA | Talaka, Karavalli svarasa, Palasa tvak ksata | Fine green-brown powder | Astringent, antispasmodic; cough soother; hepatic disorders<br>65 - 130 mg with honey, once a day |
| 7 | THAMBIRA PARPAM | Suththi seitha thaamira thakadu or paththram, Induppu, Elumichcham pazha saru, Adutheenda palai saru, Azhinjil ver kiyazham | Fine black powder | Astringent, antispasmodic; ulcer<br>3 mg/Kg<br>with honey and ghee<br>once a day |
| 8 | PANCHAMRIT PARPATI | Thambra bhasma (copper), Abhraka bhasma (mica), Loha bhasma (iron), Shudda bhasma (mercury), Shudda gandhaka (sulfur) | Fine black/ brown powder | Anorexia, diarrhea, discomfort in general, eye diseases<br>125 - 500 mg<br>twice a day. |
| 9 | JAHARMOHRA KATAI PISTI | Not reported | Grey powder | Tonic; palpitation, spasms, tetanus, hypertension<br>200 - 400 mg<br>twice a day |

**Table 3**

Total concentrations of As, Cr, Cu, Hg, Mn and Pb in the investigated samples (mg/kg).

| Sample | As | Cr | Cu | Hg | Mn | Pb |
|---|---|---|---|---|---|---|
| 1 | ≤ 1.3 | ≤ 0.1 | 1.7 ± 3.3 | 3.6-± 1.8 | 8.4 ± 1.2 | 0.6 |
| 2 | 1.1 ± 0.2 | ≤ 0.1 | 3.2 ± 0.1 | 2.0 ± 0.04 | 53.0 ± 1.0 | 1.8-± 0.4 |
| 3 | ≤ 1.3 | ≤ 0.1 | 1.1 ± 0.01 | ≤ 1.0 | 42.7 ± 0.4 | 0.5 ± 0.1 |
| 4 | 19.1 ± 1.1 | 7.0 ± 0.7 | 0.6 ± 0. 1 | 5.3 ± 0.2 | 41.7 ± 2.6 | 0.7 ± 0.1 |
| 5 | 419000 ± 600 | 6.7-± 0.1 | 26.7 ± 0.5 | 253 ± 3 | 36.4 ± 0.1 | 12.5 ± 0.1 |
| 6 | 479000 ± 4000 | 6.0 ± 0.2 | 56.2 ± 0.2 | 126 ± 2 | 18.9 ± 0.8 | 37.3 ± 0.3 |
| 7 | 45.8 ± 4.6 | 28.6 ± 0.4 | 675000 ± 1600 | 161 ± 2 | 197 ± 6 | 132 ± 1 |
| 8 | 567 ± 31 | 11.8 ± 0.3 | 21400 ± 9200 | 15600 ± 1500 | 168 ± 1 | 3.2 ± 3.6 |
| 9 | 19.1 ± 2.9 | 643 ± 25 | 81.0 ± 4.0 | 109 ± 5 | 243 ± 4 | 248 ± 13 |

**Table 4**

Comparison of tolerable uptake levels with the amounts of PTEs ingested following the posology of each product. The values are expressed as mg/day for a 60 kg individual. Element intakes exceeding the reference levels are written in boldface.

| Sample | Dose | As | Cr | Cu | Hg | Mn | Pb |
|---|---|---|---|---|---|---|---|
| 1 | Min. | - | - | 0.017 | **0.036** | 0.084 | 0.006 |
| | Max. | - | - | 0.034 | **0.072** | 0.168 | 0.012 |
| 2 | Min. | 0.007 | - | 0.019 | 0.012 | 0.318 | 0.011 |
| | Max. | 0.014 | - | 0.038 | 0.024 | 0.636 | 0.021 |
| 3 | Min. | - | - | 0.002 | - | 0.085 | 0.001 |
| | Max. | - | - | 0.011 | - | 0.427 | 0.005 |
| 4 | Min. | 0.004 | 0.001 | $1.3 \cdot 10^{-4}$ | 0.001 | 0.008 | $1 \cdot 10^{-4}$ |
| | Max. | 0.008 | 0.003 | $2.6 \cdot 10^{-4}$ | 0.002 | 0.017 | $3 \cdot 10^{-4}$ |
| 5 | Min. | **42** | $6.66 \cdot 10^{-4}$ | 0.003 | 0.025 | 0.004 | 0.001 |
| | Max. | **84** | 0.001 | 0.005 | **0.051** | 0.007 | 0.002 |
| 6 | Min. | **31** | $3.9\ 10^{-}$ | 0.004 | 0.008 | 0.001 | 0.002 |
| | Max. | **62** | $7.8\ 10^{-4}$ | 0.007 | 0.016 | 0.002 | 0.005 |
| 7 | | 0.008 | 0.005 | **122** | 0.029 | 0.035 | 0.024 |
| 8 | Min. | **0.142** | 0.003 | 5.35 | **4** | 0.042 | $8 \cdot 10^{-4}$ |
| | Max. | **0.567** | 0.012 | 21.4 | **16** | 0.168 | 0.003 |
| 9 | Min. | 0.008 | **0.257** | 0.032 | **0.044** | 0.097 | 0.099 |
| | Max. | 0.015 | **0.514** | 0.065 | **0.087** | 0.194 | 0.198 |
| **Tolerable level[1,2]** | | 0.018 | 0.05 | 30 | 0.034 | 3 | 0.21 |

[1]Acronyms for institutions and tolerable levels: Agency for Toxic Substances and Disease Registry: ATSDR; European Food Safety Authority:EFSA; Joint FAO/WHO Expert Committee on Food Additives: JECFA; Minimal Risk Levels: MRLs; Provisional Maximum Tolerable Daily Intake: PMTDI; Provisional Tolerable Weekly Intake: PTWI; Tolerable Daily Intake: TDI

[2]Sources and original values of tolerable levels: As: $3 \cdot 10^{-4}$ mg/kg bw/day (MRL issued by ATSDR); Cr(VI): $9 \cdot 10^{-4}$ mg/kg bw/day (MRL for chronic toxicity issued by ATSDR); Cu: 0.5 mg/kg bw/day (PMTDI issued by JECFA); Hg: 4 µg/kg bw/week (PTWI issued by JECFA); Mn; 0,05 mg/kg bw/day (TDI, issued by EFSA); Pb: 0,025 mg/kg bw/week (PWTI previously issued by JEFCA and now withdrawn).

**Table 5**

Concentrations (mg/kg) and percentages of bioaccessible elements in selected ayurvedic products. GM = gastric medium; IM = intestinal medium

| Sample – Medium | Unit | As | Cr | Cu | Hg | Mn | Pb |
|---|---|---|---|---|---|---|---|
| **5 – GM** | mg/kg | 144000 ± 4000 | 1.46 ± 0.11 | 2.31 ± 0.52 | 11.9 ± 0.3 | 26.8 ± 0.5 | 0.64 ± 0.01 |
| | % | 34.4 ± 1.0 | 21.9 ± 1.7 | 8.7 ± 1.9 | 4.7 ± 0.1 | 73.6 ± 1.4 | 5.1 ± 0.1 |
| **5 – IM** | mg/kg | 388 ± 50 | ≤ 0.12 | 0.33± 0.10 | ≤ 0.10 | 0.47 ± 0.01 | ≤ 0.10 |
| | % | 0.1 ± 0.01 | - | 1.2 ± 0.4 | - | 1.3 ± 0.03 | - |
| **6 – GM** | mg/kg | 29600 ± 3900 | 1.18 ± 0.35 | 2.73 ± 0.06 | 4.31[1] | 9.35± 0.72 | 10.3± 1.6 |
| | % | 6.2 ± 0.8 | 19.6 ± 0.9 | 4.8 ± 0.1 | 0.1 | 49.5 ± 3.8 | 27.6 ± 4.2 |
| **6 – IM** | mg/kg | 21700 ± 1600 | 0.26 ± 0.18 | 1.88 ± 0.13 | ≤ 0.10 | 1.07 ± 0.14 | ≤ 0.10 |
| | % | 4.5 ± 0.3 | 4.3 ± 2.9 | 3.3 ± 0.2 | - | 5.7 ± 0.8 | - |
| **7 – GM** | mg/kg | 43.0 ± 0.6 | 0.97 ± 0.19 | 1341 ± 99 | 103 ± 7 | 12.1 ± 1.0 | 9.15[1] |
| | % | 93.9 ± 1.3 | 3.4 ± 0.7 | 0.2 ± 0.01 | 64.0 ± 4.4 | 6.1 ± 0.5 | 3.3 |
| **7 – IM** | mg/kg | 0.42 ± 0.14 | ≤ 0.12- | ≤ 0.30 | 4.89 ± 0.43 | 0.55 ± 0.32 | 0.16 ± 0.08 |
| | % | 0.9 ± 0.3 | - | - | 3.0 ± 0.3 | 0.3 ± 0.2 | 0.1 ± 0.06 |
| **8 – GM** | mg/kg | 437 ± 42 | 6.39 ± 0.17 | 519 ± 9 | 326 ± 5 | 9.89 ± 0.70 | 2.17± 0.32 |
| | % | 77.1 ± 7.4 | 54.2 ± 1.5 | 2.4 ± 0.04 | 2.1 ± 0.03 | 5.9 ± 0.4 | 67.0 ± 10.0 |
| **8 – IM** | mg/kg | 87.2 ± 0.04 | 1.13± 0.002 | 22.0 ± 0.3 | ≤ 0.10 | 0.84 ± 0.001 | 0.12± 0.0002 |
| | % | 15.4 ± 0.01 | 9.6 ± 0.02 | 0.1 ± 0.001 | - | 0.5 ± 0.001 | 3.7 ± 0.01 |
| **9 – GM** | mg/kg | 3.68 ± 0.18 | 25.1 ± 2.1 | 34.2 ± 6.1 | 44.8 ± 1.2 | 93.1 ± 1.7 | 246 ± 5 |
| | % | 19.3 ± 0.9 | 3.9 ± 0.3 | 42.2 ± 7.5 | 41.1 ± 1.1 | 38.3 ± 0.7 | 99.2 ± 2.0 |
| **9 – IM** | mg/kg | 1.45 ± 0.16 | 4.16 ± 0.32 | 1.27 ± 1.16 | ≤ 0.10 | 2.35 ± 0.42 | ≤ 0.10 |
| | % | 7.6 ± 0.8 | 0.6 ± 0.05 | 1.6 ± 1.4 | - | 1.0 ± 0.2 | - |

[1]The concentrations of Hg in sample 6 (GM) and Pb in sample 7 (GM) were detectable only in one of the investigated specimens, owing to the heterogeneity of the material, as observed in our previous studies [44].

**Table 6**

Concentrations (mg/kg) and percentages of bioaccessible elements in selected ayurvedic products after mixing with *ghee.*

GM = gastric medium; IM = intestinal medium.

| Sample | Medium | As | | Cr | | Cu | | Hg | | Mn | | Pb | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg | % |
| **5** | GM | 9469 | 2.3 | ≤ 0.05 | - | 1.54 | 5.8 | 2.97 | 24.9 | 0.52 | 1.4 | ≤ 0.10 | - |
| | IM | 406 | 1.0 | ≤ 0.05 | - | 0.16 | 0.6 | 7.99 | 3.2 | 0.35 | 1.0 | ≤ 0.10 | - |
| **6** | GM | 526 | 0.1 | 0.07 | 1.2 | 2.58 | 4.6 | 11.7 | 9.3 | 2.58 | 13.6 | ≤ 0.10 | - |
| | IM | 483 | 0.1 | 1.8 | 0.02 | 1.8 | 3.2 | ≤ 0.10 | - | 0.12 | 0.7 | ≤ 0.10 | - |
| **7** | GM | 7.22 | 15.8 | ≤ 0.05 | - | 6757 | 1.0 | 0.71 | 0.5 | 3.99 | 2.0 | 1.52 | 0.6 |
| | IM | 3.11 | 6.8 | ≤ 0.05 | - | 5727 | 0.8 | 0.28 | 0.2 | 0.75 | 0.8 | ≤ 0.10 | - |

**Table 7**

Percentages and concentrations of bioaccessible elements in sample 6 (*Talaka Bhasma*) inserted in a dialysis tube, with *ghee* ("Dialysis + ghee") and without it ("Dialysis") . GM = gastric medium; IM = intestinal medium.

| **Procedure** | | **As** | | **Cr** | | **Cu** | | **Hg** | | **Mn** | | **Pb** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg | % |
| **Dialysis** | GM | 23500 | 4.9 | 0.68 | 11.1 | 3.69 | 6.6 | 2.51 | 2.0 | 8.19 | 43.3 | 15.3 | 41.9 |
| | IM | 8060 | 1.7 | 0.18 | 2.9 | 2.62 | 4.7 | 0.25 | 0.2 | 2.00 | 10.6 | 2.85 | 7.6 |
| **Dialysis + *ghee*** | GM | 543 | 0,1 | ≤ 0.05 | - | 1,95 | 3,5 | 1,68 | 1,3 | 2,58 | 13,6 | ≤ 0.10 | - |
| | IM | 483 | 0,1 | ≤ 0.05 | - | 1,81 | 3,2 | ≤ 0.10 | - | 0,12 | 0,7 | ≤ 0.10 | - |

**Table 8**

Elements exceeding the tolerable reference levels. Element intakes upon consumption of product 6 were calculated from bioaccessible concentrations. Tolerable levels are expressed in mg/day for a 60 kg individual. GM = gastric medium; IM = intestinal medium.

| Procedure | As | Cr | Cu | Hg | Mn | Pb |
|---|---|---|---|---|---|---|
| **Method A** | Sample 5 (min and max), GM<br>Sample 6 (min and max), GM<br>Sample 6 (min and max). IM | - | - | Sample 7, GM<br>Sample 8 (min and max), GM<br>Sample 9 (min and max), GM | - | - |
| **Method B** | Sample 5 (min and max), GM | - | - | - | - | - |
| **Method C** | Sample 5 (min and max), GM | - | - | - | - | - |
| **Method D** | - | - | - | - | - | - |
| **Tolerable level** | 0.018 | 0.05 | 30 | 0.034 | 3 | 0.21 |