

Article

Hazard of Contamination with Heavy Metals in *Thymus serpyllum* L. Herbs from Rural Areas

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Abstract: The aim of the research has been to assay the contents of Zn, Cu, Mn, Fe, and Pb in soil and in the organs of wild *Thymus serpyllum* L. acquired from three natural habitats from the Kuyavia-Pomerania Province (Poland) not exposed to contamination with metals. As compared with the contents of the geochemical background of the soils in the region and the value of enrichment factor (EF), there was noted a considerable content of Pb in the soil from two locations. The Pb content in plant tissues in one of the three locations was higher than the admissible content specified by the World Health Organization WHO (above 10 mg kg⁻¹ d.w.). As for the Zn content, only the plants from the locations with the relatively youngest phytocenosis met the conditions for herbs to be used for phytotherapy. The Zn content in the dry weight of the plants collected from the other two locations exceeded 50 mg kg⁻¹. The risk of an elevated content of Zn and Pb in the plants makes it necessary to monitor the soil environment and to limit the collection of the plants from natural habitats, as well as to encourage the collection from controlled habitats.

Keywords: enrichment factor; heavy metals; phytoaccumulation; phytotherapy; *Thymus serpyllum* L.



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1. Introduction

For sustainable global development, food safety is a high-priority issue. In recent decades, adverse effects of toxic substances on crop quality have threatened both food safety and human health [1]. Heavy metals and metalloids (e.g., As, Cd, Pb, and Hg) are classified as non-essential for metabolic and other biological functions as they can disturb human metabolism, contributing to a higher morbidity [2]. Trace elements and other contaminants are released into the environment by both natural and anthropogenic sources. Highly reactive, and often toxic even at low concentrations, they may enter soils and bioaccumulate in food webs. Certain heavy metals, such as Cu, Zn, and Fe, are essential components of metabolic processes in herbs, including enzymes, linked to the metabolic functioning of biota [3].

Heavy metals also may persist in the environment for years, posing long-term risks to human health [4]. For that reason, some studies have focused on understanding how to reduce these toxic elements from natural environments with an eco-friendly approach, highlighting an interesting correspondence between plant communities and heavy metal concentration in soil [5]. Recent decades have witnessed an increase in alternative medical approaches, including the use of herbal supplements, natural products, and complementary medicines. The therapeutic activity of herbs is associated with the content of bio-active

elements and organic compounds, such as vitamins, alkaloids and tannins. Herbs and spices constitute an important role in the transfer of essential metals from soil to human body [6,7]. Many trace metals are essential for the formation of constituents responsible for curative properties [8]. However, there is an increasing concern about the safety and health benefits of these traditional medicines as one of the main hazards with the use of herbs is the presence of trace elements. Many medicinal plants have been shown to bioaccumulate various heavy metals when grown near industrial areas [9]. Thus, soil-plant systems provide an example of abiotic-biotic interactions in the environment. With that in mind, this article focuses on and describes heavy metal contamination in soil-herb crop subsystems with respect to human health risks.

The pharmaceutical herbal material legislature, concerning the content of heavy metals, in the European Union (EU) countries and globally, follow the requirements of the European Pharmacopoeia [10] and the requirements of the World Health Organization [11]. The contents of trace elements, especially the contents of Fe, Mn, Zn and Cu in medicinal plant substances, are not regulated by law. In the light of the current regulations and the WHO guidelines, the admissible lead content in medicinal plants is Pb 10 mg kg⁻¹ d.w. According to the Polish Pharmacopoeia, the regulations are more restrictive and they specify the limit values of Pb concentrations below 5.0 mg kg⁻¹ d.w. in herbs [12].

One of the plants used in herbal medicine is *T. serpyllum*. This plant is a frost-hardy plant of the family *Lamiaceae*, a chamephyte with lignified creeping stems, reaching up to 30–40 cm in length. It creates low sods with a compact habit. The flowers are tiny, pink-violet in colour, with dense clusters on the top of the stems. The species occurs in the temperate climate zone; in Europe and in Asia. It is also found in India, as well as on the Kola Peninsula and in Iceland. It also occurs in North America. In Poland, it is common and it represents a native flora [13–17]. In the natural state, *T. serpyllum* plants grow in very sunny spots, in sandy permeable soil with a slightly acid reaction. It is very common in dry pine forests, well-lit shrubs, on slopes and roadsides. The species is common for the *Koelerio-Corynephoretea* class [18]. In Poland, in natural stands, one can find 12 species of the genus *Thymus*, however, only two occur frequently: *T. pulegioides* and *T. serpyllum* [17,19].

The herbal material of *T. serpyllum* is mostly collected from natural habitats [20]. The material acquisition method is part of the sustainable development of rural areas, which involves a rational and environment-friendly use of natural resources [21,22]. A rational acquisition of material from natural habitats helps limiting a negative effect of the crop protection chemicals applied throughout the cultivation on the natural environment. The herbal material collected from the areas with no agrotechnical, and phytosanitary treatments applied shows a high quality and medicinal properties. In response to a growing social demand for natural treatment methods, including herbal medicine, the number of pharmaceutical and cosmetic enterprises interested in purchasing the herbal material increases [23]. A collection from undeveloped areas, with respect to the natural resources, including woodland, supervised by qualified advisors, enhances the job market and the income of rural areas residents [24].

T. serpyllum plant has a characteristic and slightly spicy fragrance, and it has been, for a long time, used as seasoning in herbal medicine [20,25,26]. It contains luteolin, apigenin, glycosides, scutellareins, triterpenes, organic acids, mineral salts and bitter serpyllin. *Thymi oleum* essential oil contains more than 200 chemical compounds, and the main group are terpenes, especially thymol and carvacrol [10]. Their content in plants varies and it depends on the herbal material origin [27–29]. Thanks to the presence of active compounds, it has relaxant, expectorant, mucoactive, disinfecting, anti-inflammatory, and diuretic properties. Thyme essential oil shows antibacterial, antifungal, antiviral [30,31], and antineoplastic effects [27,32]. *T. serpyllum* preparations can be also used to treat rheumatic disorders and herbal infusion can prevent hair loss [33–37]. Moreover, the thyme oil shows insecticidal properties, and it can be used as an insect repellent [36,38].

The macro- and microelements found in plant tissues are indispensable for an adequate plant growth and development as well as for the health and life of the people

and animals consuming the plants. The group includes Zn, Cu, Mn, and Fe, while other metals, especially As, Cd, and Pb, have a negative effect on the course of metabolic processes [1]. The impact of heavy metals on plants is very complex. One of the effects is a decreased content of active medicinal compounds, which deteriorates the herbal material quality [39,40]. The amount of microelements and essential oils in wild herbal material depends on growth habitat [41] and on the development stage that is species-specific [42]. The uptake of trace elements by plants is complex and it is conditioned by the interaction of environmental factors. Wild *T. serpyllum* shows a specific capacity for trace elements phytoaccumulation [43]. There are three stages of the process of metal hyperaccumulation in plants: the uptake with soil solution by the root system, the transport of the ions uptaken by vascular bundles, and the compartmentation and detoxication of metals in the stem [44]. The translocation of metals from the roots to the stem decreases their availability in root cells [45]. It is related to the specific features of root cell tonoplast and to the increased capacity for accumulating metals in leaf cell vacuoles [46,47]. However, an excessive lead content has a negative impact on many metabolic processes in plants. The Pb ions decrease the accumulation of potassium, calcium, magnesium, manganese, zinc and iron cations in plant cells. Lead is mostly accumulated in roots. However, it can be also transported to aboveground parts [48,49]. The lead availability and uptake from soil is affected by pH, the root system area and by the chemicals released by roots [40].

The factors determining the occurrence of many plant species in soils with a high content of heavy metals also include a capacity for forming the arbuscular mycorrhiza with fungi representing the assemblage *Glomeromycota* [50,51]. That type of mycorrhiza is found in most herbal plants from the ecosystems of the temperate and semidesert zones [52]. The presence of mycorrhizal fungi affects not only the root system of the host but also the functioning of the stem by modifying the hormonal status of the plant and the effect of stomata [53,54]. Abscisic acid can block transpiration, which results in the accumulation of elements in the aboveground plant organs [55].

The plants of the family *Lamiaceae*, represented by *T. serpyllum*, cover the species known as heavy metal hyperaccumulators [56] which, in the aboveground parts, can accumulate from 10 to 500 times more metal than the representatives of the same species growing in the uncontaminated stands. The group includes also the plants for which the ratio of metal content in the aboveground parts to the content in roots and in soil is higher than 1 [57].

The aim of the research has been to assay the content of Zn, Cu, Mn, Fe, and Pb in soil and in the organs of *T. serpyllum* acquired from three natural habitats not exposed to contamination with metals. There was determined the content of metals in soil and in plant tissues, as compared to their biologically permissible contents. The possibilities of using the herbal material derived from rural stands were evaluated as well as a possibility of using herbal material from rural areas from undeveloped agricultural stands, with protection and sustainable use of biological diversity in mind. Assaying the content of metals indispensable to plants, including Zn, Cu, Mn, and Fe, in soil facilitates the evaluation of the soil environment and estimating a potential threat of pollution with those metals. For that reason, the contents of the forms of those metals available to plants were assayed. There is a lack of Cd, Hg, or As pollution emitters in the neighbourhood of the sampling locations; hence no assays of the content of those metals. The reports by other authors did not point to the presence of those metals in the parent material of the soils in the region. There was assayed a total Pb content due to the earlier results of herbal plants [23,58,59], pointing to a threat of the accumulation of that metal in plant material despite a relatively low total content in soil.

2. Materials and Methods

The total content of Zn, Cu, Mn, Fe and Pb and their forms available to plants were assayed in the soil samples and their content in *T. serpyllum* organs. Soil and plants were sampled in July 2018 in three locations from the Kuyavia-Pomerania Province (north-central Poland). They were as follows: A–Łosiny (53°37'22" N 17°59'08" E), B–Okole (53°17'52" N

17°56'37" E) and C–Łochowo (53°07'19" N 17°50'19" E) (Figure 1). For the purpose of representing the geographic locations, the WGS-84 reference system was used. The distance from the source of pollution was considered when selecting the study area. Sampling locations B and C were chosen as the nearest, and A as the most distant from the urban agglomeration. Soil and plant were sampled from the edge of the pine forest. The locations were most representative in terms of the plant habitat.



Figure 1. Localization study area.

Soil samples were taken from two depths of soil profiles using a spade. The soil was sampled from the plant root zone (0–30 cm). The average soil samples were made from three individual subsamples. Some soil was also sampled from a depth of 120–150 cm to estimate the metal content of the local geochemical background. Soil samples were air-dried and screened through a 2-mm sieve for physical and chemical analysis (three replications). Soil pH was measured using a glass electrode in 1M KCl solution (1:2.5 soil-solution ratio) [60]. The total organic carbon (TOC) content was assayed using analyser vario Max CN Elementar provided by Analysensysteme GmbH (Hanau, Germany). The soil texture was measured applying the laser diffraction method with Mastersizer 2000 (Malvern Instrument, Malvern, UK). The total metal contents were determined applying digestion with HF and HClO₄ acid solutions, according to the Crock and Severson (1980) [61] method. The certified reference materials (TILL-3, the Canadian Certified Reference Materials) were used to verify the accuracy of the results. The recovery rates for the elements were as follows: 98%, 99%, 103%, 97%, and 99%, for Zn, Cu, Pb, Mn, and Fe, respectively. The contents of metals available to plant forms were determined with 1 M HCl solution (Rinkis method) [62]. To evaluate the bioavailability of metals in soil samples, there was applied a test with the solution of 1 M HCl, which is a strong extractant to facilitate assaying metals both exchangeable forms, bonded with carbonates, Fe-Mn oxides, as well as with organic matter. The content of Zn, Cu, Mn, Fe forms susceptible to extraction with 1M HCl was compared with the so-called limit numbers developed by the State Research Institute–Institute of Soil Science and Plant Cultivation (IUNG) in Puławy, Poland [63]. The method is commonly used in the research of soils in Poland to evaluate the content of the metal forms potentially available to plants [64–66]. The metal contents in soil samples and plant material were assayed applying the atomic absorption spectrometry method (AAS), with the SOLAAR S4 AA spectrometer (ThermoElemental, Cambridge, UK).

For the purpose of research, *T. serpyllum* plants were collected at the flowering stage. The aboveground plant parts, namely leaves, stems and inflorescences, were separated from the roots, and then rinsed in distilled water and dried (at 40 °C) to reach the air-dry state. Then the plant material was crushed in the agate mortar. The homogenized material (300 mg) was microwave-digested in the Speedwave Two mineraliser (Berghof, Germany) with the wet mineralization method (5 mL 65% HNO₃, 1 mL 30% H₂O₂).

The total contents of Zn, Cu, Mn, Fe, and Pb in inflorescences, leaves and stems following mineralization were determined using the atomic absorption spectrophotometry, with a SOLAAR S4 AA spectrometer (ThermoElemental, Cambridge, UK).

Statistical analyses were performed applying Statistica 12.0 software (StatSoft Inc., Tulsa, OK, USA) [67]. The contents of heavy metals in soil and in plant parts are expressed as mean \pm standard deviation (Sd). The relationship between the soil properties selected and the content of metals of *T. serpyllum* was also calculated (correlation coefficient R, coefficient of determination R²). Hierarchical cluster analysis (CA) using Ward's method (1963) [68] was used to identify the similarity groups across samples.

The coefficient of variation in the metal content in the plant was calculated as follows:

$$CV = (Sd/X) \times 100\% \quad (1)$$

where: CV is the coefficient of variation (%), Sd is the standard deviation, and X—arithmetic mean. The values, 0–15%, 16–35% and >36%, indicate low, moderate, or high variation, respectively [69].

The human impact on soils was estimated with enrichment factor (EF), based on the normalization of the metal measured against a reference metal. Generally, Al and Fe are used as reference elements. The values of enrichment factor (EF) facilitate evaluating the risk of contamination with heavy metals. The values of enrichment factor (EF) were calculated according to the Equation (1):

$$EF = [C_n/C_{nFe}]/[B_n/B_{nFe}] \quad (2)$$

where C_n—total content of metal in soil sample; C_{nFe}—total content of Fe in soil sample; B_n—content of metal for the geochemical background; B_{nFe}—content of Fe for the geochemical background as the reference element [70]. For the calculations, there was assumed the content of metals in parent material at the depth of 120–150 cm. Based on the EF value, the categories were determined as: <2—deficient to minimal enrichment, 2–5 moderate; 5–20—considerable; 20–40 very high; >40—extremely high enrichment [71].

3. Results

3.1. Physical and Chemical Properties

With the percentage content of respective grain size fractions, the soil was classified as sand for A and loamy sand for B, C [72]. The results of grain size composition and selected parameters of soil samples (0–30 cm) are presented in Table 1. They include from 77.9–87.4% of sand fraction (2.0–0.05 mm), from 11.7–20.7% of silt fraction (0.05–0.002 mm) and from 0.9–1.4% of clay fraction (<0.002 mm). The soil reaction ranged from very acid for location A to slightly acid for location C. The soils demonstrated a varied content of organic carbon. The highest value was found in the soil from sampling location B (13.8 g kg⁻¹).

Table 1. Selected properties of soil samples (0–30 cm).

Sampling Location *	pH H ₂ O _{dest}	pH 1M KCl	TOC g kg ⁻¹	Grain Size Composition [%]			Textural Class USDA
				Sand 2.0–0.05 mm	Silt 0.05–0.002 mm	Clay < 0.002 mm	
A	4.97 \pm 0.05	4.34 \pm 0.10	8.51 \pm 0.22	87.4	11.7	0.9	** S
B	5.56 \pm 0.03	4.59 \pm 0.04	13.8 \pm 0.31	79.1	19.6	1.3	LS
C	6.94 \pm 0.03	6.39 \pm 0.06	9.31 \pm 0.21	77.9	20.7	1.4	LS

* location vicinity: A—Łosiny, B—Okole, C—Łochowo; TOC—total organic carbon; USDA—United States Department of Agriculture; ** S—sand, LS—loamy sand.

The soil and *T. serpyllum* plant sampling locations are the stands with sandy texture (Table 1). The soil sample from location A was most acid and it recorded the lowest content of clay fraction. However, the soil reaction in location C was slightly acid (pH in 1 M KCl) and the soil revealed the highest content of clay fraction, thus creating better plant

development conditions. The soil sampled from location B recorded the highest total organic carbon (13.8 g kg^{-1}).

3.2. Heavy Metal Content in Soil Samples

The soil from locations A and C recorded a similar Zn_t content, while the soil from location B contained over two-fold more of that metal. The content of Cu_t in the soil samples differed and it ranged from 4.03 mg kg^{-1} in location A, to 9.1 mg kg^{-1} in location B (Table 2), whereas the total lead content ranged from $45.0\text{--}54.1 \text{ mg kg}^{-1}$. The content of manganese in soils varied a lot, which is evident from a high value of the coefficient of variation (CV). The soil sampled from location A contained 87.6 mg kg^{-1} of Mn and a three-fold higher total content was noted in the soil sampled from location B, and more than six-fold higher—from location C. The least varied were the total contents of lead and iron in soil for which the CV values were 9.27% and 14.74%, respectively.

Table 2. Total content of Zn, Cu, Mn, Pb and Fe in topsoil.

Sampling Location *	Zn_t	Cu_t	Mn_t	Pb_t	Fe_t
	mg kg^{-1}				g kg^{-1}
A	25.5 ± 1.25	4.0 ± 0.08	87.6 ± 4.11	48.9 ± 2.05	4.7 ± 0.09
B	58.6 ± 2.20	9.1 ± 0.44	277.7 ± 11.12	54.1 ± 2.84	6.1 ± 0.13
C	25.6 ± 1.74	5.5 ± 0.11	536.6 ± 23.34	45.0 ± 1.76	6.2 ± 0.16
Mean	36.60	6.20	300.63	49.33	5.67
Sd	19.05	2.62	225.38	4.57	0.84
CV(%)	52.05	42.26	74.97	9.27	14.74

* location vicinity: explanations as in Table 1; Sd—standard deviation; CV—coefficient of variation; Zn_t , Cu_t , Mn_t , Pb_t , Fe_t —total forms of zinc, copper, manganese, lead and iron in soil, respectively.

The average total content of metals in soil at a depth of 120–150 cm (Table 3) was similar to the geochemical background values. Soil sampled from location B had the highest total content of Zn, Cu, Pb, and Fe content of the three habitats studied. There was also assayed the content of metal forms available to plants (Table 4). The content of Zn_a , Cu_a , Mn_a and Fe_a varied a lot across the locations. All the soil samples recorded a high content (according to the Polish norms) [63] of available-to-plants zinc forms; Zn_a ranging from 6.7 mg kg^{-1} (C) to 14.2 mg kg^{-1} (B), while the content of copper (Cu_a) available to plants in the soil sampled from location A and C was average and high in the soil from location B. In all the soil sampling locations, the content of Mn_a was average.

Table 3. Total content of metals in parent material (120–150 cm).

Sampling Location *	Zn_t	Cu_t	Mn_t	Pb_t	Fe_t
	mg kg^{-1}				g kg^{-1}
A	30.0 ± 1.65	5.10 ± 0.25	414.0 ± 22.25	15.0 ± 0.43	9.4 ± 0.34
B	35.2 ± 1.44	7.31 ± 0.31	435.1 ± 24.10	17.2 ± 0.51	10.2 ± 0.54
C	29.4 ± 1.12	6.10 ± 0.20	503.0 ± 26.21	15.5 ± 0.52	7.8 ± 0.40
Mean	31.50	6.17	450.7	15.90	9.13
Sd	3.19	1.11	46.61	1.15	1.22
CV(%)	10.1	18.0	10.3	7.2	13.4

* location vicinity: explanations as in Table 1; Sd—standard deviation; CV—coefficient of variation; Zn_t , Cu_t , Mn_t , Pb_t , Fe_t —total forms of zinc, copper, manganese, lead and iron in soil, respectively.

Table 4. Content of metals available to plants (extracted with 1M HCl).

Sampling Location *	Zn_a		Cu_a		Mn_a		Fe_a		Pb_a ***
	mg kg^{-1}	Content Rating **	mg kg^{-1}	Content Rating **	mg kg^{-1}	Content Rating **	g kg^{-1}	Content Rating **	mg kg^{-1}
A	7.9 ± 0.19	high	1.7 ± 0.07	average	56.8 ± 0.34	average	0.85 ± 0.03	average	0.8 ± 0.05
B	14.2 ± 0.61	high	4.3 ± 0.16	high	32.9 ± 1.27	average	0.97 ± 0.04	average	1.1 ± 0.05
C	6.7 ± 0.34	high	2.0 ± 0.13	average	65.7 ± 1.44	average	1.05 ± 0.04	average	0.7 ± 0.03

* location vicinity: explanations as in Table 1; ** Polish regulation acc. to IUNG Puławy [63] Zn_a , Cu_a , Mn_a , Fe_a , Pb_a —forms of zinc, copper, manganese, iron and lead available to plants, respectively; *** no rating in Polish regulation.

The value of enrichment factor (*EF*) amounted to, respectively: Zn from 1.20–2.78; Cu from 1.25–2.19; Mn from 0.92–1.75; Pb from 4.17–5.98 (Table 5).

Table 5. Values of enrichment factor *EF* in topsoil.

Sampling Location *	Zn (<i>EF</i>)	Cu (<i>EF</i>)	Mn (<i>EF</i>)	Pb (<i>EF</i>)
A	1.58	1.25	0.38	5.98
B	2.78	2.19	0.92	5.10
C	1.20	1.30	1.75	4.17

* location vicinity: explanations as in Table 1.

3.3. Content of Heavy Metal in *T. serpyllum*

Depending on the sampling location, there was recorded a varied content of metals in plant organs. There was no accumulation in the respective plant parts studied (Table 6). The highest Zn content was reported in the plants from location A (Table 6), collected from the edge of the pine forest, about 50 years of age, from 68.55 to 116.9 mg kg⁻¹. However, in stems and leaves, the content was, on average, 62% higher than in roots. In location B, where the secondary succession process started in the 1990s, the plants contained, on average, 2.4% zinc less, and its highest content was recorded in plant roots (41% higher than in inflorescences and 26% higher than in stems with leaves). *T. serpyllum* plants from location C, from a relatively younger phytocenosis, contained, on average, 46.92 mg kg⁻¹ of zinc, which is twice less than the plants from locations A and B. There was found a highly significantly negative correlation between the content of Zn_{L+S} and pH_{KCl} of soil; $r = -0.998$ (Table 7).

Table 6. Content of Zn, Cu, Pb, Mn, Fe mg kg⁻¹ in the dry weight of *T. serpyllum* plants.

Sampling Location *	Metal	<i>Thymus serpyllum</i> L.		
		Inflorescences	Leaves + Stems	Roots
A	Zn	106.2 ± 6.90 CV = 6.50%	116.9 ± 5.40 CV = 4.62%	68.55 ± 12.94 CV = 18.88%
	Cu	23.50 ± 1.62 CV = 6.89%	7.80 ± 0.28 CV = 3.59%	28.20 ± 1.30 CV = 4.61%
	Pb	6.80 ± 0.60 CV = 8.82%	17.90 ± 5.23 CV = 29.22%	5.00 ± 3.53 CV = 70.60%
	Mn	338.6 ± 18.20 CV = 5.38%	289.1 ± 20.93 CV = 7.23%	138.6 ± 47.16 CV = 34.01%
	Fe	605.0 ± 62.21 CV = 10.28%	207.0 ± 9.89 CV = 4.77%	303.5 ± 146.00 CV = 48.1%
	Zn	77.60 ± 10.47 CV = 6.13.49%	97.80 ± 6.22 CV = 6.36%	109.4 ± 14.9 CV = 13.61%
B	Zn	21.75 ± 1.06 CV = 4.87%	11.35 ± 0.65 CV = 5.73%	22.00 ± 4.58 CV = 20.82%
	Cu	6.75 ± 6.29 CV = 93.18%	16.30 ± 0.88 CV = 5.39%	20.80 ± 2.40 CV = 11.54%
	Pb	124.3 ± 9.47 CV = 7.62%	128.0 ± 6.89 CV = 5.38%	264.6 ± 67.30 CV = 25.42%
	Mn	619.0 ± 131.52 CV = 21.24%	878.0 ± 70.71 CV = 8.05%	852.5 ± 243.40 CV = 28.55%
	Fe	64.75 ± 12.02 CV = 18.56%	41.95 ± 2.89 CV = 6.89%	34.05 ± 0.35 CV = 1.03%
	Zn	30.85 ± 2.47 CV = 8.00%	16.20 ± 1.04 CV = 6.42%	25.00 ± 4.24 CV = 16.96%
C	Cu	4.1 ± 5.37 CV = 130.9%	12.00 ± 0.42 CV = 3.50%	10.45 ± 5.66 CV = 54.16%
	Pb	145.8 ± 4.17 CV = 2.86%	146.5 ± 7.96 CV = 5.43%	119.9 ± 9.05 CV = 7.55%
	Mn	299.5 ± 98.29 CV = 32.81%	326.5 ± 7.77 CV = 2.37%	265.0 ± 31.11 CV = 11.74%
	Fe			

* location vicinity; explanations as in Table 1; CV—coefficient of variation.

Table 7. Correlation (R), determination coefficient (R²) and linear regression models for selected properties of soils and *T. serpyllum* plants.

Variables		Regression Equation	R	R ²
Dependent *	Independent *			
Zn _{L+S}	pH _{KCl}	$y = 307.99 - 38.22x$	-0.998	0.996
Pb _{L+S}	Zn _{L+S}	$y = 8.6693 + 0.07889x$	0.999	0.998
Pb _{L+S}	pH _{KCl}	$y = 32.995 - 3.020x$	-0.999	0.998
Mn _I	Mn _{L+S}	$y = -48.56 + 1.3385x$	0.999	0.998

* Zn_{L+S}, Pb_{L+S}, Mn_{L+S}—concentration of zinc, lead, manganese in leaves and stems, respectively; Mn_I—concentration of manganese in inflorescences; y —dependent variable.

In the plants growing in all the habitats under study, the lowest contents of Cu were noted in stems with leaves from sampling locations A, B, C—7.8, 11.35, 16.2 mg kg⁻¹, respectively (Table 6). In the other organs from sampling locations A, B, and C, the content of that element was, on average, 3.3- and 1.9- as well as 1.7-fold higher, respectively. At the same time in the plants from locations A and B there was recorded a slightly higher copper content in plant roots than in inflorescences. However, in the *T. serpyllum* plants from location C the opposite tendency was identified.

The herb samples showed a high variation in the lead content, depending on the sampling location and on the plant organ. The highest Pb content was noted in the roots of *T. serpyllum* plants from location B (Table 6) 20.8 mg kg⁻¹. However, the lowest lead content was found in the inflorescences of the plants collected from location C—4.1 mg kg⁻¹. An equally low content of the element was reported in stems with leaves from the plants from location B and the roots of the plants from location A. The herbal material acquired from the stands in locations A and C, where the average lead content, 8.85 and 9.9 mg kg⁻¹, respectively, meets the conditions provided for in the WHO norm for herbs: Pb 10 mg kg⁻¹. The lead content in the leaves and stems in *T. serpyllum* plants (Pb_{L+S}) was significantly negatively correlated ($r = -0.999$) with soil reaction (pH_{KCl}) (Table 7).

The content of manganese in leaves also varied a lot and it depended on the sampling location and on the organ. There was noted a highly significantly positive correlation ($r = 0.999$) between the content of manganese in leaves and in stems (Mn_{L+S}) and the content of that element in inflorescences (Mn_I) in *T. serpyllum* (Table 7). The highest Mn content was noted in the inflorescences of *T. serpyllum* plants collected from location A (Table 6) (338.6 mg kg⁻¹). On average, in the aboveground parts of the plants from the same stand, 313.85 mg kg⁻¹ of manganese was recorded. However, the content of that element in the roots of *T. serpyllum* plants was 55.8% lower (138.65 mg kg⁻¹). In the other stands, in the stems and leaves of plants, a similar content was noted. However, in the roots of *T. serpyllum* plants from location B, the content of manganese was more than two-fold higher than in the aboveground stems.

The highest mean content of iron was identified in the plants from location B (Table 6). In the other cases, the mean content of Fe in tissues was definitely lower: 52.5% lower in the plants from location A and 62.1% lower in the plants from location C. The plants collected from location B accumulated similar amounts of iron in the aboveground and underground parts, while *T. serpyllum* plants from location A contained most of that element in inflorescences, and in the organs of *T. serpyllum* plants sampled from location C the iron content was similar.

The results of cluster analysis indicate that the soil demonstrated a similar total metal content in locations A and B in both the surface layer and the parent material, in comparison to location C (Figure 2a,b). The inflorescences of *T. serpyllum* collected from habitats A and B showed similar concentrations of metals, as compared with the content of metals in the inflorescences collected from location C (Figure 3a), whereas a similar concentration of metals in stems and leaves were found in plants collected from habitats A and C (Figure 3b). The cluster analysis showed differences in the content of metals in respective organs of the herbs across the sampling locations. The plants collected from

location B showed a slightly different habit, they were higher than the plants sampled from the other two locations, which could be due to slightly better soil conditions. Soil in location B showed a considerably higher content of organic matter (Table 1) and, as for that content, it demonstrated slightly more favorable water holding capacity, as compared with locations A and C, which could be the reason for such variation, despite a similar content of metals in soil.

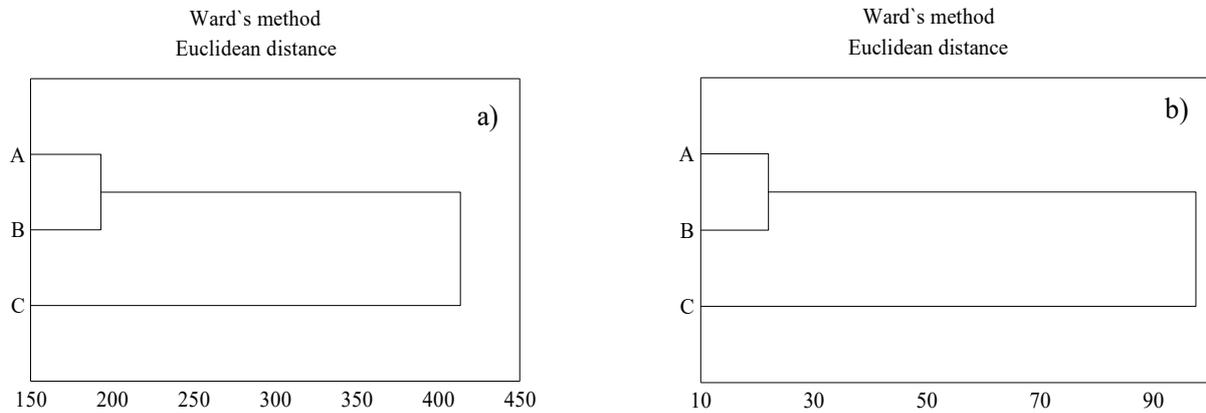


Figure 2. Total content of metals in topsoil (a); total content of metal in parent material (b). A, B, C—location vicinity: explanations as in Table 1.

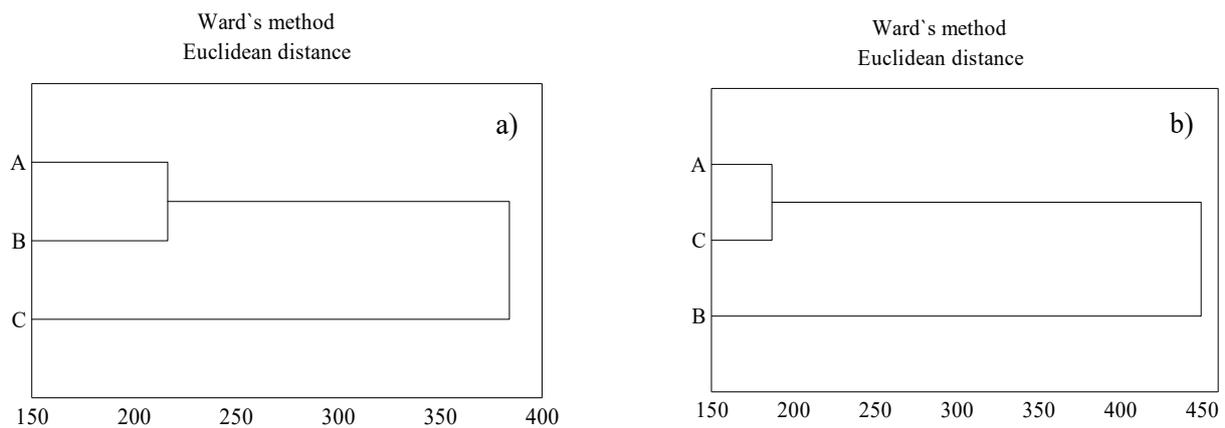


Figure 3. Concentration of metal in inflorescences (a); concentration of metals in stems and leaves (b). A, B, C—location vicinity: explanations as in Table 1.

4. Discussion

The soils were not contaminated with heavy metals [73] and their content in the parent material was typical for soils (north-central Poland) developed from Baltic glacial sediments [74]. However, as compared with the contents of the geochemical background of the soils in the region and the value of enrichment factor (EF), there was noted an average content of lead in the soil from location C, an average content of manganese in the soil from location B, and a considerable content of lead in the soil from two locations A and B (Table 5). Enrichment factor is a relatively simple and easy tool to assess the enrichment of elements in soils [58,75,76]. The content of Zn_a , Cu_a , Mn_a and Fe_a was location-specific. As reported by Mercik 2004 and Ociepa 2011 [77,78], the availability of heavy metals to plants is affected by the parent material, the soil reaction and by the content of organic substance, clay minerals and the interaction with other elements. The total content of respective metals in the soil samples was found in a decreasing series: $Fe_t \gg Mn_t > Pb_t > Zn_t > Cu_t$.

For the purpose of research, *T. serpyllum* plants were collected at the flowering stage. The concentration of heavy metals in the plant varies throughout the phenological stages

and it is different in different parts: root, leaves, stems and flowers [23,59,79]. In their roots, stems, leaves, and inflorescences, *T. serpyllum* plants accumulated relatively high contents of Zn and Pb, which is species-specific. *T. serpyllum* is a species with a natural tolerance to high contents of heavy metals in the environment and it is considered an metallophyte [80]. *T. serpyllum* is a plant which can grow in soils with a very high content of metals [81,82]. The soil sampling locations were classified as dry psammophytic grasses with sandy soils, easily drying, with an acid reaction [18]. The differences were recorded for the secondary succession period in those stands. With the age of the stands neighbouring with location A and own observations, that phytocenosis was considered the oldest one, with more than 50 years of age. The other two phytocenoses were estimated to be around 30 years of age. It could have determined the richness of the soil in mycorrhizal mycelia and, at the same time, it could have affected the accumulation of metals in plant tissues. Importantly, the village of Łosiny is found on the border of the Tuchola Landscape Park and it is adjacent to the “Jelenia Wyspa” reserve and so it is not a neighbour of any pollution emitters. Nevertheless, in the *T. serpyllum* plants there was found a relatively high content of Pb and Zn. In the present studies, the Zn content in the cells of the aboveground part of the plants from locations A and C was higher than in the roots.

The average content of zinc in crops ranges from 10–100 mg kg⁻¹ d.w. [83]. However, to meet the physiological requirements of most plants, the content of zinc in stems ranging from 15–30 mg kg⁻¹ is enough [48]. It means that in the *T. serpyllum* plants acquired from natural locations A and B, the zinc content was higher than average. In all the *T. serpyllum* plants, the zinc content exceeded their average physiological requirements. Only the herbal material acquired from location C satisfied the conditions provided for in the WHO norm for the herbs applied in herbal medicine according to which the Zn content cannot exceed 50 mg kg⁻¹.

Copper is a metal which is considered indispensable for the plant life and functioning, however, its excess is toxic. It shows a low mobility in the plant and it is mostly accumulated in roots [40]. The content of copper in plants is usually below 4–5 mg kg⁻¹, and its mean content in the aboveground parts of plants ranges from 5 to 20 mg kg⁻¹ [84]. In the *T. serpyllum* plants under study, a definitely higher Cu content was noted in the roots and the inflorescences.

The content of Zn, Cu, Mn, and Fe in *T. serpyllum* plants can determine their applicability for herbal medicine. *T. serpyllum* plants collected as herbal material from location B (considering the average Pb value in the entire plant) are not suitable for herbal medicine applications. The risk of an increased lead content in plants (above 10 mg kg⁻¹ d.w.) makes soil environment monitoring necessary. The collection of that plant from natural stands must be limited and the collection from controlled plantations—increased.

5. Conclusions

The soils studied from the Kuyavia-Pomerania Province (Poland) were not contaminated with heavy metals and their content in the parent material was typical for soils (north-central Poland) developed from Baltic glacial sediments. However, as compared with the contents of the geochemical background of the soils in the region and the value of enrichment factor (EF), there was noted a considerable content of lead in the soil from two locations A (Łosiny) and B (Okole). The content of Zn, Cu, Mn, and Fe in *T. serpyllum* plants can determine their applicability for herbal medicine. *T. serpyllum* plants collected as herbal material from location B, when considering the average Pb value in the entire plant, are not suitable for herbal medicine applications. The risk of an increased Pb content in plants (above 10 mg kg⁻¹ d.w.) makes soil environment monitoring necessary. The collection of that plant from natural stands must be limited and the collection from controlled plantations—increased. In their roots, stems, leaves, and inflorescences, the *T. serpyllum* plants accumulated relatively high contents of Zn and Pb, which is species-specific. A relatively high lead content in the *T. serpyllum* cells makes it impossible for plants to be used in herbal medicine. The Pb content above 10 mg kg⁻¹ d.w. suggests abandoning the

acquisition of herbal material from natural stands. As for the Zn content, only the *T. serpyllum* plants from the habitat of the youngest phytocenosis from the vicinity of Łochowo (C) satisfied the conditions for herbs allocated to phytotherapy. Special control measures are required for the materials from natural stands and if the permissible heavy metal content norms are exceeded, the collection of the plant must be limited and replaced with a collection from controlled plantations. Thus, we will be working to facilitate combining the effect of enhancing the material quality and the incomes generated from growing herbs with a rational use of natural resources, with the sustainable development of rural areas in mind. The results are preliminary and so further research is required.

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