

Annual Variability of Some Toxic Element Contents (Cd, Cr, Co, Ni, and Pb) and Response of Two Jerusalem Artichoke Varieties to Increasing Nitrogen Fertilizer at Constant PK Levels

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Abstract

Contents of cadmium, cobalt, chromium, nickel, and lead were determined in tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) obtained from a field experiment carried out in 2003-05. The experimental factors consisted of two cultivars of Jerusalem artichoke (Albik and Rubik), plus different nitrogen nutrition levels on a background of constant phosphorus-potassium fertilization and of manure (FYM). The following experimental objects were formed: 1) control $N_0P_0K_0$, and the following mineral objects: 2) $N_0P_{44}K_{125}$, 3) $N_{50}P_{44}K_{125}$, 4) $N_{100}P_{44}K_{125}$, 5) $N_{150}P_{44}K_{125}$, and 6) $N_{200}P_{44}K_{125}$. Mineral fertilizers were not applied to the control object, but only manure (natural fertilizer) at the rate of 30 t·ha⁻¹. The Rubik cv. accumulated more heavy metals than Albik cv.; the latter was characterized by less variability of heavy metals content. When applying constant phosphorus-potassium fertilization, the lowest cadmium content was recorded in combinations including 150 kg N·ha⁻¹, cobalt – 100 kg N·ha⁻¹, chromium (only phosphorus-potassium fertilization was used), as well as nickel and lead – in control object with no mineral nutrition. In both varieties of Jerusalem artichoke different amounts of heavy metals were collected due to the different mineral fertilization.

Keywords: cultivars, fertilization, heavy metals, Jerusalem artichoke

Introduction

Many heavy metals that can be toxic for plants penetrate the environment due to economic and industrial activities. They are mainly metals (within the group of trace elements) with atomic number greater than 20, as well as density higher than 4.5 g·cm⁻³, that in chemical reactions show tendencies to donate electrons, thus building up simple cations. Elements essential for plants (among the microelements

group: Cu, Zn, and Ni, which are uptaken at larger amounts) and those that are unnecessary for plants from a physiological point of view (Cd, Hg, Pb, As, etc.) are counted to toxic ones [1-5]. The usefulness for a living organism is determined by the form in which a given element occurs. Metals in readily soluble and exchangeable bindings are the most dangerous, because they are uptaken by plants, easily penetrate the cellular membranes, and enter the internal organs. Different human activities provide opportunities to expose the natural environment to the toxic properties of trace elements. The anthropogenic source of these elements'

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contamination includes fertilizers applied in agriculture. Morgan et al. [6], Wuana and Okieimen [7], Jasiewicz and Antonkiewicz [8], Baran et al. [9], Kuziemska [10], and Xiao et al. [11] reported that plants uptake considerable quantities of heavy metals from contaminated subsoil and accumulate them in their biomass. The agrotechnical operations on Jerusalem artichoke plantations, including fertilization and chemical plant protection, also may influence heavy metals contents in tubers.

Our study aimed at evaluating the influence of varied nitrogen nutrition (on a background of a constant phosphorus-potassium fertilization) on chromium, cadmium, cobalt, nickel, and lead contents in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers.

Material and Methods

The study material was composed of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers originating from a field experiment carried out in 2003-05 at the field experimental station in Parzew, Poland. The experiment was set by means of randomized sub-blocks in three replications. Cultivars Albik and Rubik were the first-order factor, while mineral nutrition was the second-order factor. The following experimental objects were formed: 1) control $N_0P_0K_0$, and the following mineral objects: 2) $N_0P_{44}K_{125}$, 3) $N_{50}P_{44}K_{125}$, 4) $N_{100}P_{44}K_{125}$, 5) $N_{150}P_{44}K_{125}$, and 6) $N_{200}P_{44}K_{125}$. Mineral fertilizers were not applied to the control object, but only manure (natural fertilizer) at the rate of $30 \text{ t}\cdot\text{ha}^{-1}$. The chemical composition of manure is given in Table 2. All agrotechnical operations were made in accordance to the species requirements. Nitrogen doses above $100 \text{ kg}\cdot\text{ha}^{-1}$ was used in two terms: before planting and after the emergence of plants. Tubers of *Helianthus tuberosus* L. were planted by hand, in the spacing of $62.5 \times 40 \text{ cm}$. After planting, the tubers showered. The depth to which the tubers were buried after hilling was 6 cm. Deadline for planting was dependent on the weather, especially temperatures: therefore, planting in 2003 took place 6-11 April 2004, and in 2005 to 16 April. This was performed again after emergence hilling combined with rolling. Just before the emergence of a measure intended to control dicotyledonous weeds – Afalon 80 WP at $2 \text{ kg}\cdot\text{ha}^{-1}$. After the emergence of Jerusalem artichoke, and before cover of row spacing was used for monocotyledonous weeds in the 2-4 leaf stage, the preparation Fusilade Forte 150 EC was applied at $1.0 \text{ dm}\cdot\text{ha}^{-1}$. During the growing season, in any year of the study, there was no need for insecticides and fungicides. The set of tubers was carried out in the spring, in the first half of April (in 2003 – April 9, 2004 – April 11, and in 2005 – April 16).

The soil (Albic Luvisols [12]) was subjected to the following determinations: granulometric composition – gravimetric method, pH in $1 \text{ mol KCl}\cdot\text{dm}^{-3}$ solution, percentage of organic carbon (C_{org}) – Tiurin's method helpful for determining humus content in the soil. The results were compared to the limit [13] values worked out by the Institute of Soil Science and Plants. Samples of 50 intact tubers from 10 plants at each of the plots were collected during the

Jerusalem artichoke harvest and subjected to chemical analyses. Directly after the harvest, the tuber dry matter was the subject of the following determinations (in three replicates): cadmium, chromium, cobalt, nickel, and lead contents. Samples of the plant material (1g) were combusted in a muffle furnace at 450°C . Achieved crude ash (to decompose carbonates and separate silicates) was treated with HCl solution (1:1) and evaporated till dry. The pure ash – after dissolving in 10 cm^3 10% HCl solution – was transferred to the measuring flask of 100 ml capacity through a funnel with hard filter paper, to filtrate the silica. The precipitate was washed using 10% HCl solution and the volume in flask was adjusted with de-ionized water. Such achieved solutions were used to determine the total contents of heavy metals by means of atomic emission spectrophotometry with inductively-coupled plasma technique (ICP-AES), applying an Optima 3200RL device (Perkin Elmer). The device working parameters: RF power – 1300 W, plasma argon flow rate – $15 \text{ L}\cdot\text{min}^{-1}$, auxiliary argon flow rate – $0.5 \text{ L}\cdot\text{min}^{-1}$, nebulizing argon flow rate – $0.8 \text{ L}\cdot\text{min}^{-1}$, sample pass rate – $1.5 \text{ mL}\cdot\text{min}^{-1}$, integration time – 10 s. The following analytical wavelengths were used in determinations: Pb – 220.353 nm, Cd – 228.802 nm, Cr – 267.716 nm, Co – 228.616 nm, and Ni – 231.604 nm.

Statistical results processing was performed by applying variance and polynomial regression analysis. The variability sources significance was verified using “F” Fischer-Snedecor test, while $LSD_{0.05}$ using Tukey test. Results of heavy metals contents in Jerusalem artichoke tubers, depending on mineral nutrition level, were subject to polynomial regression analysis with insignificant variables reduction. The estimation of a model's parameters was made using the least squares method (LSM), maintaining statistical rules for variables selection, the significance of estimated parameters, and general correctness of the model. Significance verification was conducted with the help of *t*-Student test. The variability coefficients for every feature of a tuber's chemical composition were calculated according to the formula:

$$V = \frac{S}{x} \times 100\%$$

...where: *S* – standard deviation, *x* – arithmetic mean.

Air temperature and rainfall distribution during the analyzed years varied (Fig. 1). In 2003 the first half of vegetation period was wet and warm, while the second was dry. In 2004 the vegetation beginning (April-May) was wet and cool; June and July were extremely dry, whereas August-October was average regarding rainfall and air temperature. In 2005, May-June appeared to be wet and cool, while other months were dry or extremely dry and warm (Fig. 1).

Results

Soil Conditions

The field experiment was carried out on soils, the surface humus layers of which were composed of light sandy forms – light loamy sands and/or strong loamy sands

Table 1. Characterization of soils according to agronomic categories.

Agronomic category of soil	Year	Percentage content of fraction in diameter, mm			Soil texture	Organic matter [g·kg ⁻¹]	pH _{KCl}
		1-0.1	0.1-0.02	<0.02			
Light	2003	57	24	19	Loamy sand	15.3	5.5
	2004	62	25	13		15.9	5.9
	2005	66	21	13		12.4	5.1

Table 2. Dry matter content (%) and select macronutrients (g·kg⁻¹ d.m.) and micronutrients (mg·kg⁻¹ d.m.) in the manure used for fertilization.

Years	d.m. (%)	N	P	K	Ca	Cd	Co	Cr	Ni	Pb
		g·kg ⁻¹ d.m.					mg·kg ⁻¹ d.m.			
2003	23.0	19.0	5.62	14.4	7.04	0.145	0.136	1.46	42.0	3.86
2004	24.7	22.4	6.80	15.6	8.20	0.108	0.162	4.08	39.4	5.06
2005	24.2	23.0	5.94	17.2	8.16	0.094	0.148	2.96	54.4	9.62
Mean	24.0	21.5	6.12	15.7	7.80	0.116	0.149	2.83	45.3	6.18

(according to the Polish Soil Science Society), acidic or slightly acidic reaction (pH 5.1-5.9), and moderate contents of carbon in organic compounds (Table 1). The abundance of these soil horizons in available phosphorus and potassium was high and very high, while in magnesium it was low to high [14]. The content of heavy metals in the soil was as follows: Cd – 0.71, Co – 0.82, Cr – 1.21, Ni – 2.71, and Pb – 30 mg·kg⁻¹ soil. Therefore, the soil can be considered as moderately contaminated [1]. The chemical composition of the soil is determined by the nature of starting materials (rocks and rock-forming minerals, which founded the soil), and by processes it underwent in the past. The chemical composition of the soil alone is not particularly useful because it does not indicate whether the elements constitute the components of the mineral soil, or whether their pres-

ence is due to surface adsorption. According to Grant [15], such elements should not pass from silicate minerals into solution and therefore they do not play a significant role in relation to plant growth or their participation in key environmental processes. These elements, which occupy ion-exchange centers on the particles of soil or are poorly adsorbed, are usually more chemically and biologically available [14].

The content of determined elements in natural manure (FYM) used in this experiment was typical for this fertilizer produced in Poland (Table 2). According to Gruca-Królikowska and Waclawek [2], a mixed content of heavy metal (Cd – 0.3-0.8 mg, Pb – 6.6-15.0 mg, Zn – 15-250 mg, Cu – 2-60 mg·kg⁻¹ dry weight) is considered as standard for the manure. Nicholson [16] showed that cattle manure

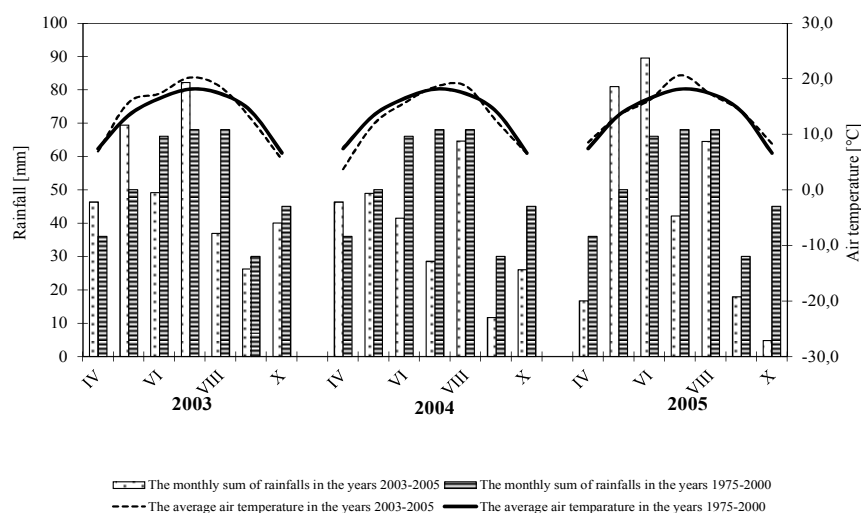


Fig. 1. Rainfall and air temperature in vegetation periods of *Helianthus tuberosus* L. acc. to meteorological station of COBORU in Uhnin (years 2003-05).

Table 3. Heavy metals content in dry matter of *Helianthus tuberosus* L. tubers [mg·kg⁻¹].

Heavy metals	Cultivar				
	Albik		Rubik		
	Mean	V*	Mean	V	LSD _{0.05}
Cd	0.141	40.92	0.156	29.52	0.005
Co	0.228	11.04	0.199	31.17	0.008
Cr	0.737	21.01	0.770	25.33	0.025
Ni	1.939	33.06	2.077	44.97	0.060
Pb	1.157	17.71	0.871	25.33	0.034

* Variability coefficient

(FYM) containing 18.4% dry matter has the following heavy metals: 153 mg Zn, 37.4 mg – Cu, Ni 3.7 mg, 3.61 mg – Pb, 0.38 mg – Cd, 1.63 mg – As, and 5.32 mg – Cr mg·kg⁻¹ dry weight of manure.

Effect of Cultivars and Nitrogen Fertilization on Heavy Metal Content in Tubers of Jerusalem Artichoke

Genetic properties of examined Jerusalem artichoke cultivars had significant impact on all studied metals contents in their tubers (Table 3). Rubik cv. accumulated significantly more Cd, Cr, and Ni in tubers, whereas Albik cv. took in more cobalt and lead. This could be attributed to the length of vegetation period as well as a cultivar's ability to accumulate heavy metals in tubers. Albik cv. appeared to be more stable, referring to Co and Pb, while Rubik cv. to Cr and Pb accumulation.

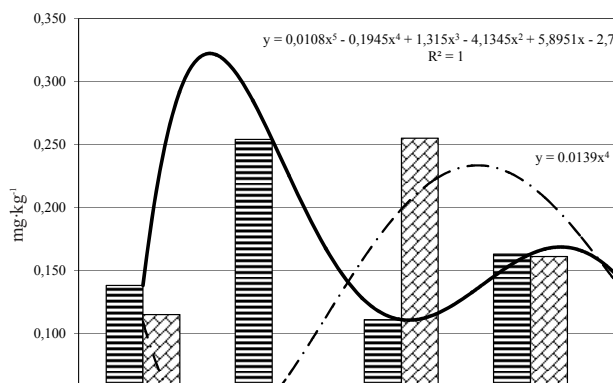
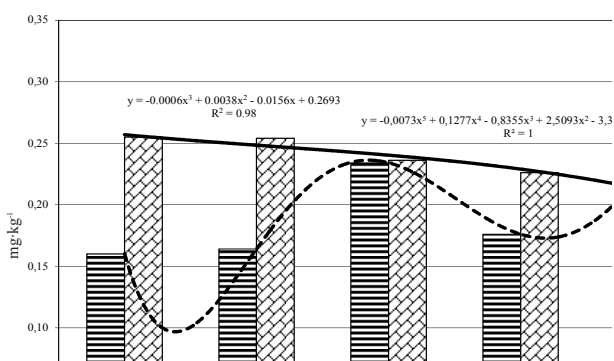
Both tested cultivars of Jerusalem artichoke accumulated different amounts of select heavy metals in their tubers due to mineral nutrition (Table 4). Applying the polynomial regression model allowed for better explaining of these dependencies and proof that cadmium content in tubers could be fitted to the fourth-order curve for Albik cv. (D = 72.6%) or fifth-order polynomial for Rubik cv. (D = 100.0%) due to fertilization (Fig. 2).

Cobalt content could be described by the third-order line (Fig. 3) for Albik cv. (D = 96.7%), and fifth-order for Rubik cv. (D = 100%). Contents of Cr, Ni, and Pb in tubers of two Jerusalem artichoke cultivars were arranged in a non-univocal manner. The largest amounts of chromium in Rubik cv. tubers were recorded in combination with the highest nitrogen dose, although significant increases of the metal were observed only to 150 kg N·ha⁻¹ level (Table 4). The content of this element in the tubers of cv. Rubik under the influence of nitrogen fertilization could be modeled with second-degree parabolic regression (D = 92%), while in the case of cv. Albik – according to the third-degree curve (Fig. 4).

The highest quantities of nickel were found in tubers of Albik cv. from objects fertilized with 100 kg N·ha⁻¹, while for Rubik cv. – 50 kg N·ha⁻¹ (Table 4). Polynomial regression

Table 4. Influence of cultivars and mineral fertilization on the content of chromium, cobalt, cadmium, nickel, and lead in dry matter *Helianthus tuberosus* L. tubers mg·kg⁻¹.

Cultivar	Fertilization	Cd	Co	Cr	Ni	Pb
		mg·kg ⁻¹				
Albik	N ₀ P ₀ K ₀	0.115	0.255	0.900	1.010	0.647
	P ₄₄ K ₁₂₅	0.021	0.254	0.709	1.911	1.227
	N ₅₀ P ₄₄ K ₁₂₅	0.255	0.236	0.632	1.790	1.149
	N ₁₀₀ P ₄₄ K ₁₂₅	0.161	0.226	0.585	2.535	1.941
	N ₁₅₀ P ₄₄ K ₁₂₅	0.127	0.216	0.730	2.347	1.306
	N ₂₀₀ P ₄₄ K ₁₂₅	0.165	0.181	0.868	2.044	0.670
Rubik	N ₀ P ₀ K ₀	0.138	0.160	0.474	1.512	1.004
	P ₄₄ K ₁₂₅	0.254	0.164	0.592	1.696	0.476
	N ₅₀ P ₄₄ K ₁₂₅	0.111	0.234	0.731	4.354	0.629
	N ₁₀₀ P ₄₄ K ₁₂₅	0.163	0.176	0.699	1.594	0.912
	N ₁₅₀ P ₄₄ K ₁₂₅	0.103	0.236	1.038	1.981	0.921
	N ₂₀₀ P ₄₄ K ₁₂₅	0.165	0.225	1.084	1.322	1.283
LSD _{0.05}		0.029	0.046	0.152	0.362	0.201

Fig. 2. Cadmium content in *Helianthus tuberosus* L. tubers, depending on fertilization and cultivar.Fig. 3. Cobalt content in *Helianthus tuberosus* L. tubers, depending on fertilization and cultivar.

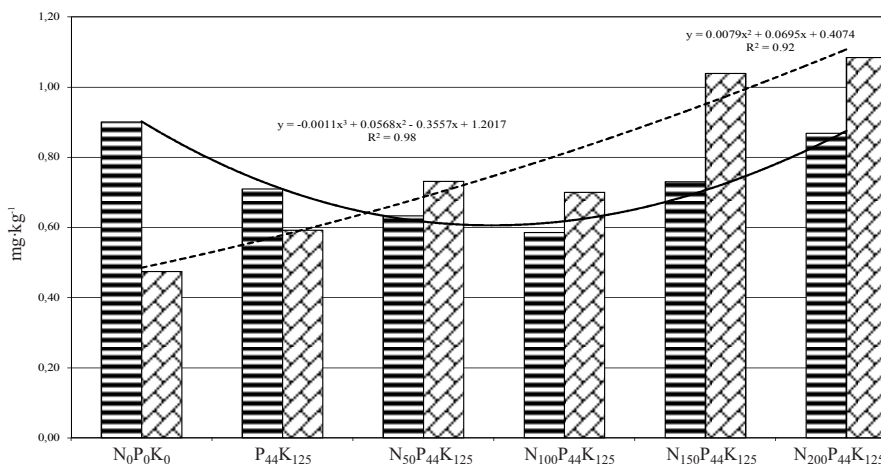


Fig. 4. Chromium content in *Helianthus tuberosus* L. tubers, depending on fertilization and cultivar.

analysis helped to determine the relationship between fertilization and the content of this element: in the tubers of cultivar Albik – the second-degree polynomial (D = 86%), and for a cultivar Rubik – the third-degree polynomial (D = 40%) (Fig. 5).

Albik cv. accumulated the largest amounts of lead due to 100 kg N·ha⁻¹, whereas Rubik cv. – 200 kg N·ha⁻¹ (Table 4). The application of the polynomial regression model allowed us to demonstrate that under the influence of nitrogen fertilization, the lead content in tubers is shaped according to the third-degree polynomial for cultivar Albik (D = 76%), and second-degree for cultivar Rubik (D = 80%) (Fig. 6).

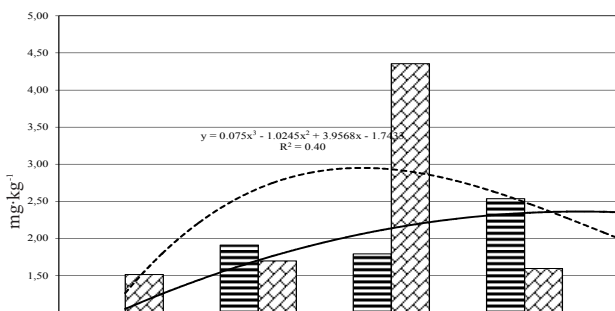


Fig. 5. Nickel content in *Helianthus tuberosus* L. tubers, depending on fertilization and cultivar.

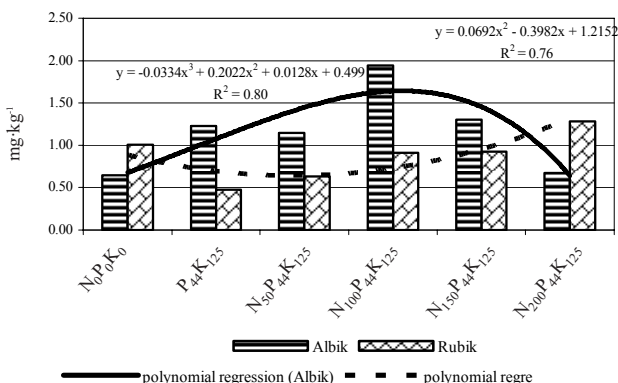


Fig. 6. Lead content in *Helianthus tuberosus* L. tubers, depending on fertilization and cultivar.

Regression model selection was made on the basis of the determination coefficient (D), the greater the value of D, the better fit of the regression model, and thus the better description of the change of trace elements (y), depending on fertilization (x).

Influence of Meteorological Conditions on the Accumulation of Heavy Metals in the Tubers of Jerusalem Artichoke

The weather conditions during the experiment had different influences on the accumulation of heavy metals by Jerusalem artichoke tubers. Higher rainfalls in the first six months of 2003 affected the slightly higher accumulation of cadmium, and the lower concentrations of cobalt, chromium, and lead. The highest amounts of Co, Cr, Ni, and Pb were determined in tubers of Jerusalem artichoke grown in 2005, which was extremely dry in September and October, i.e. crucial months for the yield of these plants (Table 5).

Nickel accumulation by *Helianthus tuberosus* L. tubers depended on a plant's reaction toward nitrogen doses, as well as weather conditions during the experiment (Table 6). Its highest contents in tubers in wet and cool 2003 were recorded only on the object with phosphorus-potassium fertilization in a warmer 2004 – when 150 kg N·ha⁻¹ was

Table 5. The annual variability the content of chromium, cobalt, cadmium, nickel, and lead in the dry matter of *Helianthus tuberosus* L. tubers, mg·kg⁻¹.

Heavy metals	Year			LSD _{0.05}
	2003	2004	2005	
Cd	0.179	0.150	0.116	0.007
Co	0.189	0.214	0.237	0.012
Cr	0.286	0.756	1.206	0.038
Ni	1.830	1.342	2.851	0.091
Pb	0.374	1.310	1.347	0.050

Table 6. The influence of annual variability and mineral fertilization on the content of nickel in the dry matter of tubers, $\text{mg}\cdot\text{kg}^{-1}$.

Fertilization*	Year		
	2003	2004	2005
$\text{N}_0\text{P}_0\text{K}_0$	1.063	1.168	1.553
$\text{P}_{44}\text{K}_{125}$	2.891	0.542	1.977
$\text{N}_{50}\text{P}_{44}\text{K}_{125}$	1.751	1.129	6.336
$\text{N}_{100}\text{P}_{44}\text{K}_{125}$	1.985	1.811	2.397
$\text{N}_{150}\text{P}_{44}\text{K}_{125}$	2.170	1.936	2.386
$\text{N}_{200}\text{P}_{44}\text{K}_{125}$	1.121	1.470	2.459
$\text{LSD}_{0.05}$	0.544		

applied, and in 2005 with extremely dry second part of vegetation period, on objects with $50 \text{ kg N}\cdot\text{ha}^{-1}$.

Discussion

The health safety of *Helianthus tuberosus* L. tubers as a food component or material for food, pharmaceutical, and fodder processing is determined by mineral composition, namely heavy metals content. Producers of food are responsible for food safety, and they should take care of it as well as taking into account the HACCP system requirements and requirements of Decree WE No. 852/2004 of the European Parliament and the Council of 29 April 2004 on the Hygiene of Foodstuffs [17]. The system also aims to evaluate methods of threat reduction [18, 19]. Biological, chemical, or physical factors during production can be a threat to food products; some of them may be dangerous for living organism's health [19-22]. The presence of chemical contaminants, including trace metals, is a principle criterion in food safety assessment [21]. Mean contents of determined heavy metals in dry matter of Jerusalem artichoke tubers were as follows ($\text{mg}\cdot\text{kg}^{-1}$): cadmium – 0.149, cobalt – 0.214, chromium – 0.753, nickel – 2.008, and lead – 1.014. The harmful influence of heavy metals can appear at their particular concentrations in a plant's environment [21, 23].

The threats of heavy metals can be divided into three groups: the most dangerous – lead ($25 \mu\text{g}$) and cadmium ($7 \mu\text{g}$), dangerous – mercury ($5 \mu\text{g}$) and arsenic ($10 \mu\text{g}$), as well as toxic, e.g. chromium [20]. Research made by the U.S. Centers for Disease Control (CDC) have revealed that the first group bears specific danger for human health at concentrations above $10 \mu\text{g}/\text{dl}$ of blood (causing brain, liver, and kidney damage, as well as reproduction system disturbances). Considering animals, it is accepted that fodder from green areas should contain $50\text{-}100 \text{ mg Zn}$, $7.1\text{-}10 \text{ mg Cu}$, $< 20 \text{ mg Cr}$, $\leq 0.5 \text{ mg Cd}$, and $\leq 10 \text{ mg Pb}$ [1]. Plants sensitive to chromium manifest toxicity symptoms even at $1\text{-}2 \text{ mg Cr}\cdot\text{kg}^{-1}$ DM concentration, while resistant ones tolerate the concentration of chromium up to 24

$\text{mg}\cdot\text{kg}^{-1}$ [24]. The toxicity symptoms in plants consist first of all in water balance disturbances, fading, young leaf chlorosis, and damage to the root apex [25]. Lead, chromium, cadmium, and nickel are on the list of 10 priority environmental contaminants in Poland, which was worked out by the Environmental Toxicology Commission of the Human Ecology Committee, Polish Academy of Sciences. Metal ions may penetrate the cells through a “fake transport” mechanism, along calcium or manganese channels. For instance, cadmium may be introduced into a cell by manganese transport channel and also be removed out of a cell the same way. Heavy metals – under particular concentrations and conditions – may affect the changes in a living organism's population composition by reducing it even to mono-species systems.

Organisms with poor sensitivity toward heavy metals are equipped with protecting mechanisms that allow for removing them. Metal-microorganism interactions can be divided into three groups:

- extracellular, when organisms have no direct contact with metal, because complexes with polymers, proteins, polysaccharides, and organic acids released out of the cell are formed, or precipitation of insoluble compounds occurs (sulfides, carbonates, phosphates)
- interaction with a cell surface, when metal is bound by functional groups of the cell-wall-membrane system and by the capsule
- intracellular, when the organism transports metal into the cell interior, where it is detoxicated and accumulated in insoluble form; another protecting mechanism consists of removing the metal ions from the cell due to the so-called $\text{Cd}^{2+}/\text{H}^+$ efflux system [2, 24-26]

Among heavy metals, cadmium is characterized by extremely high toxicity. In the atmosphere, it is readily oxidized to cadmium oxide in the presence of carbon dioxide, sulphur dioxide, and trioxide; it often takes part in reactions resulting in cadmium carbonate or sulphate formation. These compounds may be further emitted into the environment. Compared with other heavy metals, inorganic cadmium compounds are fairly water-soluble, hence they are more mobile and bio-available. In consequence, it leads to its bio-accumulation. Cadmium toxicity manifests itself at very low exposure levels. It cannot be degraded; once introduced, it remains within the environment in continuous circulation. Anthropogenic sources are the main reason for cadmium contamination [26]. Neutralization of harmful cadmium influence may be realized by ionic antagonism – antagonistic action of some salts (e.g. Ca and Mg) to other ones (Cd, Li, Ba). Enhancing the toxicity occurs in combinations of salts forming redox systems ($\text{Fe}^{2+}\text{-Fe}^{3+}$, $\text{Sn}^{2+}\text{-Sn}^{4+}$). The heavy metals ions flow from water or soil to plants, depending on the mechanism of metal ion flow through cell membranes [27]. There are several types of transport through the cytoplasmic membrane:

- transport due to simple diffusion with no energy efforts (e.g. Na^+ or Cl^- ions)
- facilitated diffusion with no energy inputs, but supported by specific proteins forming characteristic channels (e.g. for glycerol transport)

3) active transport that takes place with energy expenditure

The specific (secondary) active transport is propelled by a motor power of a proton pump (primary transport): symport, characteristic for sugar, amino acid, and organic acid transport along with H^+ or Mg^{2+} ions; antiport – characteristic for main ion transport: H^+ : Ca^{2+} , K^+ : NH_4^+ , K^+ : $H_2PO_4^-$; uniport, during which ion movement is realized in both directions; transport activated by ATP in the presence of protein carriers sensitive to osmotic stress – transport of ions associated with specific organic compounds with a strong and constant complexing strength. Kang et al. [28] and Şat [29] reported that inulin – the main storage component at Jerusalem artichoke tubers (along with pectins and crude fiber) binds large amounts of unnecessary and harmful compounds, among other heavy metals. Cadmium content in root plants (including *Helianthus tuberosus* L.) is a sensitive indicator of its level in an environment, because root plant species uptake over 50% of cadmium from the atmosphere [23, 30]. A plant's resistance toward toxic cadmium concentrations depend on its species and cultivar, as well as contents of other elements, which was confirmed by our earlier own studies.

Plants sensitive to cadmium show intoxication symptoms and decreased yields even at cadmium concentrations as low as 4-13 mg, while tomato and cabbage remain intact up to 170 $mg \cdot kg^{-1}$, and rice to 640 $mg \cdot kg^{-1}$. Cadmium is easily accumulated in plant tissues and is incorporated into the trophic chain [30]. The fodder quality determines the animal's health, namely that of ruminants, as well as the quality of animal-origin products. Metals, when accumulated within animal organisms, make particular parts of their carcasses useless for direct consumption and processing, and even as fodder for pets [4].

In the research of Chen et al. [31] the two Jerusalem artichoke cultivars (N2 and N5) had relatively high Cd tolerance and accumulation capacity (over 100 $mg \cdot kg^{-1}$), with N5 being more tolerant and having higher Cd accumulation than N2. Roots accumulated more Cd than stems and leaves. The bioconcentration factors (far higher than 1) and translocation factors (lower than 1) decreased with an increase in applied Cd. This research suggested that Jerusalem artichoke could be grown at relatively high Cd loads, and cultivar N5 could be an excellent candidate for phytoremediation of Cd-contaminated soils. Results of Augustynowicz et al. [32] showed that differentiated fertilization level did not affect the relative chlorophyll content in Jerusalem artichoke leaves. The simultaneous effect of mineral fertilization on the efficiency of photosynthetic apparatus measured as Area index was found at the end of the vegetation period.

Lead affects the principle living processes in plants. During photosynthesis it disturbs pigments synthesis, electron transport, and the dark phase. Inhibition of pigment synthesis refers mainly to chlorophyll *a* and *b* as well as hem. Due to a strong inhibition of nitrogenase during atmospheric nitrogen binding, lead has negative effects on nitrogen metabolism. Range 30-300 $mg \cdot kg^{-1}$ DM is proposed as critical lead concentration for plants [24, 30].

Considering vertebrate organisms, lead is accumulated mainly in their bone tissue (90%). It enters an organism, penetrates the blood, and is bound with plasma proteins [1, 15, 30]. The harm of lead intoxication refers namely to liver, kidneys, brain, and bone marrow. Average lead uptake by an adult amounts to 320-440 μg /daily, while the permissible weekly dose is 3,000 μg . In Poland, children have much exceeded lead doses that, due to WHO recommendations, amount to 54 μg /daily. Permissible lead concentration (35 $mg/100$ ml blood) may appear to be fatal both for children and adults [18, 20, 32, 33]. In our presented research, its contents did not exceed established criteria.

Under conditions of intensive cultivation technology, Jerusalem artichoke plants are exposed to many stress factors that make it impossible to realize physiological processes at the potential ability level of this species [34]. Studies allowed for confirming the variability at compared Jerusalem artichoke cultivars in view of heavy metals contents in its tubers, depending on genetic features and mineral nutrition. Achieved cadmium, cobalt, chromium, nickel, and lead contents were comparable to those quoted by other authors [7, 25, 30-32, 35-38].

The genotypic variability associated with cultivar features played a major role in variabilities of examined microelement concentrations in *Helianthus tuberosus* L. tubers. In the opinion of Danilcenko et al. [5], the highest amount of Se was identified in Jerusalem artichoke tubers cv. 'Rubik' than in cv. 'Albik' tubers. Al, Cr, Ni, Pb, and Zn in different cultivars of Jerusalem artichoke tubers were accumulated similarly. Sawicka [34] reported that the contribution of genotypic variability in total variability of discussed elements was 32.1-98.4%, though in the case of zinc the percentage of the variance in total variance was 32.1%, for copper – 61.5%, manganese – 97.2%, and iron – 98.4%. According to Sawicka [34], as well as Seiler and Campbell [39], the phenotypic variability of cultivars and wild forms of Jerusalem artichoke – referring to every feature of tuber chemical composition – was a combined effect of genetic and environmental variabilities. Seiler and Campbell [39] proved that the range of variance for genotypic components of Jerusalem artichoke was high in the case of microelements. Ranges of variance components of genotypic variability indicates that the major part of these metals' variability – due to a genotype – affects the opportunity to improve these features by means of crossbreeding and selection.

Applying organic fertilization in a form of manure did not increase the heavy metal contents in Rubik cv. tubers, whereas in the case of Albik cv., considerable cobalt and chromium level elevations were observed. In other opinion of Majewska and Kurek [40], organic fertilization at low soil redox potential may lead to an increase of soluble heavy metal forms concentration in a soil environment. Under such conditions, organic matter with wide C:N ratio may complex the heavy metal ions, thus enhancing their mobility.

Treating the Jerusalem artichoke with nitrogen on a background of a constant phosphorus-potassium nutrition revealed a differentiated reaction of examined cultivars in reference to every studied heavy metal. According to

Gruca-Królikowska and Waclawek [2], nitrogen fertilization has no direct influence on heavy metals accumulation in plants. Nitrogen indirectly – as ammonium form (ammonium nitrate, ammonium sulphate) – decreases soil pH, which enhances the heavy metals availability for plants. Mineral fertilization applied by mineral fertilization (applied at rates of: $N_0P_0K_0$, $N_0P_{44}K_{125}$, $N_{50}P_{44}K_{125}$, $N_{100}P_{44}K_{125}$, $N_{150}P_{44}K_{125}$, and $N_{200}P_{44}K_{125}$ on a background of full rate of FYM 30 t·ha⁻¹) by Sawicka and Michałek [23] at *Helianthus tuberosus* L. cultivation contributed to the decrease of primary photosynthetic reactions in photosystem II (Fv/Fm), and disturbances of the efficiency of open units PS II (Fv'/Fm'). The decrease of chlorophyll quantum efficiency (FPSII), photochemical chlorophyll fluorescence quenching, and non-photochemical chlorophyll fluorescence quenching (qN) also were observed. Disturbances in the photosynthesis of plants *Helianthus tuberosus* L. could affect the collection and accumulation of heavy metals by tubers.

The results of Augustynowicz et al. [32] showed a stimulating effect on the efficiency of the nitrogen artichoke photosynthetic apparatus at the end of the growing season, reflecting the positive effect of nitrogen on the transformation of solar energy into chemicals in photo system II (PSII). This in turn may increase the transport of electrons in thylakoid membranes and biochemical energy needed for assimilation of carbon dioxide in the light phase, independent of photosynthesis. Research made by Szymańska and Matraszek [38] showed that *Helianthus tuberosus* L. plants treated with nickel rather for roots than leaves, revealed higher nickel contents in their roots than leaves. Regardless of the penetration method, increasing nickel concentration affects the significant decrease of root physiological activity parameters: volume, total and active adsorption surface, as well as surface of 1 cm³ (although a higher drop of root indices was recorded after root than foliar metal application). Environmental contamination due to nickel also caused a decrease of chlorophyll content in leaves, though the foliar nickel application, unlike the root one, contributed to a larger decrease of chlorophyll *b* concentration than *a*. Therefore, older leaves have higher sensitivity to nickel applied for roots as compared to younger ones, whereas roots are more resistant to foliar applied nickel.

The cadmium, chromium, nickel, and lead contents were significantly affected by phosphorus-potassium fertilization. These trace metals concentrations were considerably lower in tubers in combinations with only phosphorus-potassium, than also with the high nitrogen dose; in the case of nickel – significantly higher as compared to the control object. Applied phosphorus fertilizers might be a major source of soil contamination with heavy metals, namely cadmium. Levels of solubility and availability for plants of particular heavy metals are different in various phosphorus fertilizers. Grant [15] reported higher coefficient of cadmium transfer to plants from superphosphate and NPK fertilizers than phosphorite powder. Grzebisz et al. [41] reported on a slight phosphorus availability on mineral acidified soils, which resulted from considerable concentrations of aluminum, iron, and manganese ions. The contents of

examined metals of Jerusalem artichoke tubers may also be influenced by phosphorus availability in a soil. Availability of the element contained in organic compounds depends on their solubility and the acidity soil solution. Phosphorus compounds are best soluble in soil with reaction close to neutral or slightly acidic [42]. In soils with slightly acidic reaction – like these in the present experiment – insoluble iron and aluminum phosphates can be formed, thus the amount of phosphorus available for plants could decrease. In the opinion of Gruca-Królikowska and Waclawek [2], phosphorus fertilizers – depending on the origin of phosphorites and apatites used for their production – may contain considerable amounts of heavy metals. Also, potassium fertilizers may enhance the heavy metals availability for plants. Doses of phosphorus and potassium fertilizers should in future be more precisely adjusted in accordance with a plant's requirements (suitable agriculture).

According to Sawicka [34], existing differences in mineral composition result from the phenotypic variability of Jerusalem artichoke cultivars, which is a combined effect of genetic and environmental variabilities. Different plant's reaction to fertilization and vegetation conditions in the case of nickel suggests that the stress may invoke an excessive accumulation of the metal in tubers during extreme drought. That suggestion was confirmed in research made by Baran and Jasiewicz [36], who reported that stress (e.g. prolonged drought or excessive rainfalls) might cause a disorganization of chloroplasts membranes, which affected directly the lowering of PS II photosystem efficiency – the most sensitive indicator of various stresses for plants. Variability of the environment, where *Helianthus tuberosus* L. grows, causes some modifications in internal regulation processes, both within a plant and its particular shoots. Recognizing the phenotypic variability of Jerusalem artichoke cultivars and separating the genetic and environmental variabilities would facilitate selection of those cultivars with the best stability of desired feature for future cultivation.

Weather conditions during the study had different influences on heavy metals accumulation in *Helianthus tuberosus* L. tubers. Tubers accumulated smaller quantities of these elements in years with the heaviest rainfalls during vegetation period than in drier years. In the opinion of Acton [43], cadmium is uptaken by plant root systems and uniformly distributed within a tuber. Its content in tubers might be affected by metal concentration in the air, water, and soil. Kays and Nottingham [44] reported that the effects of soil Cd forms in acidic soil types on its levels at plants were highly significant. The influence of vegetation conditions on nickel content in tubers were confirmed by studies performed by Acton [43]. However, Baran et al. [9] suggested that a slight amount of humus and loamy fraction in sandy soil (like that in the present experiment), determine a negligible share of potassium ions in heavy metals desorption. According to Gruca-Królikowska and Waclawek [2], the deficiency or excess of rainfalls determines a metal's availability in a soil solution. In water deficiency, the soil solution gets concentrated, leading to plant fading due to the decrease of root's sucking force, while in excess water,

metabolic processes in roots are inhibited due to oxygen deficiency, which leads to disturbances of passive and active metals uptake. Baran et al. [9] proved that *Helianthus tuberosus* L. plants are distinguished by considerable tolerance toward high heavy metals concentrations in a subsoil. Nevertheless, there is a need to recognize the dependence between soil sorption complex saturation with exchangeable cations and a plant's chemical composition. According to Gruca-Królikowska and Waławek [2] and Xiao et al. [11], the following factors can counteract excessive amounts of heavy metals in plants: cultivar, heavy metals contents in a soil, soil acidity, organic matter content, and soil moisture level. Sady and Smoleń [45] insist that following soil properties play a major role in affecting the amount of phyto-available heavy metals forms: soil type, granulometric composition, organic matter content, sorption properties, acidity, and redox potential. In the opinion of Kabata-Pendias and Pendias [1], a univocal correlation between heavy metal ions contents in soils and their presence in plants (that are able to selectively accumulate elements) cannot be found, because these elements circulating within the environment are put through various transformations.

Heavy metals and their compounds generally mean higher levels of danger to humans, plants, and animals that may have both acute and chronic impacts [46]. The effects of heavy metals on health can be complex: they can cause poisoning, and have carcinogenic, mutagenic, teratogenic, or embryotoxic effects. However, heavy metals (as other elements) are necessary for the human body, but most importantly, not to exceed the permissible limits of metal concentrations [5, 28, 47]. Our study investigated microelements such as Cd, Ni, and Pb, but their amounts do not exceed the limits specified in the Polish Hygiene Norms [18].

According to research, food quality can be characterized by the content of mineral elements. The elements are involved in many important body enzymatic reactions and bone mineralization, as well as in protection of cells and lipids in biological membranes. They also are construction elements of teeth, provide electrical signal transfers, and are co-factors in oxygen transport [28, 47]. The World Health Organization published recommendations for the daily intake (RDI) of minerals [48]. Some of the elements may constitute a potential health risk when they are consumed in amounts above the permitted ones [46]. Calcium, magnesium, sodium, potassium, sulphur, phosphorus, and chlorine have a recommended daily intake above 100 mg per body. Microelements, i.e. minerals required in small amounts (less than 100 mg per day), include copper, zinc, iron, manganese, chromium, selenium, boron, cobalt, and molybdenum. Arsenic, beryllium, mercury, cadmium, lead, and aluminium are toxic elements in high doses [15, 25, 28, 43].

Results

1. In the conditions of experience on sandy soils, genetic characteristics of cultivars of *Helianthus tuberosus* L. determined the greater accumulation of heavy metals

than mineral fertilization. A stronger tendency to accumulate cadmium, chromium and nickel had a Rubik, and more cobalt and lead in the tubers accumulated cultivar Albik.

2. The tested heavy metals, in terms of stability, arranged in the following ranks of decreasing values: in the tuber cultivar Albik: Co > Pb > Cr > Ni > Cd, and Pb; cultivar Rubik: Cr > Cd > Co > Ni. Albik was more stable in the accumulation of heavy metals than Rubik.
3. Achieving greater health security of *Helianthus tuberosus* L. tubers can be achieved using rational fertilization, especially nitrogen. When applying a constant level of phosphorus-potassium fertilization, the lowest cadmium content in the tubers were found in the object fertilized with 150 kg N·ha⁻¹, cobalt – 100 kg N·ha⁻¹, chrome – just for a phosphorus-potassium fertilization, while the lowest content of nickel and lead – in the object control, fertilized with manure only.
4. Designated polynomial regression equations allow predicting changes of the analyzed metal content in tubers of *Helianthus tuberosus* L. under the influence of mineral fertilizers and providing for their excessive concentration.
5. In years with higher rainfall in the first half of vegetation, the plants accumulated less cobalt, chromium, lead, and more cadmium. In the conditions deficiency of precipitation, in the second half of vegetation of *Helianthus tuberosus* L., the plants accumulated more nickel.
6. In order to produce a safe plant, material must take control at the stage of cultivation. Hence the need to continue research on the content of heavy metals in the tubers of *Helianthus tuberosus* L. and assess the suitability of material for the production of foods or drugs.

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