applied sciences

Review

A Review of Chemical Variability and Metal Contamination of Herbaceous Plants in Terms of Health Safety—A Case Study of *Tanacetum vulgare* L.

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Abstract: The article presents a review of studies on the chemical diversity of essential oils and extracts from *Tanacetum vulgare* L. under the influence of environmental factors and the bioaccumulation of heavy metals in the plant. These issues are crucial for consumer safety, especially when they concern plants widely used in alternative medicine. Stress factors to which plants are subjected may result in a decrease in their biological activity or toxic effects (as in the case of *Tanacetum vulgare* L.). The issue is particularly important in the context of increasing environmental pollution, e.g., with heavy metals, and climate change, which is also a stress factor. The presented review clearly shows that detailed research is required both to identify the components of *T. vulgare* with specific therapeutic potential and to assess their quantitative changes under the influence of a specific stressor. It was found that there is a need to standardize analytical procedures used in research on the bioaccumulation of metals by medicinal plants. The importance of environmental monitoring in herb-growing areas, as an important element of health policy, is also emphasized.

Keywords: *Tanacetum vulgare* L.; metals; essential oil; extract; thujone; environmental pollution; health risk; composition variability; bioaccumulation

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Academic Editor: Burkhard Poeggeler

Received: 12 November 2024
Revised: 12 January 2025
Accepted: 14 January 2025
Published: 17 January 2025

Citation: Turek, A. A Review of Chemical Variability and Metal Contamination of Herbaceous Plants in Terms of Health Safety—A Case Study of *Tanacetum vulgare* L. *Appl. Sci.* **2025**, *15*, 911. https://doi.org/10.3390/app15020911

1. Introduction

Modern medicine is largely based on phytotherapy. It is estimated that over a quarter of antibacterial agents used in industrialized countries are obtained directly or indirectly from plants. However, the healing properties of essential oils (EOs) were known and used already in the Neolithic period, and the "Father of medicine"—Hippocrates recommended EOs in the treatment of plague. Currently, great hopes are associated with the use of plant extracts in infections with drug-resistant bacteria [1]. According to the recommendations of the World Health Organization (WHO), the use of antibiotics in animal breeding and food crops should be limited [2]. Plants are a source of many biologically active substances, as well as easily available forms of micro- and macroelements, so if they are used correctly and early enough, they can effectively treat and even prevent many diseases. Herbs can be used as medicines in chronic diseases, but also as complementary or synergistic agents in acute and subacute conditions [3,4]. The advantage of herbal medicines is the presence of the vitamins and essential oils necessary for the proper functioning of the human body.

The development of industry has led to a rapid increase in demand for raw materials, including metals. The exploitation of metal ores causes more intensive weathering of deposits, which in turn increases the mobility of metal ions compared to the susceptibility to the migration of metals from geogenic sources [5]. Compounds of many metals from anthropogenic sources are usually easier to dissolve, which makes them available to plants [6].

In order to survive in a polluted environment, plants had to develop appropriate defense mechanisms against stress, e.g., consisting of pollutant absorption and their detoxification and accumulation in specialized organs. Considering the great importance of herbs, the chemical purity of the raw material is very important [7]. The high content of some substances in plant raw materials may reduce their medicinal properties and even pose a threat to human and animal health. In the case of plants with a high variability of composition (e.g., *Tanacetum vulgare* L.), the location of the site from which the raw material is collected is of great importance. Therefore, in order to reduce the risk for consumers, systematic control of the chemical composition of plants and soil is necessary [8].

The family *Asteraceae* (formerly known as *Compositae*) belongs to the order of *Asterales*. It has about 25,000 species and is one of the largest families among angiosperms. Representatives of the *Asteraceae* family are mainly herbaceous plants [9,10]. Based on the diversity of flowers, two subfamilies can be distinguished among the *Asteraceae*: *Asteroideae* (*Compositae—Tubuliflorae*) and *Cichorioideae* (*Liguliflorae*) [11]. The genus *Tanacetum* includes over 200 species of plants, some of which have a long history of use in folk medicine [12,13]. One of the species is *Tanacetum vulgare* L., commonly known as tansy.

The presented paper discusses studies on the chemical variability of *T. vulgare* under the influence of environmental factors. This work concerns the composition of essential oils, extracts, and the content of heavy metals that are potentially toxic to consumers. Stress factors, both chemical and environmental (e.g., climate change) can cause a decrease in the biological activity of plants or the synthesis of toxic metabolites. This issue is of particular importance when the plants studied are commonly used not only in professional medical practice, which is associated with more restrictive cultivation methods and quality control but also in alternative medicine. The study of the accumulation capacity of plants may also have application potential, providing a basis for the development of methods for the phytoremediation of soils contaminated with metals. However, this requires a wider range of studies in various experimental conditions and using a uniform methodology both in the preparation of plant and soil samples for analysis, as well as the analytical procedures themselves. The aim of the article was to (i) summarize the current state of knowledge on the influence of environmental factors on the composition of *T. vulgare* and on the contamination of plants with heavy metals; (ii) to demonstrate the importance of the methodology of chemical analyses of plant material in the context of the safety of plant products for consumers and subsequent application work; and (iii) to highlight the importance of plant and soil monitoring used in pharmacy and environmental control in herbal cultivation areas.

2. Characteristics of the *T. vulgare*

T. vulgare is a perennial aromatic herbaceous plant widely distributed in the temperate climate of North and South America, North Africa, Europe, and Asia [14]. *T. vulgare* has two varieties, *vulgare* and *boreale*, differing in the composition of flavonoid glycosides. Uehara et al. [15] found that var. *boreale* is distinguished by the presence of scutellarein 7-O-glucoside, eriodictyol 7-O-glucuronide, and pectolinarigenin. *T. vulgare* is considered an invasive plant. It grows wild in many places along roads and river banks, in meadows, and in wastelands and is also cultivated in gardens as an ornamental plant. It contains up to 1.9% of essential oil (EO), the composition and amount of which depends on genetic factors and environmental conditions (soil pH, humidity, chemical pollution, exposure to light) and the climate and geographical origin of plants. Due to the genetic and regional variability in chemical composition, there are about 30 chemotypes of *T. vulgare*, defined on the basis of the first dominant component of essential oils with a content of at least 40% [16–18]. It was found that stress also has a significant impact on the chemical composition of EOs; hence, the variability of its composition is observed in different environmental conditions [1,13,14,19,20]. Dussarrat et al. [21] suggest that maternal genotype determines the significant chemodiversity of *T. vulgare* more than chemotype. The amount of EO and the content of toxic β-thujone in it are higher in plants growing in polluted areas. It can be concluded that the amount of EOs is an indicator of plant adaptation to stressful conditions [8]. The opposite relationship was observed for chlorophyll, the higher content of which was determined in plants collected from the unpolluted area. However, regardless of the location, the same proportion of chlorophyll a and b was found in the plant [22].

T. vulgare has numerous applications in medicine due to its antispasmodic, anti-inflammatory, antioxidant, antibacterial, anticancer, diuretic, antihypertensive, antidiabetic, and other therapeutic properties [1,12–14,19,20]. The use of *T. vulgare* in traditional and complementary medicine is so common that it may even threaten the degradation of its genetic resources and cause irreversible changes in ecosystems [14]. Pharmaceutical raw materials are the above-ground parts of the plant—*Tanaceti herba* and *Tanaceti flos* [23]. Studies have shown that essential oils and extracts obtained from *T. vulgare* containing substances with antibacterial properties can be applied alone or in combination with antibiotics [1,23]. It has been found that ethanolic extracts of *T. vulgare* have antiprotozoal (amoebicidal) activity and, in combination with antibiotics, can effectively treat Acanthamoeba keratitis [23]. The toxic effects of β-thujone are, however, the reason for restrictions on the use of EOs [20,24]. Plants containing this compound are used as flavoring agents in foods [1] and also as a spice instead of cinnamon and nutmeg [17]. On the other hand, there are reports that thujone has a neurotoxic effect, with the α form showing greater toxicity than β one [1,12]. Therefore, a daily dose of thujone in food products has been established (3.5–5.0 mg/person). In some countries *T. vulgare* cannot be used in flavorings, food products [20], and alcoholic beverages [25]. Due to the possibility of poisoning, the plant is now more often used externally for medical purposes, e.g., as an antiparasitic agent [26,27]. For thujone, the European Medicinal Agency and European Commission have established a permissible exposure limit of 3–7 mg/day for beverages [25]. According to the EU Regulation, thujone may not be added to food and its content in beverages may not exceed 0.5 mg/kg (non-alcoholic beverages produced from *Artemisia* species), 35 mg/kg (alcoholic beverages produced from *Artemisia* species), or 10 mg/kg (alcoholic beverages except those produced from *Artemisia* species) [28]. Laboratory experiments on *Artemia* larvae by Shutevska et al. [29] show that the safe concentration of *T. vulgare* EOs in eukaryotic cells is <0.01 mg/mL. *T. vulgare* is also used in cosmetics, pesticides, and preservatives (insecticide, fungicidal, and acaricidal effects) [1,19,20].

3. *T. vulgare* Essential Oil and Extracts—Variability of Composition and Properties

A number of papers have been devoted to the study of organic substances including essential oils (EOs) present in *T. vulgare* and their effect on the properties of the plant. EOs contain chemical compounds from the group of terpenes, terpenoids, alkaloids, and phenols. It has been found that the total content of terpenes determines the biological activity of essential oils [1,18]. In terms of practical application, research on the variability of the qualitative and quantitative composition of essential oils is of great importance. Unfortunately, there are relatively few studies on this issue.

The content and composition of EOs, apart from the location of the site and environmental and stress factors are influenced by the methods of preliminary preparation of the plant material (e.g., drying temperature). From the perspective of producers and users of plant-based products, the safety and repeatability of the raw material composition, especially bioactive substances, are important. Research is being undertaken to identify the EO components responsible for their specific features. The most frequently mentioned main components of *T. vulgare* EOs are α-thujone, β-thujone, camphor, *trans*-chrysanthenyl acetate, 1,8-cineole, pinene, and sabinene [1,17,20]. Nurzyńska-Wierdak et al. [26] studied the chemical composition of essential oils from *T. vulgare* collected from four sites with different parameters (e.g., land use, pH, sunlight, humidity). They found that in sunny and humid sites, the plants had a *trans*-chrysanthenyl acetate/camphor profile and a higher content of *trans*-chrysanthenyl acetate in EOs is associated with neutral or alkaline soil pH, while higher contents of camphor and 1,8-cineole were determined in *T. vulgare* growing in acidic soil. Thujone content did not exceed 10% (sunny site), and in two sites, it was not determined at all. The authors emphasized that in the literature, there are also inverse relationships between EO composition and environmental conditions compared to the results presented in their work.

Analysis of the essential oil composition in plants grown in polluted (industrial) and unpolluted (green) areas in Serbia showed that some components of EOs can be used as indicators of plant adaptation to environmental conditions [8]. The content of EOs in plants collected from industrial areas was significantly higher than from green areas. The authors suggest that the formation of oil reduces stress caused by environmental pollution, high temperatures, or radiation. One of the substances whose content increased in plants from industrial areas is β-thujone. Therefore, it should be assumed that stress stimuli can increase the toxicity of medicinal products.

Goudarzi et al. [30] examined the content of EOs obtained from plants collected at different stages of development. The main components of oils were *trans*-thujone, camphor, and *trans*-chrysanthenyl acetate. The changes in their content are as follows:

(a) *Trans*-thujone rosette ≈ beginning of fruit set > stem initiation > full flowering >> floral budding;
(b) Camphor floral budding > rosette > full flowering > stem initiation >> beginning of fruit set;
(c) *Trans*-chrysanthenyl acetate stem initiation ≈ full flowering > beginning of fruit set > floral budding >> rosette.

Different concentrations at subsequent stages of plant development were also observed for secondary components, e.g., camphene or sabinene. The authors note that according to the literature data, the maximum of camphor occurred at the flowering stage.

T. vulgare shows regional variability, which results in the different biological activity of plants collected in different locations. Coté et al. [31] found that the essential oil from plants collected in northern Quebec consists mainly of monoterpenes (camphor, borneol, 1,8-cineole), while according to other reports these are artemisia ketone and 1,8-cineole (Finland and Lithuania) or β-thujone (Hungary, Argentina). They also identified the components of the oil responsible for the biological activity of plants from Canada. The substances below have been found to have the following properties:

(a) Anti-inflammatory α-humulene, α-pinene, β-pinene, α-terpinene, β-terpinene, β-caryophyllene, caryophyllene oxide;
(b) Antioxidant activity α-pinene, caryophyllene oxide;
(c) Antibacterial activity camphor, caryophyllene oxide (*E. coli* and *S. aureus*), α-terpinene (*S. aureus*);
(d) Moderate cytotoxicity toward DLD1, A-549, and WS1 cell lines α-pinene, β-pinene, α-caryophyllene, caryophyllene oxide, α-humulene, α-terpinene, camphene, limonene, and α-terpinene (except DLD1).

T. vulgare essential oil has antibacterial effects on drug-resistant Gram-positive and Gram-negative bacteria, and synergism between EOs and antibiotics has also been found [1].

The action of EO consists of increasing the permeability of the bacterial cell membrane, which facilitates the penetration of antibiotics into cells. The EO component that requires attention is thujone. The precursor of thujone is sabinene, which is commonly found in EOs, but numerous metabolic interactions can cause diverse effects (the formation of thujone or its absence in EOs). Thujone has been shown to have anticancer effects on choriocarcinoma and glioblastoma multiforme.

The insecticidal properties of *T. vulgare* have been used in the organic cultivation of black chokeberry [13]. Foliar insecticide based on essential oil was used. It was observed that solutions with a concentration of 1.5–4.5% of oils caused changes in the content of potassium and magnesium compared to the control sample. It was found that low doses of oil (1.5%) increased the content of potassium and vitamin C in plants compared to the control sample, while the use of higher doses resulted in a decrease in the content of potassium and magnesium. It was also found that foliar application of EO solution increases the content of substances with antioxidant properties in chokeberry. Antibacterial and antioxidant properties of *T. vulgare* EO were used to improve the properties of food packaging made of biodegradable polymer based on sodium alginate [16]. The addition of EO changed the structure and strength of the polymer, increased its roughness and porosity, and gave it antioxidant properties. The polymer obtained in this way showed activity against *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* bacteria.

The composition of *T. vulgaris* extracts, apart from the previously mentioned environmental factors, is influenced by the extraction method and especially by the properties of the extractant. The most commonly used extraction techniques include steam distillation, supercritical and subcritical carbon dioxide extraction, and solvent extraction [1,18]. For example, the total phenol content in the extract depends, among others, on the polarity of the solvent [24].

Detailed studies of the composition and properties of the essential oil (EO) and methanol extract (ME) from different parts of *T. vulgare* were performed by Devrnja et al. [25]. These studies showed that the essential oil contained over 40% of *trans*-chrysanthenyl acetate, which is responsible for the antimicrobial activity of the oil. However, the antibacterial activity of EO against Gram-negative bacteria (*E. coli*, *E. cloacae*) is weaker than that of conventional agents, while the antifungal activity of EO is comparable to that of typical medicines. The cytostatic activity of the oil in HeLa cancer cells is twice as strong as in non-cancerous MRC-5 cells. The authors found that ME of roots has the highest antioxidant and reducing effect. They attribute this to the highest content of phenols (especially derivatives of hydroxy cinnamic acid) in this extract. In turn, the content of isoquercetin in the ME of flowers also causes some antioxidant effects. The antibacterial activity of ME toward Gram-positive bacteria (*S. aureus*, *B. cereus*, *M. flavus*) is greater than that of reference antibiotics, while the antifungal effect depends on the species of the microorganisms. MEs of leaves and flowers inhibit the growth of cancer cells (HeLa) and non-cancer cells (MRC-5). In the case of ME from leaves, this effect was attributed to the presence of nepetin, hispidulin, and eupalitin. Godinho et al. [32] studied the anthelmintic activity of the essential oil and the water-ethanol extract of *T. vulgare*. They found that both preparations caused 100% mortality of adult Schistosoma worms, but the doses of the extract necessary to achieve this effect were 200 and 50 μg/mL for EO and extract, respectively. Of the seven identified EO components, the anthelmintic activity was attributed to the monoterpene thujones, whereas the composition of the hydroalcoholic extract was not identified.

Babich et al. [20] found that, among the components extracted with methanol, the strongest antioxidant activity was demonstrated by chlorogenic acid and was slightly lower by luteolin-7-glycoside and rosmarinic acid, with this activity also depending on the habitat of *T. vulgare*. According to Bączek et al. [33], caffeic, chlorogenic, chicoric, and ferulic acids (isolated from hydroethanolic extract) also have antioxidant properties. Antioxidant properties of *T. vulgare* extracts were also studied by Badaturuge et al. [34]. The crude methanol extract and its fractions isolated using ethyl acetate, petroleum ether, water, chloroform, and n-butanol scavenge free radicals. However, the authors found that the last three fractions have lower activity compared to the crude extract. Further fractionation of the ethyl acetate fraction allowed the isolation of three main components responsible for antioxidant activity. Their activity decreases in the series 3,5-O-dicaffeoylquinic acid (3,5-DCQA) >> axillarin > luteolin. In the cited work, the presence of 3,5-DCQA was confirmed for the first time in *T. vulgare*. Álvarez et al. [35] found that methanol-extractable axillarin and 3,5-DCQA revealed activity against Herpes simplex viruses HSV-2 and HSV-1/HSV-2, respectively. The material studied in this case was the aerial parts of *T. vulgare*. Continuing work on the antiviral activity, Alvarez et al. [36] studied methanol extract from the rhizomes of *T. vulgare*. The authors found that the petroleum ether fraction of this extract inhibited the entry of HSV-1 and HSV-2 viruses. The compound identified to be responsible for the antiviral activity is (E)-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4.5]dec-3-ene. Mishchenko et al. [37] demonstrated anti-cytolytic activity associated with antioxidant properties of flavonoids and hydroxycinnamic acids in ethanol extract of *T. vulgare* flowers. In studies conducted on rats, the hepatoprotective effect of the extract was comparable to the standardly used silymarin but weaker in terms of bile formation and secretion. The authors did not identify the components of the extract, limiting themselves to determining the optimal doses of the preparation. However, they assumed that the known components of *T. vulgare* extracts, especially rutin, apigenin, luteolin, and hydroxycinnamic acids, are responsible for the therapeutic effects. Lechkova et al. [38] studied the effect of the alcoholic extract of *T. vulgare* on cognitive functions. The authors observed a positive effect of the extract on the learning processes and spatial memory of Wistar rats with no histopathological changes in the internal organs. These effects have been attributed to chlorogenic acid and rutin.

T. vulgare is used in organic farming. Judžentienė et al. [39] studied the allopathic properties of *T. vulgare* in terms of the possibility of using plant extracts to control weeds, phytopathogens, and pests. The authors determined the composition of volatile compounds in extracts from roots, leaves, and flowers of plants and also examined the effect of pH on the composition of extracts. Extracts from leaves and flowers exhibited a greater inhibitory effect on the germination and growth of lettuce and pepper cress seeds than those obtained from roots. Considering the pH of extracts, the inhibitory effect decreases in the series: acidic extract > neutral extract > alkaline extract. Parus et al. [40] studied the effect of aqueous extracts of *T. vulgare* flowers, leaves, and roots on the germination and growth of maize. The flower extract contained the highest concentration of thujone. This compound was not found in the root extract. Thujone, as well as caffeic and chlorogenic acids, tannins, and saponins, have a negative effect on the germination and development of maize seeds. Aqueous extracts of flowers and leaves inhibited the growth of roots and, to a lesser extent, shoots, while the root extract inhibited the development of shoots. The aqueous extract is used as an antiviral agent in tomato cultivation and to control the peach-potato aphid [19]. The insecticidal activity of an aqueous extract of *T. vulgare* was tested on the pea leaf weevil (*Sitona lineatus* L.) and black bean aphid (*Aphis fabae* Scop.) [41]. The properties of the aqueous extract of *T. vulgare* were studied already in the 1980s and 1990s. In the cited work, Kwiecień et al. found the effectiveness of the preparation used as a substance repelling both pest species. They also emphasized the advantages of the aqueous extract (with lower environmental toxicity) in comparison with synthetic preparations.

The nutritional preferences of aphids were studied in relation to the intraspecific and intraindividual variability of the chemical composition of *T. vulgare* [18,42]. It was noted that the chemical composition of plants of one species varies in individual parts of a given plant and depending on the age of the tissues and its offspring may belong to particular chemotypes. Differences in the content of sugars, organic acids, amino acids, or terpenoids cause aphids to specialize in feeding on specific parts of *T. vulgare* [42]. Individual species of aphids show different preferences with respect to *T. vulgare* chemotypes. The attractiveness of the plants most likely results from the presence of terpenoids. However, the authors [18] did not associate this phenomenon with the occurrence of specific substances, but rather saw its cause in interactions between individual compounds from the terpenoid group. They also suggested a possible influence of secondary components on the attractiveness of *T. vulgare* for the aphid species studied. *M. tanacetaria* and *U. tanaceti* were found to prefer the thujone chemotype (*M. tanacetaria* also *trans*-carvyl acetate) while avoiding the α-pinene/sabinene chemotype.

4. Contamination of *T. vulgare* by Metals

Plants are a link between abiotic elements of the environment and animals. By absorbing macro- and microelements, they simultaneously accumulate undesirable elements, including toxic heavy metals. *T. vulgare* extracts have been found to have chelating properties [17]. In areas with strong anthropopressure, the amount of pollutants accumulated in plant tissues may exceed critical values for a long time without showing visible symptoms of poisoning. Such a situation is dangerous for consumers, and medicinal plants pose a particular threat. In relation to fresh herbs, Regulation [43] recommends only the control of cadmium content (concentration limit up to 0.2 mg/kg). The metal content limits in herbal medicines, specified in the regulations of WHO and the United States Food and Drug Administration (US FDA), are 10 mg/kg (lead, arsenic), 1 mg/kg (mercury), 20 mg/kg (copper), and 50 mg/kg (zinc) [44]. Vinogradova et al. [45] included a summary of the permissible concentration values of four elements from the group of metals and metalloids in raw materials and medicinal products of plant origin applicable under the regulations of ISO, WHO, Pharmacopoeia (published in eight countries in Europe and Asia). The given values vary and are in the range of 0.3–2.0 mg/kg (cadmium), 5.0–20.0 mg/kg (lead), 0.1–3.0 (mercury), and 0.5–5.0 mg/kg (arsenic). The presence of metals potentially toxic to organisms in plants sometimes stimulates the synthesis of metabolites with bioactive properties, which can be used in pharmacy and other industries. An increase in the content of bioactive components in medicinal plants was found under the influence of copper, zinc, and cadmium, as well as iron, silver, and copper nanoparticles [46].

4.1. Research Methodology

The study of accumulation capacities and processes responsible for the translocation of pollutants allows the use of some plant species as bioindicators of soil contamination. Consequently, this helps to limit the migration of potentially toxic metals in the food chain. However, such studies are complicated and do not always provide unambiguous information because the transport of elements in soil and plants is complex and depends on many factors related to the morphology and physiology of plants, as well as environmental factors and exposure routes. The problem of metal accumulation increases in the case of cultivated plants and wild plants in industrial areas. Studies become more complicated when plants exhibit high variability, as is the case with *T. vulgare*. Unfortunately, the problem of *T. vulgare* contamination with potentially toxic metals is not of interest to many scientific centers. The conducted studies are not systematic in terms of methodology and different techniques of sample preparation (Tables 1 and 2), which may affect the differences in the obtained results, the final assessment of the degree of *T. vulgare* contamination, and the potential risk to consumers.

The complete decomposition of the analyzed sample is an important issue from the point of view of chemical analysis. This is one of the most important stages of the analytical procedure, requiring special attention. Correct sampling and digestion of the sample are crucial in the analytical process. Errors made in these stages of analysis have the greatest consequences for the final results and conclusions. One of the most popular decomposition methods used for plant samples is the microwave technique. In the case of *T. vulgare*, the authors used the concentrated acids HCl, HNO_3, or a mixture of HNO_3 with another oxidant ($HClO_4$ or H_2O_2). These are typical methods of digestion but it should be remembered that plants accumulate various amounts of silica, which is an adsorbent of metal cations. Its presence can significantly reduce the content of analytes in the solution after mineralization, which is particularly unfavorable in the case of determining the content of trace metals. An effective method of removing silica from samples is the addition of hydrofluoric acid, which forms a volatile compound SiF_4 with silicon. During the decomposition of silicon compounds, adsorbed ions are released into solution. The mineralization of plants with the addition of hydrofluoric acid therefore enables the complete transfer of the plant sample into solution and subsequent determination of the total metal content. It should also be noted that some of the results given in Table 1 refer to samples from which the analytes were extracted without decomposition (or after partial decomposition—ash) of the plants.

An even greater problem is the soil digestion methodology used to determine metal content. Soil itself is a complex heterogeneous system. Depending on the location, it contains a variety of minerals and organic substances. Metals occurring in the form of minerals (geogenic origin) are usually more difficult to isolate than those introduced into the soil as a result of human activity (anthropogenic origin). The identification of heavy metal origin and migration in soil is crucial for reliable environmental risk assessment. However, metal origin and further pathways of migration are not easy to determine or interpret. The mobility of metal cations in the soil, and consequently their uptake by plants, is influenced by, for example, the chemical properties of a given substance, redox conditions in the soil, soil pH and grain size, precipitation, temperature, properties of accumulating plants, or soil fauna. The total metal content is usually determined in solutions obtained after mineralization with a mixture of concentrated HF and oxidizing acids. Unfortunately, the total content does not account for the solubilities of individual chemical compounds and the further availability of their components to plants. To assess the threat posed to plants by heavy metals, a number of soil extraction methods were developed (single or multi-step extractions). The extractants used are inorganic salt solutions, buffers, or complexing substances. The high variability of extraction methods causes analytical results that are not comparable or consistent. The different procedures for metal extraction from soil presented in Table 2 were then used by the authors to calculate the bioaccumulation capacity of *T. vulgare* (Table 3). However, due to the previously mentioned complexity of the soil environment, the obtained results are difficult to interpret and are incomparable.

4.2. Accumulation of Heavy Metals in T. vulgare

Chaplygin et al. [47] studied the accumulation of manganese, zinc, chromium, copper, lead, nickel, and cadmium in *T. vulgare* growing in industrial areas (energy, metallurgical, and mining industries). They found a strong correlation between the content of manganese, zinc, chromium, nickel, and cadmium in plant shoots and in the soil. Bioconcentration factors (BCFs defined as the ratio of the concentration of a metal in a plant tissue to the content of its bioavailable forms in the soil) did not exceed one at most sites. The authors attribute a BCF value of <1 to the inhibitory effect of the root barrier on the process of metal uptake from the soil. In turn, BCF > 1 indicates the significant uptake of metals by plants from the soil or atmosphere. In the cited work only the BCF values for Cd at most sites are above 1 (in the range of 1.1–1.9). Translocation factors (TFs defined as the ratio of the concentration of a metal in a shoot tissue to the roots) exceeded the value of one for all the tested metals. For manganese, zinc, lead, and cadmium, these indices were 3.5–4.0, which indicates the potential possibility of using *T. vulgare* for soil phytoextraction. The content of metals in the shoots decreased in the series manganese > zinc > chromium > copper > lead > nickel > cadmium. According to Łaszewska et al. [48], *T. vulgare* accumulates iron and cadmium in all tested parts of the plant (leaf, flower, root, stem). BCFs > 1 were also found in leaves and roots for manganese and lead, in leaves for zinc, and in roots for nickel. The highest bioconcentration coefficients were recorded for iron. BCF values < 1 for copper and chromium were determined in all parts of *T. vulgare*. The authors attributed the accumulation of metals in stems and flowers to plant pollution with atmospheric dust.

T. vulgare is a common ruderal plant that easily adapts to unfavorable environmental conditions, including high heavy metal contents. Therefore, research is being conducted on the possibility of its use in environmental biomonitoring and the phytoremediation of areas contaminated with metals. Studies on the properties of *T. vulgare* in terms of the possibility of heavy metals accumulation were conducted by Vaverková et al. [49]. The work was carried out at the municipal and hazardous waste landfill in Štěpánovice. Plant samples were collected from two locations. Based on the values of enrichment coefficients (ECs) calculated as the ratio of metal concentration in the plant to that in the soil, it was found that *T. vulgare* has the ability to increase the uptake of cadmium, chromium, copper, nickel, lead, and zinc. The results obtained are not repeatable. It was found that at one site, plants accumulated chromium, copper, nickel, and lead to a low degree and cadmium and zinc to a moderate one, while at the other site, ECs corresponded to a high (for cadmium) or moderate degree of accumulation. It was concluded that the plant could be used as a bioindicator, but it did not meet the criteria for hyperaccumulators. Adamcová et al. [50] continued the analysis of soil contamination at the waste disposal site in Štěpánovice. The degree of contamination of the area was assessed using *T. vulgare* as a bioindicator. Plants exposed to direct contact with waste and growing on the outskirts of the disposal site were collected. Enrichment coefficients were calculated, defined as the ratio of the metal content in a specific part of the plant (root, stem or leaf) to the content of this element in the soil. Only in the case of cadmium did the ECs values in all parts of *T. vulgare* at all locations significantly exceed one. The authors assessed that this element accumulates to the greatest extent in the leaves. The ranges of ECs for the remaining metals were very diverse; however, it was assumed that chromium, nickel, and zinc also accumulate mainly in the leaves, while the place of cobalt, copper, iron, mercury, manganese, and lead accumulation is mostly the root. The research conducted at the same landfill by Vaverková et al. [51] aimed to assess the suitability of *T. vulgare* for the analysis of environmental pollution by waste, leachates, and dust emitted by the landfill. Based on the enrichment coefficient (EC) values, where $EC = (M_{plant})/(M_{soil})$, the ability of the plant to absorb selected metals was determined. A particularly high ability of *T. vulgare* to the accumulation of cadmium was demonstrated. In terms of zinc, cobalt, copper, and lead uptake, *T. vulgare* was classified as a moderate accumulator plant. For chromium, iron, manganese, and nickel, the results were ambiguous (moderately or low accumulator plant). Analysis of *T. vulgare* collected from a lignite mine site showed that *T. vulgare* easily adapts to changes in environmental conditions and is a good bioindicator of cadmium but also manganese and zinc [52]. The BCF values of cadmium were 45 (for plants from the mine) and 15 (in control samples). Slightly lower BCFs (but significantly exceeding one) were also recorded for copper and zinc. The conducted studies did not confirm the literature reports, indicating that nonessential metals accumulate in the roots, which protects the organs participating in photosynthesis. The authors observed a significant translocation of cadmium, cobalt, nickel, lead, manganese, and zinc to leaves (TFs > 1). They also confirmed the earlier reports that fly ash from brown coal accumulates significant amounts of heavy metals. Plants collected from the vicinity of the fly ash storage site were characterized by the highest concentrations of the analyzed metals, except for iron and manganese. The latter metals were accumulated in plants growing in areas where the influence of drainage water was noticeable. In turn, the content of metals in *T. vulgare* from areas reclaimed with clay limestone was lower. The authors explained this by the influence of calcium on the reduction in the uptake of other metals by the plant (antagonism).

Konieczny et al. [53] analyzed the content of potassium, phosphorus, copper, zinc, and molybdenum in *T. vulgare* leaves collected from industrial areas, the vicinity of roads with heavy traffic, and areas not significantly affected by human activity. In addition to the effect of soil use on the content of toxic thujone, a threat to consumers may be plant contamination with metals taken from the soil but also from the air, through the leaves' surfaces. The dose of pollution from the atmosphere depends on the size of the leaf surface and the content of hydrophobic substances (e.g., waxes), which prevent metals from being washed away by rainwater. The authors analyzed the composition of plants collected from four sites located near sources of pollutant emission and eight points in areas with low anthropogenic pressure. A positive correlation was found between the total copper and zinc content and negative relationships for the zinc/potassium and molybdenum/phosphorus pairs. Considering the average content of elements, a clear effect of industrial areas and roads was observed on the increase in the content of copper, molybdenum, and especially zinc compared to unpolluted areas. In the case of the latter element, its concentration was more than twice as high in industrial areas and by roads compared to non-anthropogenic areas. However, no effect of land use on the contents of potassium and phosphorus was found. The same authors also studied the accumulation of cadmium, chromium, nickel, and lead in *T. vulgare* leaves [27]. It was shown that industrial activity (iron smelting and processing) and transport (distribution and combustion of fuels) cause a significant increase in the content of the above-mentioned elements in *T. vulgare*. A high correlation (r 0.96) occurs between the content of lead and chromium, while a medium one occurs for nickel and cadmium.

Table 1. Description of analytical methods for the determination of metals in *T. vulgare*.

Part of Plant	Preparation and Decomposition Methods	Determination Methods	Ref.
shoot, root	dry digestion and extraction of heavy metals from ash (20% HCl)	AAS	[47]
flower, leaf, stalk, root	dried in the air at room temp., microwave digestion (conc. HNO_3)	FAAS	[48]
whole plant	dried in the air (1 month), microwave digestion (conc. HNO_3 and H_2O_2)	ICP-OES	[49,51]
leaf, stalk, root	dried in the air at room temp. (2 weeks), microwave digestion (HNO_3 (1:1))	ETAAS (Cd, Co, Cr, Pb) 1% Pd/Mg(NO_3) modifier; FAAS (Cu, Fe, Mn, Ni, Zn); CVAAS (Hg)	[50]
flower, leaf, stalk, root	dried at 50 °C, microwave digestion (conc. HNO_3 and $HClO_4$)	ETAAS (Cd, Co, Cr, Cu, Ni, Pb); FAAS (Fe, Mn, Zn)	[52]
leaf	dried at room temp. with air circulation, in the dark (5 days), microwave digestion (aqua regia)	ICP-MS	[27,53]
leaf, stalk, root	dried at 60 °C in a force-air oven, microwave digestion (conc. HNO_3 and $HClO_4$), for total Hg: dried at 70 °C, sample with conc. HNO_3 left overnight and heated in a water bath at 80 °C for 1 h	AAS	[7]
leaf, stalk, root	dried in the air (1 month), boiling with conc. HNO_3	ICP-OES	[22]

Table 2. Description of analytical methods for the determination of metals in soil.

Preparation Methods	Decomposition/Extraction Methods	Determination Methods	Ref.
no data	1 mol/L ammonium acetate buffer pH 4.8 18 h—exchangeable (actual mobile) forms; 1% EDTA in NH_4Ac pH 4.8 18 h—exchangeable and relatively unstable complexes; 1 mol/L HCl 1 h—acid soluble compounds	AAS	[47]
	total metals	XRF	
dried in the air at room temp. sieved (diameter < 1 mm)	KNO_3—exchangeable forms; KF—adsorbed forms; $Na_4P_2O_7$—organic matter bound forms; EDTA—carbonate bound forms; 6.0 mol/L HNO_3—sulfide bound forms; 65% HNO_3—residual forms	FAAS	[48]
dried at 105 °C, ground to fine powder	microwave digestion (aqua regia)	ICP-OES	[49,51]
dried in the air at room temp. (2 weeks), sieved (diameter < 2 mm)	microwave digestion (aqua regia)	ETAAS (Cd, Co, Cr, Pb) 1% Pd/Mg(NO_3) modifier; FAAS (Cu, Fe, Mn, Ni, Zn); CVAAS (Hg) *	[50]
dried at 50 °C, sieved (diameter < 2 mm)	microwave digestion (conc. HNO_3 and $HClO_4$)	ETAAS (Cd, Co, Cr, Cu, Ni, Pb); FAAS (Fe, Mn, Zn) *	[52]
not determined	no data	no data	[27,53]
dried in the air at 25 °C, sieved (diameter < 2 mm)	stirred with conc. HNO_3 (8 h, 96 °C)	AAS	[7]
dried in the air at 25 °C, sieved (diameter < 0.1 mm)	no data	no data	[22]

* Cd (cadmium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Hg (mercury), Mn (manganese), Ni (nickel), Pb (lead), Pd (palladium), Zn (zinc).

Table 3. BCFs (or ECs) and TFs determined in *T. vulgare*.

Metal	BCF (or EC)	TF	Ref.
cadmium	0.14–1.91	4.0	[47]
	2.68–6.51 leaf 1.05–2.52 flower 2.19–4.86 stalk 2.05–3.77 root	-	[48]
	3.86–37.65 leaf 3.95–30.11 stalk 2.531–13.785 root	-	[50]
	15–45 root	1.2–2.0	[52]
cobalt	0.074–0.827 leaf 0.02–0.519 stalk 0.261–1.715 root	-	[50]
	0.08–0.2 root	1.1–2.0	[52]
chromium	0.11–0.97	1.1	[47]
	0.04–0.06 leaf 0.04 flower 0.04 stalk 0.06–0.13 root	-	[48]
	0.532–2.659 leaf 0.059–1.021 stalk 0.252–0.956 root	-	[50]
	0.06–0.4 root	0.8–0.9	[52]

Table 3. *Cont.*

Metal	BCF (or EC)	TF	Ref.
copper	0.13–1.17	1.4	[47]
	0.40–0.41 leaf 0.37–0.45 flower 0.16–0.20 stalk 0.51–0.54 root	-	[48]
	0.349–1.440 leaf 0.065–0.721 stalk 0.571–2.220 root	-	[50]
	3–12 root	1.3–2	[52]
iron	7.56–12.80 leaf 4.02–5.21 flower 2.10–2.36 stalk 45.64–50.31 root	-	[48]
	0.017–20.397 leaf 0.003–0.234 stalk 0.255–1.163 root	-	[50]
	0.03–0.2 root	0.2–0.4	[52]
mercury	0.373–1.513 leaf 0.287–1.97 stalk 0.674–2.274 root	-	[50]
manganese	0.11–0.39	3.6	[47]
	2.09–4.67 leaf 0.74–1.94 flower 0.43–0.98 stalk 1.00–1.88 root	-	[48]
	0.276–0.686 leaf 0.055–0.338 stalk 0.236–0.919 root	-	[50]
	0.7–3 root	6–13	[52]
nickel	0.04–0.52	2.4	[47]
	0.27–0.53 leaf 0.91–1.53 flower 0.12–0.16 stalk 1.84–1.92 root	-	[48]
	0.132–3.713 leaf 0.007–1.604 stalk 0.226–1.763 root	-	[50]
	0.2–0.1 root	6–7	[52]
lead	0.03–1.12	3.5	[47]
	3.49–12.69 leaf 0.51–3.39 flower 0.26–0.39 stalk 1.73–12.73 root	-	[48]
	0.131–0.732 leaf 0.006–0.486 stalk 0.470–2.114 root	-	[50]
	0.1–0.3 root	2	[52]
zinc	0.31–2.80	3.7	[47]
	1.21–1.66 leaf 0.51–0.54 flower 0.27–0.34 stalk 0.43–0.54 root	-	[48]
	1.484–22.909 leaf 0.563–7.319 stalk 0.751–6.477 root	-	[50]
	7–10 root	3–5	[52]

The content of mercury, lead, cadmium, chromium, and nickel in parts of *T. vulgare* (root, stem, leaf) was analyzed [7]. The plants were collected from five sites with different land uses. High concentrations of metals were found in samples growing in industrial areas and by the motorway. This was particularly true for the area of a chemical factory, where eight tons of mercury spilled into the soil during an accident. Only 6 years after the incident did *T. vulgare* re-colonize the premises of the company. After 9 years, the mercury content in *T. vulgare* was 10 times higher compared to other industrial areas and the motorway, and in relation to green areas (city recreation area and park), the concentration was even 200 times higher. Similarly, the amount of mercury in essential oil from leaves was 10 times the level determined in the industrial area. The highest concentrations of cadmium, chromium, and nickel in EO and plants were determined in all industrial areas. The maximum amounts of lead were found in plants collected from the vicinity of the motorway and in the essential oil from leaves in this location. It was found that the content of mercury, as well as chromium and cadmium, decreases in the order root > stem > leaf. In turn, the distribution of cadmium and lead has a different pattern: leaf > root > stem. Differences in the content of metals were found in parts of plants from "polluted" areas (industry, motorway) in comparison with "unpolluted" recreational areas and parks. The authors also noted the lack of necessary legal regulations regarding the permissible content of heavy metals in medicinal plants. Continuing the research [22], the properties of *T. vulgare* growing in an "anthropogenic" (industrial zone of Belgrade) and "non-anthropogenic" (park) site were compared. It was found that in the case of both mercury and lead, there is a relationship between the content of these elements in the soil and the plant. The concentrations of mercury, lead, cadmium, chromium, nickel, aluminum, iron, and calcium in *T. vulgare* collected from the contaminated area were higher, while the magnesium content was five times lower than in the non-anthropogenic site. The concentrations of potassium, sodium, and manganese were similar in plants in both locations. It was shown that the place of potassium, magnesium, and manganese accumulation is primarily the leaf, followed by the root, while the opposite relationship occurs in the case of calcium, sodium, iron, and aluminum. In essential oils in plants from the anthropogenic area, the content of cadmium, chromium, nickel, and lead was significantly higher compared to the second location; the level of lead corresponded to the maximum permissible concentration, and in the case of nickel, it was even exceeded. Table 3 summarizes the bioaccumulation factors (BCF), enrichment coefficients (ECs), and translocation factors in plants (TFs) given in the above-mentioned papers.

5. Conclusions

T. vulgare has a wide range of therapeutic properties; therefore, the main beneficiary of current and future research on the plant is the pharmaceutical industry. However, the analytical results presented in this paper prove that the plant has a much greater potential for use. The antioxidant and antibacterial properties of *T. vulgare* are used in biodegradable packaging technologies, which can be an alternative to conventional polymer packaging. In line with the sustainable development policy, this limits environmental pollution both with non-degradable polymers and with synthetic toxic antioxidants and biocidal preparations. The results of analyses of allelopathic activity and plant-insect interactions also look promising in terms of their possible use in organic farming. In the longer term, this is an opportunity to reduce the load of xenobiotics introduced into the environment as a result of agricultural activities. Understanding the plant's response to stress related to climate change and soil conditions (pH, redox) allows us to limit the accumulation of undesirable substances (mainly heavy metals) and the production of toxic metabolites (e.g., thujone). However, it would also be necessary to take advantage of experiments carried out on other plants regarding stress related to plant–metal interactions and to investigate the possibility of using such reactions to stimulate the biosynthesis of desired biologically active compounds. The high resistance of *T. vulgare* to environmental conditions, e.g., soil pollution, and the observed tendency to accumulate potentially toxic metals provide hope for the use of the plant as a bioindicator or in the processes of phytoextraction or metal immobilization. Removing pollutants from soil using chemical methods is an expensive undertaking and often causes social protests. Phytoremediation does not arouse such emotions and is usually acceptable, although in the case of a significant level of contamination, land reclamation is a slow process.

In summary, the high variability of the chemical composition of *T. vulgare* creates prospects for the use of the plant or its components. *T. vulgare* cultivation can bring tangible benefits to the economy. However, the effective use of the plant requires a holistic approach to research. It is crucial to systematize research and standardize the methodology of soil and plant analysis. The main problems related to the use of *T. vulgare*, proposed solutions, and conclusions are listed below.

1. Insufficient knowledge about the variability of the composition of *T. vulgare* requires the continuation of detailed research, primarily the identification of components with therapeutic potential and the assessment of their quantitative changes under the influence of a specific stress factor;
2. Health safety requires constant monitoring of both plants and soil. This applies not only to areas with confirmed chemical contamination but also to crops in which other stress factors could have occurred (e.g., long-term drought). This is a particularly important issue in the context of the currently observed rapidly progressing climate changes;
3. The problem of toxicity of metabolites synthesized under the influence of stress and pollutants accumulated by plants primarily concerns consumers who prepare medicinal preparations on their own from untested raw materials and the use of these substances by people unaware of the risk;
4. Systematic control of the quantitative composition of EOs at different stages of *T. vulgare* ontogeny allows the selection of plants with the optimal content of desired components for a given purpose or limiting the content of undesirable (toxic) substances;
5. A limitation in monitoring the quality of medicinal products of plant origin, especially in relation to heavy metals, is the lack of uniform analytical procedures. Due to the complex chemical composition and enormous diversity, the development of a methodology for testing contamination of agricultural soils is a major challenge;
6. The high tolerance of *T. vulgare* to soil contamination with metals may pose a threat to consumers but at the same time creates hope for the use of the plant in phytoremediation and industrial processes and in agriculture.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The author declares no conflicts of interest.

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