Biological Trace Element Research (2023) 201:5848–5860
https://doi.org/10.1007/s12011-023-03642-y

Correlation Between Toxic Elements and Pesticide Residues in Medicinal Herbs Available in Pharmaceutical Market

Mihaela Mirela Bratu[1] · Semaghiul Birghila[2] · Corina Birghila[3] · Valentina Coatu[4] · Diana Andreea Danilov[4] · Naliana Lupascu[5] · Dan Vasiliu[5] · Marius Daniel Radu[6]

Received: 24 February 2023 / Accepted: 17 March 2023 / Published online: 25 March 2023

Abstract

The use of medicinal plants for self-medication of minor health conditions has become a widespread practice in contemporary society. Few consumes, however, question the contamination of these products with toxic factors resulting from the planet's increasingly polluted environment. This paper presents the levels of five toxic elements (As, Cr, Pb, Cd, and Hg) and nine organochlorine pesticides (hexachlorobenzene (HCB), lindane, heptachor, aldrin, dieldrin, endrin, p,p'DDE, p,p'DDD, and p,p'DDT) in 14 brands of regularly consumed medicinal products in Romania. The toxic elements content was determined using energy-dispersive X-ray fluorescence (EDXRF) technique, and organochlorine pesticide residues (OPCs) were quantified using gas-chromatographic method, equipped with electron capture detector (GC-ECD). The results show that in the case of Cr, Cd, and Hg, the concentrations exceeded the limit values established by World Health Organisation (WHO) for raw herbal material. The higher level of OPCs (such as p,p'DDD, p,p'DDT, aldrin, and dieldrin) was found in the samples of *Hypericum perforatum*-St. John's wort, *Crataegus monogyna*-hawthorn, and *Epilobium parviflorum*-hoary willowherb. The correlations between the content of toxic elements and pesticides were determined by statistical analysis. Hierarchical clustering technique was used to detect natural grouping between the toxic elements and pesticides. For herb samples, four clusters were identified, the strongest correlated cluster consisting of Pb, HCB, Cr, and Hg. A further analysis within this cluster suggested that Cr levels are statistically different from the rest of the elements.

Keywords Herbal medicines · Toxic elements · OPCs · Clustering analysis

✉ Semaghiul Birghila
sbirghila@univ-ovidius.ro

Mihaela Mirela Bratu
mmbratu@hotmail.com

Corina Birghila
corina.birghila@ovgu.de

Valentina Coatu
vcoatu@alpha.rmri.ro

Diana Andreea Danilov
ddanilov@alpha.rmri.ro

Naliana Lupascu
naliana.lupascu@geoecomar.ro

Dan Vasiliu
dan.vasiliu@geoecomar.ro

Marius Daniel Radu
drd_maryus@yahoo.com

1 Department of Pharmaceutical Sciences II, Ovidius University of Constanta, 6, Aleea Universitatii, Campus 1, 900470 Constanta, Romania

2 Department of Chemistry and Chemical Engineering, Ovidius University of Constanta, 124, Mamaia Avenue, 900527 9 Constanta, Romania

3 Faculty of Mathematics, Institute for Mathematical Stochastics, Otto-von-Guericke University Magdeburg, Universitätsplatz 2, 39106 Magdeburg, Germany

4 NIMRD "Grigore Antipa" National Institute for Marine Research and Development, Bvd.Mamaia, nr. 300, RO-900581 Constanta, Romania

5 National Institute for Research and Development on Marine Geology and Geoecology – GeoEcoMar, 23-25 Dimitrie Onciul Str, Bucharest, Romania

6 Department of Biological Sciences II, Ovidius University of Constanta, Aleea University Avenue, Campus 1, 900470 Constanta, Romania

Introduction

The use of herbal remedies as first choice in the empirical treatment of minor health conditions is widespread worldwide today.

The World Health Organisation (WHO) estimated that about 70–80% of the world's population trusts alternative therapies, in which herbal medicines are predominantly used [1]. These percentages are expected to increase with the globalisation process and given the current socio-economic conditions. Most of these plants are grown in the wild, with only a few being cultivated. This requires rigorous toxicological control of herbal products for therapy, including pollutants absorbed by the plants from the environment in which they grow. Otherwise, instead of the expected benefits, herbal cures could cause serious damage to human health.

Medicinal plants can be easily contaminated with toxic pollutants from soil, water, and air. Soil is usually contaminated by intensive agricultural activities using pesticides to control pests, by various industrial activities, by rainwater, or by atmospheric dust. The accumulation of pollutants such as toxic elements and pesticides in the soil is increasing with the accelerating growth of pollution in the natural environment [2].

The uptake of toxic elements from polluted soils by plant roots depends on both soil characteristics (pH, organic load) and species [3]. These are metals that are more rapidly taken up by the plants, such as Cd, Tl, and Zn, which have a higher transfer coefficient. Other metals such as Cu, Cr, Co, and Pb are strongly bound to soil structures and have low transfer coefficients [4].

Some of these elements, when in concentrations above the permitted limit, can cause cancers and central nervous system damage (Hg, Pb, and As), while others can cause liver or kidney damage (Pb, Cd, and Cu) or affect the development of bones and teeth (Ni, Cd, Cu, and Cr) [5, 6].

Organochlorine pesticides (OPCs) have been widely used over the last century for agricultural and non-agricultural purposes worldwide. Even though they have been banned in most countries, due to their high persistence in the environment, their residues are still found in many matrices, including medicinal herbs [7].

Pesticides contained in medicinal herbs from contaminated soil and water can also lead to hepatotoxicity, nephrotoxicity, developmental disorders in children, and respiratory and cardiovascular system damage [8, 9].

Taghizadeh et al. [10] evaluated the potential poisoning risk to consumers of some food products (dates), in which the authors identified 22 pesticides, 6 toxic elements, and 16 polycyclic aromatic hydrocarbons.

A study conducted by Luo et al. [11] on medicinal plant crops in China showed that more than half of the samples analysed had pesticide content above the European Pharmacopoeia (EP) limit, with some of the products contain 35 types of banned, highly toxic pesticides detected in concentrations well above the maximum permitted level.

As for toxic elements contained in herbal therapeutic products, Yang et al. [12] quantified the metals contained in 279 traditional Chinese herbal remedies; the authors reached the alarming conclusion that some of the analysed products contained amounts above the permitted limits of Pb, As, Cd, and Hg. Other authors, Filipiak-Szok et al. [13], also investigated the content of toxic elements in European herbal medicines and herbal nutritional supplements and showed low levels of contamination. Also, Kulhari et al. [14] investigated the content of nine mineral elements (Mn, Cr, Pb, Fe, Cd, Co Zn, Ni, and Hg) in ten medicinal herbs collected from natural areas in northwest India, concluding that, except for Cr, all other elements were within permissible concentrations.

Currently, there are few studies attempting to correlate toxic elements content with pesticides in natural therapeutic products.

In this paper, was investigated the content of five elements (Hg, Pb, As, Cd, and Cr) and nine organochlorine pesticides (HCB, lindane, heptachor, aldrin, dieldrin, endrin, p,p'DDE, p,p'DDD, and p,p'DDT) in herbs available in Romanian pharmacies. The correlations between the concentrations of toxic elements and pesticides were determined by a statistical analysis. A hierarchical clustering method was used to detect the natural grouping between toxic elements and pesticides in herb samples. Clustering analysis is a statistical technique of partitioning variables or observations based on a given measure of similarity. The method was applied to illustrate the source of higher mineral levels in rice herbs [15], agricultural soil [16, 17], urban soil [18], or groundwater and soil samples affected by surgical instrument industries discharges [19].

Therefore, we consider that the evaluation of pollutants in herbs as raw materials applied in the pharmaceutical industry is necessary from a regulatory point of view. Considering the fact that these pollutants do not offer any therapeutic benefit, their content in the final products is important from a toxicological point of view.

Experimental

Sampling

The analysed samples consisted of 14 medicinal plants preserved by drying, known and commonly used in Europe, having monographs in the European Pharmacopoeia [20]. They were purchased only from Romanian pharmacies and come from certified Romanian producers. Table 1 presents information on the studied plants. In order to reduce particle size and satisfy the conditions for homogeneity, the samples were ground in an electric grinder, using a grinding time of 2–3 min. Once plants were powdered, they were kept in capped polypropylene flasks until analysis.

Toxic Element Analysis

The energy dispersive X-ray fluorescence (EDXRF) is a technique used to identify mineral elements, being considered a simple and non-destructive method for multielement analysis [21]. The concentrations of mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd), and chromium (Cr) were determined according the method proposed by Jyothsna et al. [22] using EDXRF technique (XEPOS C spectrometer, Na-U, Spectro Germany). The instrument is equipped with an X-ray tube using Rh anode and a silicon drift detector (SDD) operated at 50 kV and 1000 uA. The quantitative analysis is carried out using the X-LAB Pro software. The accuracy and precision of the EDXRF analysis were checked by testing a certified reference material (NIST SRM 1571-Orchard leaves). The results for the recoveries ranged from 90 to 104%, which indicated good correlation between the certified and measured values.

Organochlorine Pesticide Analysis

Two grams of homogenised sample was placed in a 50-mL recipient. Thirty millilitre hexan were added, and the mixtures were submitted to microwave extraction for 50 min at 120 °C. Subsequently, the extracts were cleaned up and fractionated by passing through a florisil column, previously conditioned with solvent. Extracts were then concentrated using the Kuderna-Danish concentrator to 1 mL before injection in gas chromatograph.

The analytical determination of organochlorine pesticide content was conducted by the gas-chromatographic method with a Perkin Elmer gas chromatograph CLARUS 500, equipped with electron capture detector. This model was equipped with a Perkin Elmer column, Elite 5MS having the following dimensions 30 m × 0.25 mm × 0.50 µm, and temperature range −60 to 330 °C. The carrier gas is helium; sample injection volume is 1.0 µl. Detector and injector temperature was initially set at 180 °C for 0 min, ramped 1–7 °C/min to 230 °C for 10 min, and ramped 2–15°/minute to 250°C for 10 min.

The identification of each compound was done by comparing the retention time of the peaks in the chromatograms with the retention time of the standards. Quantification was carried out by interpolation from the calibration curves, obtained by analysis of five different standard concentrations. The correlation coefficients derived from the linear regressions were higher than 0.9800, showing the

Table 1 General information on analysed medicinal herbs

No	Latin name	Common name	Herbal medicine uses
1	*Centaurea cyanus* L.	Cornflower	Treatment of the ocular inflammations, as diuretic
2	*Verbascum thapsus* L.	Common mullein	Treatment of cough, respiratory infections
3	*Artemisia absinthium* L.	Wormwood, Absinthe, Mugwort	For appetite increasing, external for mycosis, dermatitis, infections
4	*Chelidonium majus* L.	Swallow wort	As a mild sedative, antispasmodic and detoxifying, hepatobiliary affections, antiviral
5	*Taraxacum officinale* Webb.	Dandelion	As a diuretic, cholagogue, laxative, stomachic, and tonic
6	*Betula pendula* Roth.	Silver birch	Anti-inflammatory, cholagogue, diaphoretic
7	*Tilia cordata* Mill.	Small leaved lime	Lime flowers are a popular remedy in the treatment of colds and other ailments where sweating is desirable
8	*Matricaria chamomilla* L.	Chamomile	For a sore stomach, irritable bowel syndrome, and as an anti-inflammatory effect
9	*Hypericum perforatum* L.	St. John's wort	Used in treating pulmonary complaints, bladder problems, nervous depression
10	*Crataegus monogyna*	Hawthorn, oneseed hawthorn	For treating disorders of the heart and circulation system, especially angina pectoralis
11	*Xanthium spinosum* L.	Spiny cocklebur	Styptic, diaphoretic, diuretic and sedative
12	*Calendula officinalis* L.	Pot marigold	For skin problems (insects bites and stings, sprains, wounds, sore eyes, varicose veins)
13	*Epilobium parviflorum* Schreb.	Smallflower hairy willowherb	In disorders of the prostate gland, bladder and kidney, having an antioxidant and antiinflammatory effect
14	*Rosa canina* L.	Dog rosehip	For the treatment of colds, influenza, minor infectious diseases, scurvy, diarrhoea

relationships between peak area and analyte concentration. The detection limits were ranged between 0.002 and 0.003 $\mu g \cdot g^{-1}$, and recovery percentages corresponding to each compound were found in the range 89–103, indicating the accuracy of the method.

Statistical Analysis

The statistical software used in the analysis is R version 3.4.3 [23]. The Pearson correlation matrix between metals and pesticides was computed using the function *cor*, while the function *hclust* was applied for the hierarchical clustering based on the correlation matrix. Both functions are found in the R package *stats*. The chosen similarity measure for clustering is:

$$d(i,j) = 1 - \left|\rho_{i,j}\right| \quad (1)$$

where $|\rho_{i,j}|$is the absolute value of the Pearson correlation coefficient between two elements *i* and *j* and. Further properties of this similarity measure can be found in study of Gu and Wang [24]. To decide the optimal number of clusters, the function *kgs* in R package *maptree* was used to compute the Kelley-Gardner-Sutcliffe penalty function. For the cluster with the larger number of nonzero values, the statistical significance difference between the components of the clusters was assessed based on Kruskal-Wallis Rank Sum test at 5% level, implemented in *kruskal.test* in the package *stats*. Before applying the test, the normality of the samples was statistically checked using the Shapiro-Wilk Normality test *shapiro.test* at level $\alpha = 0.05$. Furthermore, the Wilcoxon Rank sum test with Benjamini and Hochberg correction [25] *pairwise.wilcox.test* was employed to decide which element in the cluster is statistically different from the others.

Results and Discussion

Toxic Elements in Medicinal Herbs

A number of mineral elements important for nutrition can accumulate in medicinal herbs [26], as well as toxic elements such as Cd, Hg, and Pb, which are not directly used by plant and are harmful to human health [27]. Table 2 shows the results of determinations made on 14 of the most popular herbal medicinal products sold in Romanian pharmacies. As show in Table 2, the herbs contained toxic elements in the following order Cr > Pb > Cd > Hg > As.

Arsenic is considered a non-essential element. Its contamination is caused by geological sources, pesticide application, and some industrial processes. In this study, As was present only in *Calendula officinalis L*; in the rest of the herbs, arsenic was not detected, indicating that the activities carried out in the areas where plants were harvested do not lead to arsenic accumulation in the soil. Similar results were reported by Sindhu and Beena [29] in medicinal herbs (*Aloe vera L*) available in the local market.

Cadmium is an element with unknown essential functions in both plants and humans [30]. Major sources of Cd in soil and plants are phosphate fertilisers, sewage sludge

Table 2 Toxic elements content in herbs ($mg \cdot kg^{-1}$)

No	Herbs	Cr	As	Cd	Hg	Pb
1	*Centaurea cyanus* L.	9.32 ± 1.09	nd	1.25 ± 0.12	0.285 ± 0.01	0.527 ± 0.03
2	*Verbascum thapsus* L.	4.88 ± 0.45	nd	0.798 ± 0.05	0.650 ± 0.05	1.25 ± 0.10
3	*Artemisia absinthium* L.	4.36 ± 0.70	nd	0.778 ± 0.07	0.692 ± 0.03	1.27 ± 0.28
4	*Chelidonium majus* L.	2.40 ± 0.34	nd	nd	nd	0.689 ± 0.05
5	*Taraxacum officinale* Webb.	9.43 ± 1.56	nd	0.454 ± 0.02	0.688 ± 0.02	2.93 ± 0.20
6	*Betula pendula* Roth.	3.10 ± 0.70	nd	2.34 ± 0.35	nd	0.752 ± 0.08
7	*Tilia cordata* Mill.	2.68 ± 0.52	nd	nd	nd	0.515 ± 0.02
8	*Matricaria chamomilla* L.	1.72 ± 0.81	nd	nd	0.19 ± 0.01	1.09 ± 0.15
9	*Hypericum perforatum* L.	1.90 ± 0.26	nd	1.19 ± 0.42	0.129 ± 0.01	0.697 ± 0.05
10	*Crataegus monogyna* Jacq.	3.45 ± 0.85	nd	0.900 ± 0.37	nd	1.03 ± 0.09
11	*Xanthium spinosum* L.	nd	nd	nd	nd	0.64 ± 0.04
12	*Calendula officinalis* L.	1.89 ± 0.75	0.141±0.02	nd	nd	0.938 ± 0.10
13	*Epilobium parviflorum* Schreb.	1.68 ± 0.42	nd	1.03 ± 0.08	0.503 ± 0.04	1.58 ± 0.21
14	*Rosa canina* L.	0.87 ± 0.09	nd	1.67 ± 0.07	nd	0.120 ± 0.01
Mean ± sd		3.40 ± 0.57	0.01 ± 0.00	0.74 ± 0.05	0.22 ± 0.01	1.02 ± 0.08
% of all samples above permissible limit, WHO, 2007		57%	nd	64%	28%	nd
Maximum limit ($mg \cdot kg^{-1}$) WHO, 2007 [28]		2.0	5.0	0.3	0.2	10

nd not detected, *sd* standard deviation application, and fossil fuel combustion. It has been reported that the toxicity of Cd on the plants can be observed in the form of stunt growth [31]. In this study, the level of cadmium in 64% of herbs exceeded the WHO maximum permissible limit of 0.3 mg·kg^{-1} [28], while in the rest of plants, the content of this element were below the detectable minimum values (<0.001). Similar results of high levels of cadmium have been reported in Iranian medicinal herbs and some medicinal plants commercialised in Turkey [32, 33].

Lead is known as the most common and stable toxic element in nature, being very dangerous for plants, animals, and organisms [34]. Continuous fertiliser application, fuel burning, and sewage sludge are major sources leading to increased Pb pollution. The WHO maximum permissible limit of lead in medicinal herbs is 10 mg·kg^{-1} [28]. In our study, Pb was detected in all herb samples and the values were around 1 mg·kg^{-1}. It is possible that the low value of the soil-plant transfer coefficient of this metal leads to the accumulation of lower amounts of Pb in the aerial parts of plants. It was reported that some of factors such as root surface area and rood exudation can affect the availability and uptake of Pb [35]. Contrary to the data from this study, high level of Pb has been reported in medicinal plants in Jordan and traditional herbs consumed in the United Arab Emirates [32, 36].

Chromium is another toxic element, being released by steel industries, sewage sludge applications, and tanneries [37, 38]. The permissible level for this metal in raw herbals is 2.0 mg·kg^{-1} [28]. In the present study, 57% of samples contained Cr concentrations above the permissible limits defined by WHO. The highest contents have been obtained for *Centaurea cyanus* L. and *Taraxacum officinale* Webb, while the lowest ones for *Rosa canina* L.

Due to its multiple uses (gold mining, batteries, industrial products, and pesticides), mercury is accumulated in different sites and represents a global pollutant. It has been described that the major symptoms of Hg toxicity in plants are decrease of growth process, reduced root development, and inhibition of photosynthesis activity [39]. In the investigated samples, 28% of the plants contained Hg above the limit proposed by WHO, of 0.2 mg·kg^{-1}. Previous researchers, Sindhu and Beena, [29] and Begum et al. [40], reported the presence of mercury below limit levels in medicinal plants.

Minerals can get into medicinal plants and their products from contaminated agricultural sources (soil, water, agrochemicals), during processing or deliberate addition for their supposed medicinal value [41].

In the present work, the data show that toxic elements were present in varying concentrations in the herbs, in some cases exceeding the maximum permitted levels for medicinal plants [28]. Similarly, ten medicinal plants analysed by Ajasa et al. [42] collected from different areas of Ogbomoso (Nigeria) showed variable element levels. In addition, Gajalakshmi et al. [43] studied the presence of toxic elements in some medicinal herb species and revealed similar results. They reported that Cr content was at higher level in all plants.

Some researchers have been reported that these content differences can be attributed to the differences between the absorption and translocation capacities of minerals in plants [36, 44]. The uptake, accumulation, and amount of metals in plants can be influenced by a number of factors, such as atmospheric (traffic density, metal smelting operations), bioavailability of metals in the soil (through the addition of pesticides, sewage sludge or manure), the nature of the soil in which grasses grow (pH, organic matter), plant species (plant maturity, time of harvest), and plant processing conditions [14, 45, 46].

In their research, Srivastava et al. [47] compared the mineral content of different species of herbs collected from their natural habitats and from market samples, in India. Results showed that market samples were more contaminated with toxic elements than natural samples, which may indicate contamination during processing or storage.

At the same time, there are studies which indicate that metal content varies in different parts if the same plant [48]. Thus, Xu et al. [49] showed that accumulation of metals is higher in the leaves than in the root, while Bawa et al. [50] reported higher concentrations of metals in the root than in the shoots.

However, the risk of toxic elements entering a food chain depends on the metal mobility and availability in the soil [51]. In soil, metal cations are bound to negatively charged particles such as clay and organic matter; when metals detach from these particles and enter the soil solution, they become bioavailable with the potential to accumulate in plants and other soil-dwelling/growing organisms [52].

In order to favour the decontamination process of polluted soils, there are now concerns about phytoremediation by growing species capable of extracting and concentrating certain toxic elements [2]. The example of plants of the *family Asteraceae*, alternatively *Compositae*, capable of accumulating and concentrating Cd, Zn, and Cu from soil is known [53].

These results confirm these hyperaccumulative properties of the plants of this family, as both *Centaurea cyanus* and *Taraxacum officinale* were found to contain the highest amounts of toxic elements of all the plants analysed, being the most popular and frequently used medicinal plants. There are several plants of the *Asteraceae* family frequently used in therapy (e.g., *Tagetes patula*, *Achillea millefolium*, *Cichorium intybus*) that could have a decontamination effect through bioconcentration of toxic elements. Therefore, it becomes necessary to introduce control protocols on the toxic element content of herbs and standardised plant extracts for therapeutic use [54]. In addition, the investigation of the specific bioaccumulation properties of each of the herbs used in therapy would be of great value for the improvement of the control protocols developed by the EP [20].

In the case of *Taraxacum officinale Webb* species, studies show that this plant not only accumulates and concentrates toxic elements in soils but its growth and mineral accumulation are stimulated by the addition of fungicides such as thiuram [55]. In nature, fungi and microorganisms are largely responsible for the immobilisation of toxic elements in soil. The application of fungicides thus facilitates the release of these metals, which become bioavailable to plants growing on these treated soils. Recent studies on Asian phytotherapeutic products have shown that toxic elements (As, Cd, Pb) are present in the composition of these products, but below the toxicity limit [56].

Organochlorine Pesticides

OCPs have been widely used in the last century for agricultural and non-agricultural purposes worldwide. Even though they have been banned in most countries due to their persistence, OPCs are still found in areas where they were previously used and often contaminate plants growing nearby. Some of these substances are also still used for public health purposes, for example to control disease vectors such as mosquitoes and are often applied near agricultural fields. Pesticide residues can then pass through the air to crops, contaminating medicinal plants growing in nearly fields.

OPCs contain in their structure carbon, chlorine and hydrogen, and the carbon-halogen bond being highly resistant to degradation. Therefore, organochlorine compounds are degraded very slowly and may remain in the environment and inside organisms after exposure for a longer time [57].

Table 3 presents the content of organochlorine pesticides in the herbs selected for the study as commonly used by the population. The results show the presence of OPCs, such p,p'DDD, p,p'DDT, aldrin, and dieldrin in concentrations above the maximum permitted limit established by the European Pharmapoeia (EP), in *Hypericum perforatum*, *Crataegus monogyna*, and *Epilobium parviflorum* herbs [58]. These plants are known to grow in natural areas, and contamination may be due to plant-specific bioaccumulation processes. The case of *Epilobium parviflorum* species, which became more abundant and vigorous after herbicide (glyphosate) application, is presented in the literature by Matulevičiūtė, [59].

Profile of individual pesticide was γ- HCH (lindane) > p,p'DDT > p,p'DDD > HCB > endrin > aldrin > dieldrin> heptachlor> p,p'DDE. Environmental factors can influence their levels and distribution. Thus, it has been reported that the application of the pesticides in the wet season leads to an easier uptake by the plant [60]. Furthermore, different physicochemical characteristics of soils (organic matter, microorganism activity), as well as properties of pesticide compounds (hydrophobicity), can influence the degradation, adsorption, and bioaccumulation of pesticides in plants [61]. It was reported that DDT is more hydrophobic than HCH; hydrophobic compounds are strongly bound to root and soil organic colloidal surfaces [62].

Some compounds such as heptachlor, dieldrin, and pp'-DDE were detected in high concentrations in some plants (*Epilobium parviflorum Schreb*, *Hypericum perforatum L.*, *Xanthium spinosum L.*), while in others they were in very low levels or not detected. This indicates that some herbs came from areas with lower levels of contamination, or their processing may have contributed to lower pesticide concentrations. Plant processing such as drying, cleaning, grinding, and storage has been reported to reduce pesticide residues in final products [63].

Of the HCHs group, the γ- HCH isomer (Lindane) was detected in most herbs, being above the limit established by EP, in 78% of the plants (Table 3). The results showed variations in lindane concentration from 0.06 ± 0.00 mg·kg^{-1} to 5.30 ± 1.24 mg·kg^{-1}, with a mean value of 1.34 ± 0.09 mg·kg^{-1}, depending on the degree of exposure of herbs to this compound. The primary source of lindane in the environment is associated with contaminated water runoff containing the compound; it is associated with the management of common insecticides on agricultural land (vector control) and the use of animal pest control products. The results obtained by us are lower than those reported by Murtaj et al. [64] in medicinal plants collected in Albania, where α- and β-HCH isomers predominate. The presence of γ-HCH isomer in most of the herbs analysed can also be confirmed by evaluation the ratio α/β-HCH. Thus, the ratio of α/β-HCH > 3 is an indication of an input of technical HCH and long-range atmospheric transport and deposition, while a ratio of close to <1 is characteristic of lindane sources [65]. In the present study, the α/β-HCH mean ratio was closed, indicated the presence of lindane as source of HCHs.

Due to its persistent nature and hydrophobicity, aldrin is readily metabolised to dieldrin. Aldrin has low phytotoxicity; plants are only affected at high level of concentrations. Dieldrin was present in 48% of the samples, and higher amounts were identified in *Artemisia absinthium* L., *Hypericum perforatum* L., and *Epilobium parviflorum* Schreb.

Hexachlorobenzene (HCB) is considered a very persistent compound due to its chemical stability and resistance to biodegradation. In the soil, HCB binds strongly to the organic part, accumulating in plants and crops. Although HCB was detected in most samples, its concentration was above the permissible limit of 0.1 mg·kg^{-1} [58], in 14% of the plants. Its commercial production has been banned in the most of the world; however, recent sources of HCB may include waste and sewage sludge incineration processes, production of chlorinated organic solvents, and fossil fuel combustion

Table 3 Organochlorine pesticides (OPC) in herbs (mg·kg^{-1})

	Herbs	HCB	Lindan	Hepta chlor	Aldrin	Dieldrin	Endrin	p,p'DDE	p,p'DDD	p,p'DDT
1	*Centaurea cyanus* L.	0.04 ± 0.00	1.00 ± 0.17	n.d	0.01 ± 0.00	n.d	n.d	n.d	1.17 ± 0.20	n.d
2	*Verbascum thapsus* L.	0.09 ± 0.00	0.93 ± 0.10	n.d	0.03 ± 0.0	n.d	n.d	n.d	0.27 ± 0.01	n.d
3	*Artemisia absinthium* L.	0.04 ± 0.00	0.94 ± 0.85	n.d	0.14 ± 0.01	0.18 ± 0.01	n.d	0.006 ± 0.00	1.56 ± 0.12	n.d
4	*Chelidonium majus* L.	0.02 ± 0.00	1.00 ± 0.07	n.d	0.10 ± 0.01	n.d	0.16 ± 0.02	n.d	n.d	n.d
5	*Taraxacum officinale* Webb.	1.95 ± 0.18	0.06 ± 0.00	n.d	n.d	n.d	0.005 ± 0.00	n.d	n.d	n.d
6	*Betula pendula* Roth.	0.14 ± 0.01	1.03 ± 0.05	n.d	n.d	0.03 ± 0.00	0.07 ± 0.00	n.d	n.d	n.d
7	*Tilia cordata* Mill.	n.d	0.14 ± 0.01	n.d	0.003 ± 0.00	n.d	0	n.d	n.d	n.d
8	*Matricaria chamomilla* L.	n.d	0.88 ± 0.05	n.d	0.45 ± 0.06	n.d	0.35 ± 0.01	0	1.84 ± 0.31	n.d
9	*Hypericum perforatum* L.	0.03 ± 0.00	3.24 ± 0.55	n.d	0.07 ± 0.00	0.41 ± 0.03	0.06 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	1.16 ± 0.20
10	*Crataegus monogyna* Jacq.	0.08 ± 0.01	5.30 ± 1.24	n.d	0.13 ± 0.01	0.02 ± 0.00	0.32 ± 0.03	n.d	0.16 ± 0.01	1.64 ± 0.42
11	*Xanthium spinosum* L.	n.d	0.21 ± 0.03	n.d	0.004 ± 0.00	0.07 ± 0.00	0	0.14 ± 0.01	0	0.91 ± 0.39
12	*Calendula officinalis* L.	0.005 ± 0.00	1.73 ± 0.15	n.d	0.05 ± 0.00	n.d	1.52 ± 0.21	n.d	n.d	n.d
13	*Epilobium parviflorum* Schreb.	0.04 ± 0.00	1.36 ± 0.10	0.64 ± 0.02	0.21 ± 0.03	0.22 ± 0.05	0.007 ± 0.00	n.d	n.d	9.56 ± 1.05
14	*Rosa canina* L.	0.06 ± 0.0-	0.93 ± 0.08	n.d	0.03 ± 0.00	n.d	0	n.d	n.d	n.d
Range		n.d–1.95	0.06–5.30	n.d–0.64	n.d–0.45	n.d–0.41	n.d–1.52	n.d–0.14	n.d–1.84	n.d–9.56
ΣOCP		2.49	18.75	0.64	2.23	0.93	2.49	0.16	5.02	13.27
Mean ± sd		0.18 ± 0.01	1.34 ± 0.09	0.045 ± 0.01	0.087 ± 0.01	0.066 ± 0.01	0.18 ± 0.02	0.011 ± 0.01	0.358 ± 0.01	0.948 ± 0.08
Maximum permissible limit (mg/kg), EP [58]		0.1	0.6	0.05	0.05		0.05	0.2	0.2	0.2

nd not detected, *sd* standard deviation, *EP* European Pharmacopoeia: applicable to medicinal plant materials included in the European Pharmacopoeia, 5th ed, Pharmeuropa 18 (4) 2006

[66]. It has been mentioned that currently, HCB contamination mainly comes from chemical production processes (where it is used as a chemical intermediate or polymer additive) and incineration processes [67].

Heptachlor is a pesticide that has been widely used mainly against termites and soil insects. It is metabolised by soil bacteria and fungi into different products via independent metabolic pathways [68]. A widespread reaction in the environment is the conversion of heptachlor to the more persistent heptachlor epoxide [69]. In the analysed plants, heptachlor was detected only in *Epilobium parviflorum Schreb*., at levels above the permissible limit [58].

p,p'DDT was detected in 36.66% of the analysed herbs, in amounts higher than its metabolites p,p'DDD and p,p'DDE. Using the ratio between DDT and its metabolites, it is possible to identify the possible source of DDT; thus, a DDT/DDD + DDE ratio higher than 1 indicates recent use of DDT [70].

In the present study, the ratio DDT/DDD + DDE in 10 herbs were 0 as there was no detection of DDT in those plants. The rations in the other samples (*Hypericum perforatum* L., *Crataegus monogyna* Jacq., *Xanthium spinosum* L.) exceeded 1 value; this indicates that there was recent input of DDT into the agricultural areas. Also, the ratio of parent DDT to its metabolite DDE, higher than 1 in *Hypericum perforatum* L. and *Xanthium spinosum* L., plants, indicates the presence of DDT in the last years [71]. At the same time, the ratio of DDD to DDE, higher than 1 in *Hypericum perforatum* L. and *Artemisia absinthium* L species, shows anaerobic degradation of DDT [72].

The results of the present study showed higher detection of OPC residues in medicinal plants than the data reported in Ethiopia and Iran [73, 74] and lower than those in China [75].

Correlation Coefficient Matrices and Cluster Analysis

Cluster analysis was used to group the metals and pesticides into groups with homogeneous structures. The technique reveals hidden clusters among the objects, based on a similarity measure. The advantage of clustering analysis is that the number of groups does not need to be known a priori, but it is instead obtained as a result of some exploratory techniques [76].

In this analysis, the aim was to empirically discover which groups of metals and pesticides are likely to be found together in herbs. The clustering is performed using the distance (1) in section Statistical Analysis. In this way, elements that are highly correlated, negative or positive, will be closed in the distance $d(i,j)$ and thus will belong to the same cluster. Further properties of this similarity measure can be found in study of Gu and Wang [24]. Since Pearson correlation is invariant under linear transformation, the data were scaled prior to computing the distance. Table 4 shows the correlation matrix for the metals and pesticide levels in herb samples. The statistically significant values, corresponding to p-values below 0.05, are indicated in bold. Based on the distance matrix obtained, a hierarchical clustering method with complete linkage was used to group the elements. The method assigns first each element in its own cluster and then, iteratively, it merges similar clusters until there is only a single cluster. The function *hclust* outputs a binary tree with elements as leaves (Fig. 1).

Since the number of clusters is not known a priori, the Kelley-Gardner-Sutcliffe penalty function helps to decide the optimal number of clusters in the hierarchical cluster tree [77]. The procedure *kgs* generated for a range of clusters k from 2 to 14, the corresponding penalty function. The

Table 4 Correlation coefficient matrix for metals and pesticides found in herb samples (n =14)

	Cr	As	Cd	Hg	Pb	HCB	Lindan	Heptaclor	Aldrin	Dieldrin	Endrin	p,p'DDE	p,p'DDD	p,p'DDT
Cr	1.00	−0.15	0.13	**0.58**	0.53	**0.63**	−0.14	−0.18	−0.28	−0.24	−0.21	−0.35	0.24	−0.23
As		1.00	−0.30	−0.23	−0.03	−0.10	0.08	−0.08	−0.09	−0.16	**0.96**	−0.09	−0.16	−0.11
Cd			1.00	0.02	−0.18	−0.05	0.22	0.11	−0.26	0.22	−0.35	−0.28	−0.11	0.12
Hg				1.00	**0.73**	0.48	−0.26	0.28	0.10	0.18	−0.32	−0.21	0.34	0.21
Pb					1.00	**0.83**	−0.12	0.25	0.11	-	−0.04	−0.16	-	0.22
HCB						1.00	−0.25	−0.08	−0.23	−0.16	−0.14	−0.11	−0.17	−0.11
Lindan							1.00	-	0.15	0.32	0.24	−0.21	−0.11	0.17
Heptaclor								1.00	0.29	0.36	−0.12	−0.09	−0.16	**0.98**
Aldrin									1.00	0.11	0.12	−0.20	**0.63**	0.28
Dieldrin										1.00	−0.20	0.08	−0.04	0.45
Endrin											1.00	−0.14	−0.06	0.45
p,p'DDE												1.00	−0.15	-
p,p'DDD													1.00	−0.21

Correlation is significant at the 0.05 level and is displayed in bold. Strong correlation is a significant correlation with value greater than 0.5 or smaller than −0.5

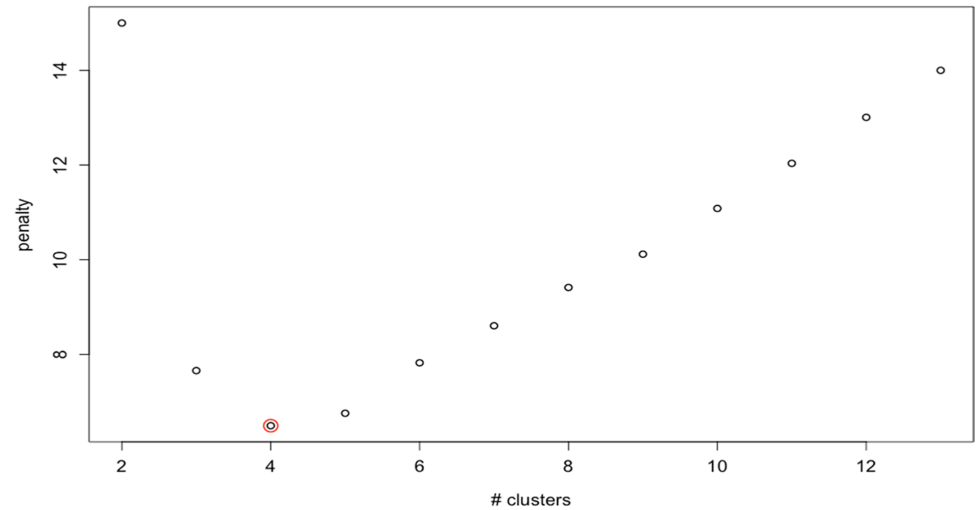

Fig. 1 Kelley-Gardner-Sutcliffe penalty for different number of clusters. The optimal number of clusters is marked in red

optimal number of clusters is the one that attains the minimum penalty. Figure 1 shows the result of the Kelley-Gardner-Sutcliffe penalty function procedure and the optimal $k = 4$. Using 4 clusters, the clustering analysis is shown in Fig. 2, where the red rectangles indicate the structure of each group.

An important step of our research was to study the correlations between selected pesticides and toxic elements in the analysed herbs. Numberless studies have reported that some metals exhibit catalytic properties and can affect the behaviour of co-existing pesticides in soil [16]. To the best of our knowledge, there is no such information at the herb level. In this study, the obtained data show a strong positive correlation between Endrin and As with r-value of 0.96, indicating an increase in Endrin concentration in the presence of higher As concentration for *Calendula officinalis L.* Similarly, a significant positive correlation was obtained between HCB and Pb with r-value of 0.83. In about 72% of the herbs, it was observed that Pb concentrations increased with increasing HCB levels, thus indicating a similar origin of the pollution source. Thus, it can be mentioned that As, Pb, and even Cr stabilise some pesticides, such as endrin and HCB; in this way, they become more persistent, therefore leading to an increase/accumulation of pesticide content. For the other elements and pesticides, the correlation coefficients were not high, indicating that the correlations are not significant; these metals may facilitate pesticide degradation [15].

The strongest connection was observed between Heptaclor and p,p'DDT, corresponding to the shortest linkage. The significant Pearson correlation coefficient between

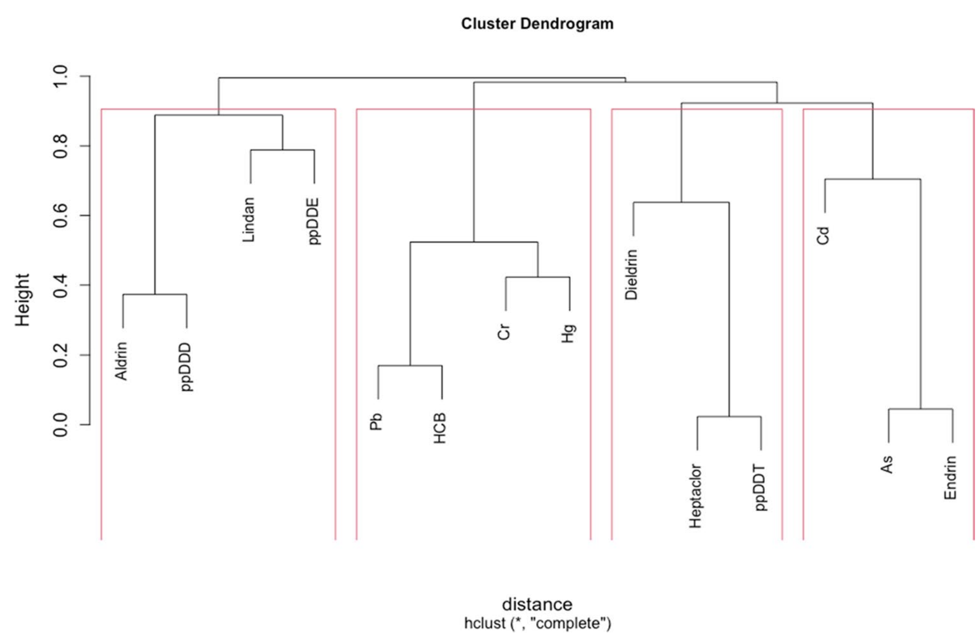

Fig. 2 Optimal number of clusters and the structure of each cluster

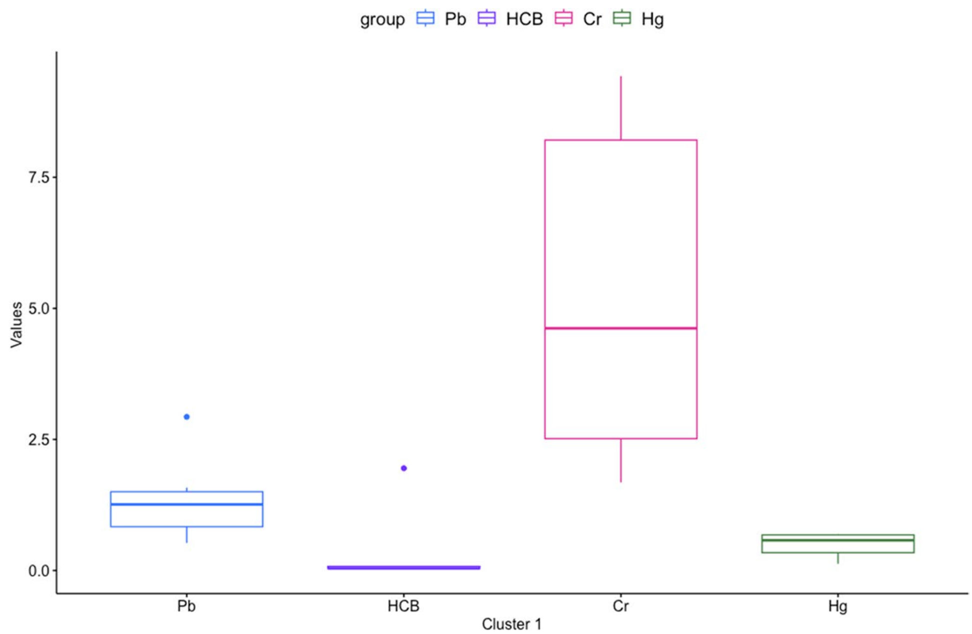

Fig. 3 Box plot for the elements in the strongest correlated cluster

them is around 0.98 (Table 4), indicating that small levels of Heptaclor in herbs are linearly correlated to small levels of p,p'DDT. This strong correlation between Heptachor and p,p'DDT indicates recent contamination. In *Epilobium parviflorum Schreb*, both compounds showed concentrations above the maximum permitted limit [57]. Similarly, a strong positive correlation was observed between Aldrin and p,p'DDD with *r*-value of 0.63.

No significant correlation was observed between the other selected pesticides, showing that the pesticide sources were the same. This may be due to the fact that the herbs show different vegetation periods, hence different contamination. Some pesticides are applied at different stages of crop production, so they reach the soil and plants at different times.

The first cluster consisting of Pb, HCB, Cr, and Hg has a linkage distance around 0.5. Within this cluster, the toxic elements and the pesticide are positively correlated, suggesting that the existence of Pb is correlated to the presence of HCB, Cr, and Hg in herbs. These correlations indicate that the compounds have similar emission sources; even if HCB is not used directly, it may be a by-product of industrial waste incineration processes or sewage sludge use. The clusters formed by Cd, As, and Endrin, on the one hand, and dieldrin, heptachlor, and p,p'DDT, on the other hand, are closer to each other than any other cluster. The relationship between these compounds, especially in the case of *Epilobium parviflorum Schreb* and *Calendula officinalis* L., shows a contamination of the area where the plants grow. The geometry of the dendrogram at the top level indicates that the cluster composed by aldrin, p,p'DDD, lindane, and p,p'DDE is substantially different from the other 3 clusters.

The first cluster (Pb, HCB, Cr, and Hg) contained the smallest number of zero values, and it was further analysed to detect any statistically significant difference between the medians of each element. First, the Shapiro-Wilk normality was applied to each component of the cluster to test the null hypothesis that the data are normally distributed. Since all *p*-values obtained were below the significance level $\alpha = 0.05$, the null hypothesis can be rejected at 5% level. Since the normality assumption did not hold, the Kruskal-Wallis test was applied to test the null hypothesis $H_0: med_{Pb} = med_{HCB} = med_{Cr} = med_{Hg}$, where med is the median of the data. The alternative hypothesis is that at least one median is different. The test *kruskal.test* produced a *p*-value of around 0.001181 below the significance level 0.05, indicating that there is at least one component in the group with the median statistically different from the others. In the boxplots of the 4 elements, shown in Fig. 3, it can be seen that the medians and the variances vary significantly among the components, with the largest difference being observed for Cr. To test this observation, a pairwise comparison was performed using the Pairwise Wilcoxon Rank Sum test with the Benjamini and Hochberg, [25], correction for multiple testing. The test confirmed that the values of Cr are statistically different from the rest of the group. However, the *p*-values obtained were approximately computed, due to the ties existing in the data.

Conclusions Among the toxic elements that have been determined, Cr, Cd, and Hg exceeded the maximum concentrations allowed for raw plant material in some of the analysed products, especially in *Centaurea cyanus L.*, *Verbascum thapsus L.*, *Artemisia absinthium L.*, *Taraxacum officinale Webb.*, and *Betula pendula Roth*. Also, some pesticides (endrin, aldrin, p,p'DDT, p,p'DDD) showed concentrations higher than European Pharmacopoeia limits in *Hypericum perforatum*, *Crataegus monogyna*, and *Epilobium parviflorum*. These results raise alarm bells for both producers and consumers. As a follow-up to these determinations, it will be necessary to quantify toxic elements and pesticides in infusions and decoctions prepared from these plant products, as recommended to be consumed for therapeutic purposes.

The results indicate the existence of significant correlation between HCB concentrations and Pb levels, as well as between endrin and As concentrations in Calendula officinalis, a plant obtained from crops. Furthermore, bioaccumulation studies may clarify the cause of the observed correlations.

Cluster analysis indicated that the elements detected are clusters in 4 groups, among which the strongest correlated group consists of Pb, HCB, Cr, and Hg. Within this cluster, the pairwise Wilcoxon rank sum test indicated that the values of Cr are statistically the most different from the other components.

Although the extent to which the transfer of the determined toxic elements and pesticides from dried plants into preparations for therapy has not yet been investigated, the results presented make it necessary to introduce the quantification of toxic elements and pesticides into the quality control protocols of products for phytotherapy.

Code Availability Not applicable.

Author Contributions MMB: sampling, formal analysis, investigation, supervision; SB: validation, visualisation, writing — original draft; CB: statistical data analysis; NL: methodology; DV: visualisation, supervision; VC: investigation, validation; DAV: methodology, validation; MDR: review and editing.

Data Availability All data generated or analysed during this study are included in this published article.

Declarations

Ethics Approval The authors declare that ethics approval was not required for this research.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. WHO, World Health Organization (2019) WHO global report on traditional and complementary medicine, Geneva; Licence CC BY-NC-SA 3.0 IGO
2. Gorelova S, Frontasyeva M (2017) Phytoremediation management of environmental contaminants: the use of higher plants in biomonitoring and environmental bioremediation. Springer International Publishing AG (eBook) 5(5). https://doi.org/10.1007/978-3-319-52381-1
3. Wang CC, Zhang QC, Kang SG, Li MY, Zhang MY, Xu WM, Xiang P, Ma LQ (2023) Heavy metal (loid) s in agricultural soil from main grain production regions of China: bioaccessibility and health risks to humans. Sci Total Environ 858(2):159819. https://doi.org/10.1016/j.scitotenv.2022.159819
4. Kloke A, Sauerback DR, Vetter H (1984) The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains. In: Nriagu JO (ed) Changing Metal Cycles and Human Health. Springer-Verlag, Berlin, pp 113–141
5. International Agency for Researcg on Cancer (IARC) (2017) Agents Classified by the IARC Monographs. 1–123
6. Taghizadeh SF, Azizi M, Hassanpourfard G, Rezaee R, Karimi G (2023) Assessment of carcinogenic and non-carcinogenic risk of exposure to metals via consumption of coffee, tea, and herbal tea in Iranians. Biol Trace Elem Res 201:1520–1537. https://doi.org/10.1007/s12011-022-03239-x
7. Zuin VG, Yariwake JH, Bicchi C (2003) Fast supercritical fluid extraction and high-resolution gas chromatography with electron-capture and flame photometric detection for multiresidue screening of organochlorine and organophosphorus pesticides in Brazil's medicinal plants. J Chromatogr A 985:159–166
8. Georgiadis N, Tsarouhas K, Tsitsimpikou C, Vardavas A, Rezaee R, Germanakis I, Tsatsakis A, Stagos D, Kouretas D (2018) Pesticides and cardiotoxicity. Where do we stand? Toxicol Appl Pharmacol 353:1–14
9. Taghizadeh SF, Goumenou M, Rezaee R, Alegakis T, Kokaraki V, Anesti O, Sarigiannis DA, Tsatsakis A, Karimi G (2019) Cumulative risk assessment of pesticide residues in different Iranian pistachio cultivars: applying the source specific HQS and adversity specific HIA approaches in Real Life Risk Simulations (RLRS). Toxicol Lett 313:91–100
10. Taghizadeh SF, Rezaee R, Azizi M, Hayes AW, Giesy JP, Karimi G (2021) Pesticides, metals and polycyclic aromatic hydrocarbons in date fruits: a probabilistic assessment of risk to health of Iranian consumers. J Food Compost Anal 98:103815
11. Luo L, Dong L, Huang Q, Ma S, Fantke P, Li J, Jiang J, Fitzgerald M, Yang J, Jia Z, Zhang J, Wang H, Dai Y, Zhu G, Xing Z, Liang Y, Li M, Wei G, Song J et al (2021) Detection and risk assessments of multi-pesticides in 1771 cultivated herbal medicines by LC/MS-MS and GC/MS-MS. Chemosphere 262:127477. https://doi.org/10.1016/j.chemosphere.2020.127477
12. Yang CM, Chien MY, Chao PC, Huang CM, Chen CH (2021) Investigation of toxic heavy metals content and estimation of potential health risks in Chinese herbal medicine. J Hazard Mater 412:125142. https://doi.org/10.1016/j.jhazmat.2021.125142
13. Filipiak-Szok A, Kurzawa M, Szłyk E (2015) Determination of toxic metals by ICP-MS in Asiatic and European medicinal plants and dietary supplements. J Trace Elem Med Biol 30:54–58
14. Kulhari A, Sheorayan A, Bajar S, Susheel Sarkar S, Chaudhury A, Kalia RK (2013) Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of north western India. Springer Plus 2:676 http://www.springerplus.com/content/2/1/676
15. Tariq SR, Rashid N (2013) Multivariate analysis of metal levels in paddy soil, rice plants, and rice grains: a case study from Shakargarh, Pakistan. J Chem 1-10:539251. https://doi.org/10.1155/2013/539251
16. Tariq SR, Shafiq M, Chotana GA (2016) Distribution of heavy metals in the soils associated with the commonly used pesticides in cotton fields. Scientifica:7575239. https://doi.org/10.1155/2016/7575239

17. Gupta N, Yadav KK, Kumar V, Cabral-Pinto MMS, Alam M, Kumar S, Prasad S (2021) Appraisal of contamination of heavy metals and health risk in agricultural soil of Jhansi city India. Environ Toxicol Pharmacol 88:103740. https://doi.org/10.1016/j.etap.2021.103740
18. Wang Y, Tan SN, Yusof MLM, Ghosh S, Ming Lam Y (2022) Assessment of heavy metal and metalloid levels and screening potential of tropical plant species for phytoremediation in Singapore. Environ Pollut 295:118681. https://doi.org/10.1016/j.envpol.2021.118681
19. Tariq SR, Iqbal F, Ijaz A (2013) Assessment and multivariate analysis of metals in surgical instrument industry affected top soils and groundwater for future reclamation. Int J Environ Pollut 1:54–71
20. European Pharmacopoeia (EP) (2019) Monographs on herbal drugs and herbal drug preparations. Council of Europe 1:1289–1675
21. Potts PJ, Ellis AT, Kregsamer P, Marshall J, Streli C, West M, Wobrauschek P (2004) Atomic spectrometry update. X-ray fluorescence spectrometry. J Anal At Spectrom 19:1397–1419
22. Jyothsna S, Manjula G, Suthari S, Nageswara Rao AS (2020) Qualitative elemental analysis of selected potential anti-asthmatic medicinal plant taxa using EDXRF technique. Heliyon 6:e03260. https://doi.org/10.1016/j.heliyon.2020.e03260
23. R version 3.4.3 (2017-11-30)Kite-eating tree, Copyright (C) 2017 The R Foundation for Statistical Computing, Platform: x86_64-pc-linux-gnu (64-bit).
24. Gu Y, Wang C (2010) A study of hierarchical correlation clustering for scientific volume data. In: International Symposium on Visual Computing. Springer, pp 437–446
25. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57(1):289–300
26. Popović-Djordjević J, Paunović D, Milić A, Krstić D, Moghaddam SS, Roje V (2021) Multi-elemental analysis, pattern recognition techniques of wild and cultivated rosehips from Serbia, and nutritional aspect. Biol Trace Elem Res 199:1110–1122. https://doi.org/10.1007/s12011-020-02199-4
27. Sui M, Kong D, Ruan H, Sun X, Gu W, Guo M, Ding S, Yang M (2023) Distribution characteristics of nutritional elements and combined health risk of heavy metals in medicinal tea from genuine producing area of China. Biol Trace Elem Res 201:984–994. https://doi.org/10.1007/s12011-022-03173-y
28. WHO, World Health Organization (2007) Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Switzerland, Geneva
29. Sindhu S, Beena C (2016) Quantification of heavy metals in the *Aloe vera* L. samples available in the market. S. Indian J of Biol Sci 2(1):81–83
30. Singh KP, Bhattacharya S, Sharma P (2014) Assessment of heavy metal contents of some Indian medicinal plants American- Eurasian. J Agric Environ Sci 14:1125–1129
31. Kumar V, Srivastava S, Chauhan RK, Thakur RK, Singh J (2017) Heavy metals and microbial contamination of certain leafy vegetables grown in abattoir effluent disposal province of Saharanpur (Uttar Pradesh) India. AESA 2(1):36–43
32. Ziarati P (2012) Determination of contaminants in some Iranian popular herbal medicines. J Anal Toxicol 2(1):1–3
33. Özden H, Özden S (2018) Levels of heavy metals and ochratoxin A in medicinal plants commercialized in Turkey. Turk J Pharm 15(3):376–381
34. Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity and detoxification in plants. Rev Environ Contam Toxicol 213:113–136
35. Sharma P, Dubey RS (2005) Lead toxicity in plants. J Plant Physiol 17:35–52
36. Dghaim R, Khatib SA, Rasool H, Khan MA (2015) Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. J Environ Health:973878. https://doi.org/10.1155/2015/973878
37. Peralta-Videa JR, Lopez ML, Narayana M, Saupea G, Gardea-Torresdeya J (2009) The biochemistry of environmen-tal heavy metal uptake by plants: implications for the food chain. Int J Biochem Cell Biol 41:1665–1677
38. Vikram A, Johri T, Tandon PK (2011) Effect of chromium (IV) on growth and metabolism of *Spinacia oleracea* (Spinach) plants. Research in Environment and Life Science 4(3):119–124
39. Israr M, Sahi SV (2006) Antioxidative responses to mercury in the cell cultures of *Sesbania drummondii*. Plant Physiol Biochem 44(10):590–595
40. Begum HA, Hamayun M, Zaman K, Shinwari ZK, Hussain A (2017) Heavy metal analysis in frequently consumable medicinal plants of Khyber Paktunkhwa. Pakistan Pak J Bot 49(3):1155–1160
41. Street RA (2012) Heavy metals in medicinal plant products—an African perspective. S Afr J Bot 82:67–74. https://doi.org/10.1016/j.sajb.2012.07.013
42. Ajasa MA, Bello OM, Ibrahim OM, Ogunwande AI, Olawore ON (2004) Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chem 85:67–71
43. Gajalakshmi S, Iswarya V, Ashwini R, Divya G, Mythili S, Sathiavelu A (2012) Evaluation of heavy metals in medicinal plants growing in Vellore District. Eur J Exp Biol 5:1457–1461
44. Chizzola R, Michitsch H, Franz C (2003) Monitoring of metallic micronutrients and heavy metals in herbs, spices and medicinal plants from Austria. Eur Food Res Technol 216(5):407–411
45. Danezis GP, Georgiou CA (2022) Elemental metabolomics: food elemental assessment could reveal geographical origin. Curr Opin Food Sci 44:100812. https://doi.org/10.1016/j.cofs.2022.100812
46. Madrid D, Cornu S, Bourennane H, Baize D, Ratié C, King D (2020) Effect of agricultural practices on trace-element distribution in soil. Commun Soil Sci Plant Anal 38:473–491
47. Srivastava SK, Rai V, Srivastava M, Rawat AKS, Mehrotra S (2006) Estimation of heavy metals in different Berberis species and its market samples. Environ Monit Assess 116(1–3):315–320
48. Angelova VR, Babrikov TD, Ivanov KI (2019) Bioaccumulation and distribution of lead, zinc, and cadmium in crops of Solanaceae family. Commun Soil Sci Plant Anal 40:2248–2263
49. Xu D, Chen Z, Sun K, Yan D, Kang M, Zhao Y (2019) Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (Solanum tuberosum L.). Ecotoxicol Environ Saf 97:147–153
50. Bawa U, Abdulhameed A, Nayaya AJ, Ezra AG, Jibrin M (2021) Bioaccumulation factor of heavy metals in some crops grown in plateau state Nigeria. Biol Life Sci Forum 4(1):12. https://doi.org/10.3390/IECPS2020-08737
51. Rajakaruna N (2018) Distribution of heavy metals in tannery effluent polluted soils of Tamil Nadu, India. Bull Environ Contamin Toxicol 60:142–150
52. Zhuang P, McBride MB, Xia H, Li N, Li Z (2009) Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Sci Total Environ 407:1551–1561
53. Giacomino A, Malandrino M, Colombo ML, Miaglia S, Maimone P, Blancato S, Conca E, Abollino O (2016) Metal content in dandelion (*Taraxacum officinale*) leaves: influence of vehicular traffic and safety upon consumption as food. J Chem:9842987. https://doi.org/10.1155/2016/9842987
54. Masarovičová E, Králová K (2017) Phytoremediation management of environmental contaminants. Essential elements and toxic metals in some crops, medicinal plants, and trees. Springer International Publishing AG (eBook) 5 (7). https://doi.org/10.1007/978-3-319-52381-1

55. Adamczyk-Szabela D, Lisowska K, Wolf WM (2021) Hysteresis of heavy metals uptake induced in Taraxacum officinale by thiuram. Sci Rep 11:20151. https://doi.org/10.1038/s41598-021-99666-2
56. Yu IS, Lee JS, Kim SD, Kim YH, Park HW, Ryu HJ, Lee JH, Lee JM, Jung K, Na C, Joung JY, Son CG (2017) Monitoring heavy metals, residual agricultural chemicals and sulfites in traditional herbal decoctions. Complement Altern Med 17:154. https://doi.org/10.1186/s12906-017-1646-y
57. Jibrin M, Abdulhameedm A, Nayayam AJ, Ezram AG (2021) Health risk effect of heavy metals from pesticides in vegetables and soils: a review. DUJOPAS 7(3b):24–32
58. European Pharmacopoeia, EP, (2006) Applicable to medicinal plant materials included in the European pharmacopoeia, 5th ed, PHARMEUROPA 18 (4).
59. Matulevičiūtė D (2016) The role of willowherbs (*Epilobium*) in the recovery of vegetation cover a year after use of herbicide: a case study from Central Lithuania. Bot Lith 22(2):101–112
60. Mumtaz M, Qadir A, Mahmood A, Mehmood A, Malik RN, Li J, Yousaf Z, Jamil N, Shaikh IA, Ali H, Zhang G (2015) Human health risk assessment, congener specific analysis and spatial distribution pattern of organochlorine pesticides (OCPs) through rice crop from selected districts of Punjab Province, Pakistan. Sci Total Environ 511:354–361
61. Sweetman AJ, Dalla Valle M, Prevedouros K, Jones KC (2005) The role of soil organic carbon in the global cycling of persistent organic pollutants (POPs): Interpreting and modelling field data. Chemosphere 60:959972. https://doi.org/10.1016/j.chemosphere.2004.12.074
62. Calvelo Pereira R, Camps-Arbestain M, Rodríguez Garrido B, Macías F, Monterroso C (2006) Behaviour of α-, β-, γ-, and δ-hexachlorocyclohexane in the soil–plant system of a contaminated site. Env Pollution 144(1):210–217
63. Kaushik G, Satya S, Naik SN (2009) Food processing a tool to pesticide residue dissipation-A review. Food Res Int 42:26–40
64. Murtaj B, Nuro A, Salihila J (2018) Organochlorinated pesticides and PCB in some medicinal plants from South-East Albania. IJEPEM 1(2):44–49
65. Walter K, Vallero DA, Lewis RG (1999) Factors influencing the distribution of lindane and other hexachlorocyclohexanes in the environment. Environ Sci Technol 33(24):4373–4378
66. Barber JL, Sweetman AJ, van Wijk D, Jones KC (2005) Hexachlorobenzene in the global environment: emissions, levels, distribution, trends and processes. Sci Total Environ 349(1-3):1–44
67. Liu CY, Jiang X, Fan JL, Noura Ziadi N (2013) Hexachlorobenzene accumulation in rice plants as affected by farm manure and urea applications in dissimilar soils. Can J Soil Sci 93:631638. https://doi.org/10.4141/CJSS2013-001
68. Sosan MB, Adeleye AO, Oyekunle JAO, Udah O, Oloruntunbi PM, Daramola MO, Saka WT (2020) Dietary risk assessment of organochlorine pesticide residues in maize-based complementary breakfast food products in Nigeria. Heliyon 6(12):e05803. https://doi.org/10.1016/j.heliyon.2020.e05803
69. Xiao P, Mori T, Kamei I, Kondo R (2011) Metabolismof organochlorine pesticide heptachlorand its metabolite heptachlor epoxide by white rot fungi, belonging to genus Phlebia. Microbiol Lett 314:140–146
70. Unyimadu JP, Osibanjo O, Babayemi JO (2019) Concentration and distribution of organochlorine pesticides in sediments of the Niger River Nigeria. J Health Pollut 9(22):190606. https://doi.org/10.5696/2156-9614-9.22.190606
71. Li Y, Zhang Q, Ji D, Wang T, Wang Y, Wang P, Ding L, Jiang G (2009) Levels and vertical distributions of PCBs, PBDEs, and OCPs in the atmospheric boundary layer: observation from the Beijing 325-m meteorological tower. Environ Sci Technol 43(4):1030–1035. https://doi.org/10.1021/es802138w
72. Doong RA, Sun YC, Liao PL, Peng CK, Wu SC (2002) Distribution and fate of organochlorine pesticide residues in sediments from the selected rivers in Taiwan. Chemosphere 48(2):237–246
73. Siraj J, Mekonen S, Astatkie H, Gure A (2021) Organochlorine pesticide residues in tea and their potential risks to consumers in Ethiopia. Heliyon 7:e07667. https://doi.org/10.1016/j.heliyon.2021.e07667
74. Amirahmadi M, Shoeibi S, Abdollahi M, Rastegar H, Khosrokhavar R, Hamedani MP (2013) Monitoring of some pesticides residue in consumed tea in Tehran market Iran. J Environ Heal Sci Eng 10(1):9. https://doi.org/10.1186/1735-2746-10-9
75. Feng J, Tang H, Chen D, Li L (2015) Monitoring and risk assessment of pesticide residues in tea samples from China. Hum Ecol Risk Assess 21(1):169–183
76. Hardy A (1996) On the number of clusters. Comput Stat Data Anal 23(1):83–96
77. Kelley LA, Gardner SP, Sutcliffe MJ (1996) An automated approach for clustering an ensemble of NMR-derived protein structures into conformationally related subfamilies. Protein Eng Des Sel 9(11):1063–1065