Analysis of the content of selected heavy metals in dietary supplements available on the Polish market

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Background. Eating highly processed foods and lacking important nutrients in the diet may increase the need for dietary supplements. Their use allows consumers to supplement deficiencies of vitamins and other minerals necessary for the proper functioning of the body. However, in addition to valuable vitamins and minerals, supplements often contain various types of plant components and may be contaminated with heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg). Preparations with a high content of toxic elements may constitute an additional source of their uptake by the body and, apart from the desired nutritional effect, cause intoxication of the organism.
Aim of the study. The aim of the study was to compare the levels of cadmium, lead and mercury in herbal and vitamin-mineral preparations available on the Polish market.
Material and methods. 93 samples belonging to vitamin and vitamin-mineral preparations (50 contained plant components, 43 did not) were evaluated for selected heavy metal content using flame atomic absorption spectrometry (FAAS) for cadmium and lead and atomic absorption spectrometry with amalgamation technique for mercury.
Results. Analyzed samples of herbal and vitamin-mineral dietary supplements contained a low contamination of cadmium and lead and did not pose a risk to public health. The content of cadmium ranged from 0.010 to 0.710 mg/kg, while the lead ranged from 0.02 to 1.55 mg/kg. The average cadmium and lead contamination in preparations containing plant raw materials was significantly higher ($p < 0.05$) compared to the average content of these elements in preparations containing only synthetic ingredients. The level of cadmium and lead in all specimens did not exceed the admissible value (1.0 mg/kg and 3.0 mg/kg respectively). Mercury concentration in the tested dietary supplements varied and ranged from 0.0005 to 0.1470 mg/kg. The average mercury content was almost 10 times higher in supplements containing plant raw materials compared

to the average content of this element in preparations containing only synthetic ingredients, but differences were not statistically significant ($p < 0.088$). Four preparations containing raw materials of plant origin exceeded the permissible mercury content (0.1 mg/kg). The tested dietary supplements were not a significant source of cadmium and mercury, but the supply of lead from supplements was much higher.
Conclusion. Heavy metals were detected in all tested preparations. However, supplements with plant raw materials contained significantly higher concentrations of cadmium, lead and mercury compared to supplements that did not contain components of plant origin. Our research showed that the permissible amounts of mercury in herbal supplements may be exceeded; moreover, supplements with plant material may be a significant source of lead. Therefore, further studies covering a larger number of samples are needed to estimate the heavy metal contamination risk of dietary supplements.

Keywords: heavy metals, atomic absorption spectrometry, contaminations, dietary supplements.

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Introduction

Eating highly processed foods and lacking important nutrients in the diet may increase the need for dietary supplements, and in fact, their sales have been constantly growing for the past few years. Modern supplements are being created; product lines dedicated to various consumer groups: children, pregnant and breastfeeding women, seniors, athletes, young learners, women and men of different ages (20+, 30+, 40+, 50+, etc.) or people who want to improve their beauty (mainly hair, nails, and skin). Their use allows consumers to supplement deficiencies of vitamins and other minerals necessary for the proper functioning of the body [1, 2]. Additionally, dietary supplements are produced in a convenient form (tablets, capsules or liquids). Due to the fact that they are classified as foodstuffs, they must meet the criteria related to food health safety. The levels of chemical and microbiological contamination should not exceed the permissible limits specified in the relevant legal acts [3].

The studies show that the most chemical contaminants (mainly heavy metals) in the daily diet are provided by plant-based foods [4]. Due to the fact that dietary supplements, in addition to valuable vitamins and minerals, often contain various types of plant components (e.g., herbs, plant extracts, isolated bioactive ingredients), they may be contaminated with heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg). Contamination of plants and plant raw materials with heavy metals is most often caused by soil, air and water contamination, as well as from equipment, and industrial and technological processes. Contamination may also come from packaging, artificial fertilizers and other substances (ingredients) added during production. There are situations where, in the finished multi-component product, the permissible level of heavy metals is exceeded, even though in individual raw materials (before their mixing), the level of these metals does not exceed permissible standards. The sale of an unproven product may pose a threat to the health of the potential consumer. Preparations with a high content of toxic elements may constitute an additional source of their uptake by the body and, apart from the desired nutritional effect, cause intoxication of the organism [5, 6].

Heavy metals are the main contaminants of food, due to their toxicity and common occurrence. Cadmium, lead and mercury are the most toxic, especially in inorganic compounds that penetrate through mucous membranes to internal organs (liver, kidneys, and pancreas) [7]. They are dangerous for humans because of their ability to accumulate in the organism and their long biological half-life (cadmium 10–30 years [8]; lead 5–10 years in the bones (even 20 years in the body) [9]; mercury from 30 to 60 days in the body, approx. 20 years in the brain)[10], which consequences in the incidence of chronic disorders [8]. They can form connections with lipids, nucleic acids and proteins, which leads to cell damage and disruption of their functions. Disease symptoms can appear even after many years. These are mainly kidneys, cardiovascular system, skeletal system and nervous system diseases; furthermore, abnormal development in children; teratogenic, mutagenic and neoplastic changes [11–13].

To protect public health, various countries have set regulations on the maximum contamination of heavy metals in food products. The European Commission has established maximum limits for cadmium, lead and mercury in dietary supplements. There is not much information in the available literature about the concentration of these elements in dietary supplements available on the Polish market. Taking into account consumer safety and the global interest in supplements, it is important to control the level of contamination of these products with heavy metals.

The aim of the research was to assess the content of lead, cadmium and mercury in dietary supplements available in Polish pharmacies.

Materials and Methods

Sample collection

The research material included 93 samples of vitamin and vitamin-mineral preparations. 50 samples contained plant components in combination with other ingredients, while the remaining 43 samples were free of plant ingredients. The supplements were in the form of tablets and capsules and were manufactured in Poland. All dietary supplement samples were purchased from Polish pharmacies.

Chemicals

Heavy metal standards, solvents, and reagents used in this study were delivered by Merck (Darmstadt, Germany). Standard aqueous solutions of lead, cadmium and mercury were prepared from separate 1000 mg/L standards. Six mixed working standards were established from the previous solutions. The heavy metal contents in the analyzed samples were calculated based on calibration graphs.

The contamination of lead and cadmium was determined by the flame atomic absorption spectrometry technique (FAAS) in accordance with PN-EN 14082:2004 "Foodstuffs. Determination of trace elements. Determination of lead, cadmium, zinc, copper, iron and chromium content by atomic absorption spectrometry (AAS) after dry mineralization" [14]. The author's own research procedure was used to prepare and mineralize the tested samples.

5 g of supplements were weighed into the quartz crucibles, burned on a heating plate, and mineralized in a muffle furnace at 420°C. 5 mL of 65% nitric acid was added to the ash, evaporated, and burned again at 420°C in a muffle furnace. White ash was dissolved in 50 mL of 1 mol/L nitric acid in the quartz crucibles. The samples were tested in duplicate. For the determination, the HITACHI Z-2000 atomic absorption spectrometer was used. Table 1 describes the conditions used for cadmium and lead determinations.

Table 1. Instrument settings applied for cadmium and lead determination.

Parameters	Cd	Pb
Wavelength, nm	228.8	283.3
Lamp current, mA	5	7
Slit width, nm	0.30	0.30
Air flow rate, dm³/h	400	400
Acetylene flow rate, dm³/h	60	60

Mercury's level was determined with an AMA 254 mercury analyzer using the atomic absorption spectrometry method with the mercury vapor generation technique based on the author's own research procedure: PS-02, edition 3, 6 July 2009. This method did not require any prior mineralization of the sample. 0.1 g or 0.1 mL of the dietary supplement sample was fed directly to the apparatus on the feeder. Inside the catalytic tube, drying took place and then burning the sample at a temperature of about 1000°C in the presence of oxygen. The mercury released from the sample was "captured" on the amalgamator, from which, after being heated, it flowed into the measuring cuvette. The absorbance was measured. Based on the calibration curve, the measurement result was read. Each sample was tested in duplicate. Table 2 shows the conditions used for mercury determinations.

Validation of methods

The content of lead, cadmium and mercury in dietary supplements was tested using validated methods. The Mixed Polish Herbs (INCT-MPH-2) reference material was applied to estimate the validation parameters (table 3). Analytical quality assurance used for the determination of lead, cadmium and mercury was

Table 2. Settings of the AMA 254 mercury analyzer.

Parameters	
System	single-beam; serial arrangement of measuring cells
Control	from an external computer
Source of light	low pressure mercury lamp
Wavelength	253.65 nm
Interference filter	254 nm, half width 9 nm
Detector	silicon UV diode
Analysis conditions	drying 90 s; decomposition 140 s; read 60 s
Amount of the analysed sample	100 μg (μL)

Table 3. Validation parameters.

Parameters	Cd	Pb	Hg
The limit of quantification LOQ), mg/kg	0.003	0.02	0.0005
The limit of detection (LOD), mg/kg	0.0025	0.012	0.0002
Recovery, (%)	99	98	99
Linearity	0.9998	0.9998	0,9998
Precision,%	2.9	3.8	3.2

confirmed in FAPAS 04309 inter-laboratory studies.

Statistical Analysis

The STATISTICA 10 statistical package software was used to statistically evaluate the experimental data. The Mann-Whitney test at $\alpha = 0.05$ was used to assess significant differences between samples containing plant components and samples without plant ingredients, taking into account the average concentration of heavy metals.

Results

Tables 4 and 5 present the cadmium, lead and mercury levels in dietary supplements.

To determine the level of contamination with heavy metals in the tested preparations, the obtained results related to the requirements of Regulation (EU) No 2023/915 of April 25, 2023 [15].

According to the above regulation, the maximum permissible limits of heavy metals in dietary supplements are as follows:

- 1.0 mg/kg for cadmium (3,0 mg/kg in the case of dietary supplements consisting mainly of dried seaweed or dried mussels);
- 3.0 mg/kg for lead;
- 0.1 mg/kg for mercury.

In the analyzed samples of vitamin and vitamin-mineral preparations, the content of cadmium ranged from 0.010 to 0.710 mg/kg. The mean cadmium level in preparations containing plant raw materials was 0.253 ± 0.193 mg/kg and was significantly higher ($p < 0.004$) compared to the average content of this element in preparations containing only synthetic ingredients, which was 0.036 ± 0.025 mg/kg. The content of cadmium in all specimens did not exceed the admissible value of this element.

The determined lead content in all dietary supplements ranged from 0.02 to 1.55 mg/kg. Similarly to the case of cadmium, supplements with plant raw materials contained a significantly higher lead amount compared to supplements that did not contain components of plant origin, and there were statistically significant differences ($p < 0.032$). The permissible lead level was not exceeded in any preparation.

The mercury content in the tested dietary supplements varied and ranged from 0.0005 to 0.1470 mg/kg. The average mercury contamination was almost 10 times higher in supplements containing plant raw materials compared to the average

Table 4. Characteristics and concentration of heavy metals in dietary supplements.

	Type of dietary supplement	Number of samples	Pharmaceutical forms	Cd [mg/kg] (min. – max.)	Cd [mg/kg] mean ± SD	Pb [mg/kg] (min. – max.)	Pb [mg/kg] mean ± SD	Hg [mg/kg] (min. – max.)	Hg [mg/kg] mean ± SD
With plant ingredients	Calming	10	tablets	0,025–0,620	0.324 ± 0.125	0.11–0.94	0.42 ± 0.21	0,0025–0,0098	0.0071 ± 0.0036
		5	capsules						
	Strenghtening hair, nails and skin	18	tablets	0,076–0,684	0.443 ± 0.154	0.41–1.55	0.94 ± 0.37	0,0010–0,1470	0.0068 ± 0.0026
		7	capsules						
	Support slimming	10	tablets	0,060–0,710	0.49± 0.182	0.32–1.34	0.75 ± 0.30	0,0052–0,1210	0.0092 ± 0.0044
Vitamin and mineral preparations		43	tablets	0.010–0.088	0.036 ± 0.025	0.02–0.80	0.29 ± 0.19	0.0005–0.0021	0.0010 ± 0.0005

Table 5. Statistical summary of the content of heavy metals in dietary supplements.

Content of the tested element [mg/kg]	Supplements containing plant ingredients (n = 50) (min.–max.)	Supplements containing plant ingredients (n = 50) mean ± SD	Supplements free from plant ingredients (n = 43) (min.–max.)	Supplements free from plant ingredients (n = 43) mean ± SD	Significance of the differences p
Cd	0.025–0.710	0.253 ± 0.193 **(A)**	0.010–0.088	0.036 ± 0.025 **(B)**	$p_{A/B} < 0.004$
Pb	0.11–1.55	0.43 ± 0.30 **(C)**	0.02–0.80	0.29 ± 0.19 **(D)**	$p_{C/D} < 0.032$
Hg	0.0010–0.1470	0.0081 ± 0.0049 **(E)**	0.0005–0.0021	0.0010 ± 0.0005 **(F)**	$P_{E/F} < 0.088$

n – number of samples; SD – standard deviation content of this element in preparations containing only synthetic ingredients, however, the differences were not statistically significant ($p < 0.088$). Four preparations containing raw materials of plant origin exceeded the permissible mercury content (0.1 mg/kg).

EFSA's Panel on Contaminants in the Food Chain established the tolerable weekly intake (TWI) for heavy metals:

- 25 µg/kg body weight for cadmium [16];
- 4 µg/kg body weight for mercury [17].

There is no recommended tolerable weekly intake level for lead. In 2010, EFSA's Panel on Contaminants in the Food Chain concluded that the TWI of 25 µg/kg body weight was no longer appropriate because there is no evidence of thresholds for a number of critical health effects [18]. In American Herbal Products Association guidance [19], the tolerable daily intake (TDI) for lead is established at 0.29 µg/kg body weight (20 µg for an average person of 70 kg).

The health risk from consumption of tested dietary supplements was also assessed in this work. Taking into account the weight of tablets and capsules and the dosage of the supplements, the weekly intake of cadmium and mercury and the daily intake of lead from preparations were calculated (table 6). Estimates were made for the highest content of heavy metals in supplements. TWI and TDI were calculated for an average person weighting 70 kg.

The calculations show that the tested dietary supplements were not a significant source of cadmium and mercury. The supply of these elements in the analyzed preparations was less than 1% TWI; values of weekly intake ranged below the permissible standards. The daily intake of lead from supplements was much higher and oscillated between 2.4 and 5.0% TDI. Consumption of these preparations for a longer period of time can pose a health risk for consumers.

Discussion

Dietary supplements are registered by the Chief Sanitary Inspectorate, and there are fewer requirements for them compared with herbal medicines. Drug manufacturers sometimes avoid pharmaceutical law by registering their products as dietary foods or dietary supplements in order to reduce the costs associated with biological activity and toxicity testing [20]. Despite the many advantages of dietary supplements, there are doubts about whether it is safe to introduce these types of products on a large scale. In some herbal plants from roadside and industrialized areas, significant amounts of toxic elements, such as lead and cadmium, were found. Exposure to these toxic elements may cause changes in the mineral composition of various plant parts, reduce the content of biologically active substances, and consequently, cause a decrease in the medicinal value of the plant raw material [21, 22]. There is not much information available in the literature regarding the heavy metal contamination of dietary supplements available in Poland. The research is mainly related to herbal plants, herbal dietary supplements and dietary supplements marketed in other countries. They show that supplements with herbal raw materials may have higher quantities of heavy metals, according to the type of plant. Drabek et al. [23] determined the heavy metal content of medicinal plant dietary supplements and assessed the potential daily burden on their consumers. 68% of all samples were contaminated with cadmium and lead, and 29% of samples were contaminated with mercury. The health risk of consumers of dietary supplements was estimated and showed that taking tested supplements for only a week poses a health risk regarding Pb. Puścion-Jakubik et al. [24] tested dietary supplements containing ingredients of plant origin available for sale in Poland. The authors found the highest mercury value (23.97 ± 38.56 µg/kg) in preparations used as adjuncts for lowering glucose levels. The highest percentage of TWI (1.143%) was recorded in supplements for improving vitality. Dopierała-Brodziak et al. [25] investigated the mercury content in the herbal supplements purchased on the Polish market. The studies showed that mercury was present in all examined herbal supplements; moreover, its amount exceeded the permissible standards (0.10 mg/kg) in two preparations (with alga and bamboo). There were statistically significant differences in the level of mercury depending on the herbal ingredient in the supplements. A study of dietary supplements available in Polish pharmacies by Socha [26] recorded the lead amount ranging from 0.35 µg/kg to 1752.3 µg/kg (average content 256.5 µg/kg). The contamination of this element in preparations containing plant raw materials was higher compared to vitamin and mineral products. None of the tested samples exceeded the permissible lead level in dietary supplements. Similar to this study, the presented research noticed significantly higher lead content in supplements containing plant raw materials; moreover,the author didn't find any exceedance of permissible limits. In other studies of herbal dietary supplements that facilitate falling asleep available in the USA, significant contamination with lead was found

Table 6. The supply of cadmium, lead and mercury from dietary supplements for weekly and daily intake.

Type of dietary supplement		Cd						
		The highest amount in product [µg/kg]	Weight of tablet/capsule [mg]	Amount in tablet/capsule [µg]	Daily dosage [µg]	Weekly intake [µg]	TWI for a person of 70 kg [µg]	% TWI
With plant ingredients	Calming	620	620	0,384	1	2,688	1750	0,15
	Strenghtening hair, nails and skin	684	700	0,479	1	3,353	1750	0,19
	Support slimming	710	660	0,469	1	3,283	1750	0,19
Vitamin and mineral preparations		88	580	0,051	1	0,357	1750	0,02
Type of dietary supplement		**Pb**						
		The highest amount in product [µg/kg]	**Weight of tablet/capsule [mg]**	**Amount in tablet/capsule [µg]**	**Daily dosage [µg]**	**Daily intake [µg]**	**TDI for a person of 70 kg [µg]**	**% TDI**
With plant ingredients	Calming	940	610	0,573	1	0,573	20	2,87
	Strenghtening hair, nails and skin	1550	650	1,008	1	1,008	20	5,04
	Support slimming	1340	620	0,832	1	0,832	20	4,16
Vitamin and mineral preparations		800	600	0,48	1	0,48	20	2,40
Type of dietary supplement		**Hg**						
		The highest amount in product [µg/kg]	**Weight of tablet/capsule [mg]**	**Amount in tablet/capsule [µg]**	**Daily dosage [µg]**	**Weekly intake [µg]**	**TWI for a person of 70 kg [µg]**	**% TWI**
With plant ingredients	Calming	9,8	650	0,006	1	0,042	280	0,02
	Strenghtening hair, nails and skin	147	670	0,098	2	1,372	280	0,49
	Support slimming	121	655	0,079	1	0,553	280	0,20
Vitamin and mineral preparations		2,1	610	0,001	1	0,007	280	0,003

TDI – tolerable daily intake; TWI – tolerable weekly intake in some of the tested preparations [27]. Jairoun et al. [28] investigated the daily exposure levels of heavy metal in dietary supplements sold in the United Arab Emirates and explored the factors associated with the heavy metals contamination of dietary supplements. The average daily intake of cadmium was 0.73 µg compared to the acceptable daily intake (ADI) of 6 µg and the daily intake of lead was 0.85 µg compared to the acceptable daily intake (ADI) of 20 µg. Keshvari et al. [29] measured the level of cadmium, lead and mercury in herbal products from the Iranian market. The heavy metal contents in the investigated samples did not show significant levels that may be related to toxicity. Similar to the research provided by Jairoun et al., the cadmium, lead and mercury concentrations in tested herbal remedies were also below the acceptable intake established by global recommendations. Also, Mustatea et al. [30] determined the cadmium and lead content of 41 food supplements purchased from the Romanian market and assessed the risk to consumer health. Estimations showed a small risk associated with the consumption of tested supplements.

The available data revelated the results of cadmium, lead and mercury levels in herbal plants and dietary supplements. Our study presents the levels of contamination with these elements in dietary supplements and compares the differences between supplements containing plant raw materials and preparations containing only synthetic ingredients regarding the amounts of heavy metals. However, due to increasing tendency of consumers to self-medicate and the increasing availability of herbal dietary supplements from other parts of the world on the Polish market, in order to protect public health, it is necessary to constantly monitor the levels of heavy metals in these preparations.

Conclusion

In this study, tested samples of herbal and vitamin-mineral dietary supplements were characterized by low values of lead and cadmium and did not exceed the permissible levels. In four preparations containing raw materials of plant origin, the mercury content was above the permissible value. Heavy metals were detected in both types of preparations. However, supplements with plant raw materials contained significantly higher concentrations of cadmium, lead and mercury compared to supplements that did not contain components of plant origin.

Moreover, dietary supplements were not a significant source of cadmium and mercury (the supply of these elements from analyzed preparations was less than 1% TWI). The daily intake of lead from supplements was much higher (oscillated between 2.4 and 5.0% TDI). Consumption of these preparations for a longer period of time can pose a health risk to consumers. In order to increase consumer health safety, the level of heavy metal contamination in dietary supplements should be constantly monitored. Therefore, further testing of larger numbers of samples is needed.

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