ASSESSMENT OF HEAVY METALS CONTENT IN SOME MEDICINAL PLANTS AND SPICES COMMONLY USED IN ROMANIA

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Abstract

The present study was designed to investigate the level of six heavy metals (arsenic, cadmium, copper, iron, mercury and lead) in both wild-growing and packaged samples from twelve commonly used medicinal plants and spices in Romania. The concentrations of all heavy metals, except mercury and lead, were within permissible limits in the analysed plant samples. The wild-growing sample of Tilia cordata and some packaged samples of Origanum vulgare and Thymus vulgaris contained higher levels of mercury than 0.1 mg/kg. The concentrations of lead exceeded the limit recommended by the EU (0.3 mg/kg) for some samples of Achillea millefolium, Matricaria chamomilla, Mentha piperita, Ocimum basilicum, Origanum majorana and Tilia cordata. The findings of the study highlight the need for constant monitoring of heavy metals residues in order to ensure the quality and safety of herbal products.

Résumé

Ce travail a été conçu pour évaluer le niveau de six métaux lourds (arsenic, cadmium, cuivre, fer, mercure et plomb) dans des échantillons de plantes médicinales et de condiments couramment utilisés en Roumanie. Les concentrations de tous les métaux lourds, excepté le mercure et le plomb, étaient à l’intérieur des limites permises dans les échantillons analysés. Les échantillons sauvages de Tilia cordata et certains échantillons conditionnés de Origanum vulgare et Thymus vulgaris contenaient plus d’arsenic que la limite de 0,1 mg/kg. Les concentrations de plomb dépassaient la limite recommandée par l’UE (0,3 mg/kg) pour certains échantillons de Achillea millefolium, Matricaria chamomilla, Mentha piperita, Ocimum basilicum, Origanum majorana et Tilia cordata. Les résultats de l’étude mettent en évidence la nécessité de faire un suivi constant des résidus de métaux lourds pour assurer la qualité et la sécurité des produits végétaux.

Keywords: heavy metals, medicinal plants, spices, contamination

Introduction

Medicinal plants and plant-derived products play a significant role in the strategies for the assessment of global health and their use is rapidly rising. WHO reported that about 80% of the population in developing countries relies on traditional herbal medicine for their primary healthcare [29]. These findings impose major concerns on the quality and safety of herbal products in order to provide appropriate and effective therapy [12, 15, 16]. Among the main safety risks related to herbal medicines is contamination with heavy metals. The medicinal plants may be contaminated with toxic heavy metals during cultivation, harvesting or processing stages [25]. Also, the environmental pollution that includes industrial waste, traffic emissions and some agricultural activities can affect significantly the heavy metals content in medicinal plants. The uptake and accumulation of heavy metals in vegetal tissues can directly affect plant growth and can clinically pose major threat for the human health. Excessive levels of these elements can cause central nervous system toxicity (mercury, lead, arsenic), produce kidney and liver damage (mercury, lead, cadmium, cooper), or respiratory dysfunction (cadmium), and have adverse effects on skin, bones and teeth (nickel, cadmium, cooper, chromium) [2, 11]. Also, large amounts of iron might cause abdominal and skin injuries [2]. Thus, the assessment of metal contamination in herbal raw materials is an important step in the control of plants’ quality. Nevertheless, there is limited data in terms of heavy metals contamination of medicinal and culinary plants used in Romanian traditional medicine or diet. Taking all this into account, the present study was designed to investigate the level of six heavy metals (arsenic-As, cadmium-Cd, cooper-Cu, iron-Fe, mercury-Hg, lead-Pb) in both wild-growing and packaged samples from twelve commonly used medicinal plants and spices in Romania.
Hg and lead-Pb) in some commonly used medicinal plants and spices in Romania.

Materials and Methods

Plant material
A total of forty-two samples belonging to six medicinal plants and six spices commonly used in Romania were analysed. For each medicinal plant species, four samples were analysed as follows: a sample collected from spontaneous flora of Galați County, Romania, and three samples provided by different indigenous producers as herbal teas (packaged samples). In the case of spices, for each plant species, three samples were analysed, namely: a sample collected from spontaneous flora of Galați County, Romania, and two samples purchased from local suppliers. Table I shows the herb samples investigated in this study and some characteristics of the plant species [26].

Table I
Samples and some characteristics of selected medicinal plants and spices

| Plant species             | Samples                  | Common name | Part used | Main beneficial effects                      |
|--------------------------|--------------------------|-------------|-----------|----------------------------------------------|
| **Medicinal plants**     |                          |             |           |                                              |
| Achillea millefolium L.  | AM1, AM2, AM3            | AM4         | yarrow    | eupeptic - cholagogue - carminative - anti-inflammatory - spasmyotic |
| Calendula officinalis L. | CO1, CO2, CO3            | CO4         | marigold  | choleric - anti-inflammatory - spasmyotic - wound healing - antimicrobial |
| Hypericum perforatum L.  | HP1, HP2, HP3            | HP4         | St. John’s wort | aerial parts |
| Matricaria chamomilla L. | MC1, MC2, MC3            | MC4         | chamomile | spasmyotic - carminative - anti-inflammatory - sedative - antiulcer |
| Ocimum basilicum L.      | OB1, OB2, OB3            | OB4         | basil     | eupeptic - spasmyotic - carminative - diuretic |
| Tilia cordata Mill.      | TC1, TC2, TC3            | TC4         | linden    | expectorant - spasmyotic - sedative |
| **Spices**               |                          |             |           |                                              |
| Anethum graveolens L.    | AG1, AG2                 | AG3         | dill      | carminative - eupeptic - diuretic |
| Mentha piperita L.       | MP1, MP2                 | MP3         | peppermint | choleric - cholagogue - spasmyotic - carminative - anti-diarrheal |
| Origanum majorana L.     | OM1, OM2                 | OM3         | marjoram  | eupeptic - spasmyotic - carminative - sedative - diuretic |
| Origanum vulgare L.      | OV1, OV2                 | OV3         | oregano   | eupeptic - spasmyotic - carminative - expectorant - antimicrobial |
Sample processing and heavy metals analysis
The content of heavy metals in selected plants and spices was determined using atomic absorption spectrometry after wet digestion of the samples with 65% HNO₃ and 30% H₂O₂ [30]. The analysis of As, Cd and Pb was done using atomic absorption spectrometry with graphite furnace atomization (SpectrAA240Z spectrophotometer with GTA120 furnace, Varian, The Netherlands) [23, 25]. Flame atomic absorption spectrometry was performed for the analysis of Fe and Cu content (SpectrAA240FS spectrophotometer, Varian, The Netherlands) [23]. The Hg concentrations were determined by cold vapour atomic absorption spectrometry (SpectrAA240FS spectrophotometer, Varian, The Netherlands) [24]. High purity standards for heavy metals (Merck, Germany) were used as reference analytes and the calibration standards were prepared by appropriate dilution of their stock solutions. The concentrations of heavy metals were expressed as mg/kg.

Statistical analysis
Four determinations were performed for each sample and the mean and standard deviation were calculated.

Results and Discussion
The regulatory guidelines for heavy metals content in plants are presented in Table II. The results of the analysis of heavy metals in the selected medicinal plants and spices are summarized in Tables III and IV, respectively.

| Plant species                  | Samples   | Common name | Part used | Main beneficial effects |
|-------------------------------|-----------|-------------|-----------|-------------------------|
| *Petroselinum crispum* (Mill.) Fuss. | PC1, PC2 | PC3         | parsley   | leaves - carminative    |
|                               |           |             |           | - diuretic              |
|                               |           |             |           | - antihypertensive      |
|                               |           |             |           | - haemostatic           |
| *Thymus vulgaris* L.           | TV1, TV2  | TV3         | thyme     | aerial parts - eupeptic |
|                               |           |             |           | - spasmylic             |
|                               |           |             |           | - carminative           |
|                               |           |             |           | - expectorant           |
|                               |           |             |           | - diuretic              |

Arsenic (As). It is a highly toxic non-essential metalloid that significantly affects human health leading to gastrointestinal and hepatic disorders, hypotension, polyneuropathy, loss of sight and carcinogenic effects [19]. Plants are exposed to As mainly from anthropogenic activities such as mining and smelting, the use of As-based pesticides in agriculture and of As-contaminated groundwater for irrigation purposes [7]. In our study, the level of As varied from less than 0.01 to 0.15 mg/kg in medicinal plants samples (Table III) and from less than 0.01 to 0.78 mg/kg in spices, respectively (Table IV). Maximum concentrations of As were found in *Tilia cordata* and *Petroselinum crispum* packaged samples, respectively (TC3; PC1); in all samples of *Hypericum perforatum*, As was undetectable. In general, we noticed that the investigated plant samples contained extremely low concentrations of As. Only some samples of spices, mainly *Petroselinum crispum* (PC1, PC2), *Thymus vulgar* (TV2) and *Origanum vulgare* (OV2, OV3) showed higher values but all were below the acceptable limit (Table II and Table IV). The levels of As identified in this work are much lower than those noticed in other studies. Oprea et al. reported for samples of some medicinal plants from two areas belonging to the Baia Mare municipality, Maramureş County (Romania), concentrations of As ranging from 0.11 to 5.33 mg/kg [17]. These large amounts of As are primarily associated with specific metallurgical industry and soil pollution of Baia Mare [17], but contamination with As is important due to the fact that Romania is one of the countries having problems due to the natural arsenic groundwater [28].

Table II

| Limits               | Arsenic (As) | Cadmium (Cd) | Mercury (Hg) | Lead (Pb) |
|----------------------|--------------|---------------|---------------|-----------|
| European Pharmacopoeia [6] | -            | 1             | 0.1           | 5         |
| FAO/WHO [30]        | 1            | 0.3           | -             | 10        |
| EU [13]             | -            | 0.2           | -             | 0.3       |

Cadmium (Cd) is a non-essential hazardous heavy metal that causes serious effects on the kidney, liver, vascular and immune system. Prolonged exposure to high-doses of Cd results in the Itai-Itai disease that is characterized by severe renal tubular dysfunction, osteomalacia and osteoporosis [20]. The combustion of fossil fuels, the use of phosphate fertilizers, non-ferrous smelters, mines and sewage sludge application are the major sources for the Cd contamination of plants [11]. This metal presents high rates of soil-to-plant transfer such that it is a contaminant found in most human foodstuffs [20]. The levels of Cd were within allowable limits (Table II) in all samples of medicinal plants (0.01 - 0.05 mg/kg) (Table III) and...
levels compared to their packaged variants of different origin (0.01 - 0.06 mg/kg vs. 0.05 - 0.12 mg/kg) (Table IV). Cd was undetected in all samples of Hypericum perforatum, the sample of wild-growing Tilia cordata (TC4) (Table III) and some packaged samples of Origanum majorana (OM1) and Petroselinum crispum (PC1) (Table IV).

The content of heavy metals in selected medicinal plants

| Plant species/samples | Mean concentration of heavy metals*standard deviation (mg/kg*) |
|-----------------------|-------------------------------------------------------------|
| Digitalis purpurea    | Arsenic (As) 0.02 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |

The content of heavy metals in selected spices

| Spices/Samples       | Mean concentration of heavy metals*standard deviation (mg/kg) |
|----------------------|-------------------------------------------------------------|
| Anethum graveolens   | Arsenic (As) 0.01 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |
| Origanum majorana    | Arsenic (As) 0.01 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |
| Origanum vulgare     | Arsenic (As) 0.01 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |
| Mentha piperita      | Arsenic (As) 0.01 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |
| Petroselinum crispum | Arsenic (As) 0.01 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |
| Thymus vulgaris      | Arsenic (As) 0.01 ± 0.00  Cadmium (Cd) 320.00 ± 7.19  Copper (Cu) 670.00 ± 7.35  Iron (Fe) 719.00 ± 6.51  Mercury (Hg) 673.00 ± 7.35  Lead (Pb) 12.00 ± 5.47 |
Cooper (Cu) is an essential trace element in humans and plants. It is a cofactor of many redox enzymes and plays a significant role in iron metabolism and many physiological processes including antioxidant defence, neuropeptide synthesis and immune function [3]. Also, Cu is essentially involved in normal growth and development of plants [31]. Elevated levels of Cu can cause gastrointestinal symptoms, hepatotoxicity, skin irritation and sensitization, and dysfunction of upper respiratory tract [4]. Sources of Cu contamination in plants could be linked to industrial and mining activities.

The Cu concentrations ranged from 4 to 24 mg/kg in samples of medicinal plants (Table III) and from 122 to 348 mg/kg in spices samples (Table IV). The highest levels were recorded in wild-growing samples of Calendula officinalis (CO4) (Table III) and Origanum majorana (OM 3) (Table IV). For similar species, the results obtained in this study are higher than other samples from Romania [8] or other regions [9, 10]. Also, all investigated samples of spices have a high Cu content (over 120 mg/kg).

Iron (Fe) is a biologically essential trace element for all living organisms. It is involved in a broad variety of cellular processes, namely electron transport and oxygen supply, cellular respiration, synthesis of DNA and proteins, cell proliferation and differentiation, regulation of gene expression and immunity [4]. Overdoses (above 20 mg/kg) of iron are associated with gastrointestinal disorders, metabolic acidosis, cardiomyopathy, and renal and hepatic failure [4].

The results from this current study revealed a large variation of Fe content in selected plant samples. It was found in a range between 180 and 21020 mg/kg in samples of medicinal plants (Table III) and between 320 and 6683 mg/kg in spices samples (Table IV). The highest Fe level was obtained for a packaged sample of Calendula officinalis (CO3). In fact, all samples of Calendula officinalis contained large amounts of Fe (over 11000 mg/kg) (Table III). Among investigated species, Anethum graveolens, Origanum majorana and Petroselinum crispum are characterized by a low accumulation of Fe. The Mentha piperita and Hypericum perforatum presented large variations between samples (even 10 times) (Tables III and IV). In the case of all samples, our results are higher than the reported Fe values for similar species from Mediterranean area [10], United Arab Emirates [5], or even Romania [8].

Mercury (Hg) is a highly toxic non-essential metal that is considered to be a dangerous xenobiotic in living cells. The consequences of Hg toxicity mainly as organic mercury compounds include central nervous system damage, alteration of motor function, neuro-behavioural, neurodevelopmental, immunological effects and cardiovascular and kidney injuries. Exposure of plants to Hg derives from natural sources (volcanic eruptions, emissions from the ocean) and anthropogenic activities (burning of fossil fuels, production of cement, mining, smelting, production of ferrous and non-ferrous metals) [21]. Hg levels were within allowable limits in our samples (Tables II, III and IV) except for a wild-growing sample of Tilia cordata (0.106 mg/kg) (TC4) and some packaged samples of Origanum vulgare (OV1) and Thymus vulgaris (TV1) spices (0.374 and 0.131 mg/kg, respectively). Hg was not detected in any of the samples of Hypericum perforatum, Anethum graveolens and Origanum majorana. Also, some packaged and wild-growing samples of Matricaria chamomilla (MC4), Ocimum basilicum (OB4) (Table III) and packaged samples of Mentha piperita (MP1, MP2) do not contained Hg (Table IV).

It seems that the accumulation of Hg in plants depends primarily on the plants species, the degree of soil and atmosphere contamination, the season for harvesting [14]. Şenilă et al. reported high concentrations of Hg in perennial plants growing in the urban area of Baia Mare, a historical mining and metal processing centre from North-western Romania. The average content exceeded the permissible limit of Hg in plants (0.22 mg/kg compared to 0.1 mg/kg) [27]. Also, Ordak et al. showed a high accumulation of Hg in the leaves of the more commonly used perennial plants collected from the vicinity of busy streets in Poland [14].

Lead (Pb) is a highly noxious metal for humans and the environment. It can affect any organ but causes toxic effects mainly on the nervous central system, the cardiovascular, haematological and renal systems. Plants’ exposure to Pb results from: mining, smelting, steel industry, electroplating, inorganic fertilizers and pesticides use, fuel combustion, exhaust of automobiles and sewage sludge [22]. The content of Pb ranged from 0.04 to 2.03 mg/kg in samples of medicinal plants used as herbal teas (Table III) and from 0.04 to 1.28 mg/kg in samples of spices (Table IV). The highest levels were found in wild-growing samples of Ocimum basilicum (2.03 mg/kg) (OB4) and Mentha piperita (1.28 mg/kg) (MP3). The concentrations of Pb were lower compared to the permissible limits defined by the European Pharmacopoeia and WHO/FAO (Table II), but they exceeded the maximum value required by the EU (0.3 mg/kg) for 75% of Ocimum basilicum samples, 66.66% of Mentha piperita samples and 33% of Origanum majorana, Matricaria chamomilla, Achillea millefolium and Tilia cordata samples, respectively (Tables III and IV). In general, the samples of plants collected from spontaneous flora are highly contaminated with lead. However, all values were inline or lower compared to those reported in other studies for similar plants from different regions of the globe [1, 5, 25], or even Romania [18].

Conclusions

In our study, forty-two samples of medicinal plants and spices commonly used in Romania were screened for six heavy metals contents (arsenic, cadmium, cooper,
The high load of iron and copper can be associated with the collection site, such as the metallurgical type industrialized area of Galați city, Romania, in the case of wild-growing plants, and additionally, the processing methods for packaged samples. The findings of the study highlight the need of constant monitoring of heavy metals residues or other pollutants in order to ensure the quality and safety of herbal products. Therefore, the present study provides baseline data useful for a comprehensive assessment of heavy metals in medicinal plants from different parts of our country.

Conflict of interest
The authors declare no conflict of interest.

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