

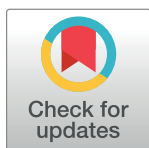
RESEARCH ARTICLE

A comprehensive analysis of chemical and biological pollutants (natural and anthropogenic origin) of soil and dandelion (*Taraxacum officinale*) samples

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Abstract

A range of analytical methods (GC-MS, LC-MS, voltammetry, microbiological and microscopic techniques, PCR) was used to assay a range of potential chemical and biological contaminants in soil and dandelion samples. The results provide the first comprehensive safety analysis of dandelion as a herbal product. Samples were collected from three different sites in Poland where the local population collects dandelion plants for their own consumption: Rudenka (a mountain meadow in the European Ecological Network of Natura 2000 protection area, free of agrotechnical treatments for over 30 years), Warszawa 1 (dense single-family housing with heavy traffic), and Warszawa 2 (recreation area with heavy traffic near a coal-fired heat and power plant). The assays of heavy metals and other chemical pollutants (PAHs, PCBs, dioxins, pesticides, mycotoxins) confirm that all collected soil and dandelion samples were chemically pure; however, 95 species of pathogenic bacteria were detected, including “carnivorous” *Vibrio vulnificus*, zoonotic *Pasteurella pneumotropica*, *Pasteurella canis*, *Staphylococcus pseudintermedius*, *Staphylococcus lentus* and *Francisella tularensis* as well as 14 species of pathogenic fungi and one protozoan parasite (*Giardia intestinalis*). The discovery of septicemia agents *V. vulnificus*, *Fusobacterium mortiferum* and *Rahnella aquatilis* in the soil surrounding dandelion roots and in the flowers, *G. intestinalis* in dandelion leaves and roots samples, all collected in Warsaw, is highly disturbing. This finding underlines the need for increased caution when collecting dandelion in densely populated areas with a large population of pets. Thorough washing of the harvested plants is necessary before using them for consumption, especially in the case of making salads from fresh dandelion leaves, which is becoming increasingly popular among people leading healthy and an environmentally friendly lifestyle.

Data Availability Statement: All relevant data are within the paper and its [Supporting information](#) files.

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Introduction

Herbs have accompanied mankind since its inception. For millennia, they have been intensively used as important foodstuffs, medicines, cosmetology and beauty treatments, and have been key components of animal nutrition and breeding [1–3]. Medicinal and aromatic plants play significant roles in meeting the demands of the traditional medicine and food markets [4–6] and according to the World Health Organisation (WHO), approximately 80% of the world's population uses herbal products for therapeutic purposes [7]. The rapidly-growing interest in healthy food and the rising ecological awareness of consumers have driven a dynamic increase in sales of ecological and organic food. The healthy lifestyle trend encourages consumers to look for natural ways to support health and prevent disease. Herbal blends, herbal and functional teas, as well as herbal dietary supplements believed to strengthen immunity and help fight overweight are very popular, and the demand for herbs on the global market has been growing dynamically in recent years.

However, the safety of herbal products, both those derived from cultivated plants and from those obtained at natural sites, is coming into question. The natural environment is subject to increasing pollution as a result of the increasing combustion of fossil fuels, for heating and industrial production, and by individual human mobility and mass tourism. Such problems are exacerbated by increasing accumulation of waste in the environment, which is still insufficiently recycled.

The greatest threats of chemical contamination concern the accumulation of heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins from natural and anthropogenic sources [6, 8–14]. In addition, the use of commonly-used pesticides, plant protection agents intended to control pests and to improve crop yields and quality, also poses a serious threat to the safety of picked herbs [15], as do mycotoxins, natural contaminants produced by pathogenic fungi which frequently attack growing plants and improperly-stored dried herbs [16–19].

Unfortunately, organic production, based on using only natural fertilizers while eliminating plant protection products, however, does not guarantee complete safety. Such food products can still be biologically contaminated by pathogenic microorganisms, as well as by parasitic protozoa and helminths. While studies have been performed on the microbiological safety of herbs and herbal products [20, 21], very few have examined the potential for parasitic contamination.

Most of the contamination by pathogenic microorganisms and parasites takes place from wild animals that act as intermediate or final hosts of many parasites, as well as the use of natural fertilizers (manure, slurry) and sewage sludge for cultivation [22, 23]. European Union directive 86/278/EEC [24] requires the sludge generated in the wastewater treatment process to be agriculturally reused. As part of a unique study, Zdybel and co-workers found living eggs of intestinal nematodes in 99% of samples of sewage sludge produced by municipal waste treatment plants; most samples were contaminated by the eggs of *Ascaris* spp. (95%), *Toxocara* spp. (96%) and *Trichuris* spp. (60%) [25]. The impressive survival rates of parasite eggs [26, 27] combined with their potential health hazards, such as disorders of the digestive, respiratory and nervous systems, allergies and, in the case of toxocarosis, visual disturbances leading to blindness, pose a significant threat for those consuming herbs grown on fields fertilized with sewage sludge. Parasites have also been increasingly implicated in cases of liver and pancreatic cancers and brain glioma [28, 29]. Polish environmental studies have reported the presence of *Echinococcus multilocularis* DNA in fruit, vegetable, and mushroom samples, underlining the potential risks associated with this very dangerous cosmopolitan tapeworm [30].

The aim of the current research was to comprehensively analyze a range of chemical and biological contaminants in dandelion (*Taraxacum officinale*), a cosmopolitan herbaceous perennial plant of the *Asteraceae* (Compositae) family. Dandelion is often considered a weed and grows worldwide in temperate regions with moist soils, though originally introduced from Eurasia, and can be found in parks, gardens, pastures, orchards, along roadsides, and among agricultural and horticultural crops [31, 32]. The plant has a range of uses. The Kazakh dandelion native to Kazakhstan, Kirghizia and Uzbekistan is notable for its production of high quality rubber [33, 34]. The dandelion is also an important plant in beekeeping, being a very efficient honey plant with pollens that are readily consumed by honey bees [35]. The plant also has many culinary uses: leaves are added to salads, roasted and ground roots can be used as coffee replacements, the flowers can be used to make a wine or are boiled to prepare a honey substitute. Dandelion also has a number of therapeutic properties: the roots are used to treat liver failure, dyspepsia and anorexia, while boiled leaves are used as diuretics. The anti-diabetic properties of dandelion are attributed to the bioactive components: chicoric acid, taraxasterol, chlorogenic acid, and sesquiterpene lactones [36], and the methanolic extracts of the roots have been found to have anti-proliferative effects on several human cancer cell lines, particularly against liver cancer [37, 38]. Dandelion is also believed to have a number of antibacterial and antifungal properties [1, 39].

For this medically-important plant to be used in therapeutic treatments, it must be free of chemical and biological contamination. Unfortunately there are many reports of chemical contamination in dandelion plant material and herbal teas. Previous studies have found *T. officinale* to demonstrate as much as 15-fold excess lead levels in plants growing near railway lines, accompanied by significant accumulations of PCB congeners [40, 41]. In addition, studies of individual PAH levels in several commercial herbal teas have recorded high values of naphthalene, pyrene, phenanthrene and benzo(b)fluoranthene in dandelion tea [42], and mycotoxin multicontamination by aflatoxins, ochratoxin A, zearalenone, deoxynivalenol and citrinin have been noted in the plant [43]. However, no data exists regarding potential hazardous biological contaminants such as bacteria or fungi, particularly those that are pathogenic to humans and farm animals, nor by parasitic worms and protozoa. The present study attempts to fill this gap.

Results

Heavy metal content in *Taraxacum officinale* and soil samples

Fig 1 shows an example voltamperogram of *Taraxacum officinale* flowers collected in Warszawa 2. Zn, Cd, Pb, Cu and Tl were determined by anodic stripping voltammetry (ASV) at hanging mercury dropping electrode (HMDE), while Co and Ni were determined by adsorptive cathodic stripping voltammetry (AdCSV) at HMDE.

Considerable differences in Cd, Co, Cu, Pb, Ni, Tl and Zn content were found between plant parts and collection sites (Table 1). The highest, and the most diverse, concentrations were found in the case of Zn (24.79–137.66 mg/kg) and Pb (14.7–80.15 mg/kg), and the lowest concentrations, with moderate differentiation, were found for Cd (0.31–4.89 mg/kg), Co (0.28–5.76 mg/kg) and Tl (0.37–23.45 mg/kg). On the other hand, the smallest variation was observed for Cu (13.56–38.14 mg/kg) and Ni (3.54–14.57 mg/kg).

The greatest amount of Cd (4.89 mg/kg) was measured in flowers collected in Warszawa 2, while the greatest amount of Co (5.76 ± 2.22 mg/kg) was found in soil samples from Rudenka. The highest Pb values were found in the flowers, leaves and roots of a dandelion harvested in Warszawa 2 (79.62, 77.34 and 80.15 mg/kg, respectively). Interestingly, the soil in this site contained the lowest measured Pb content (14.70 mg/kg). In each of the three sites, the highest Tl

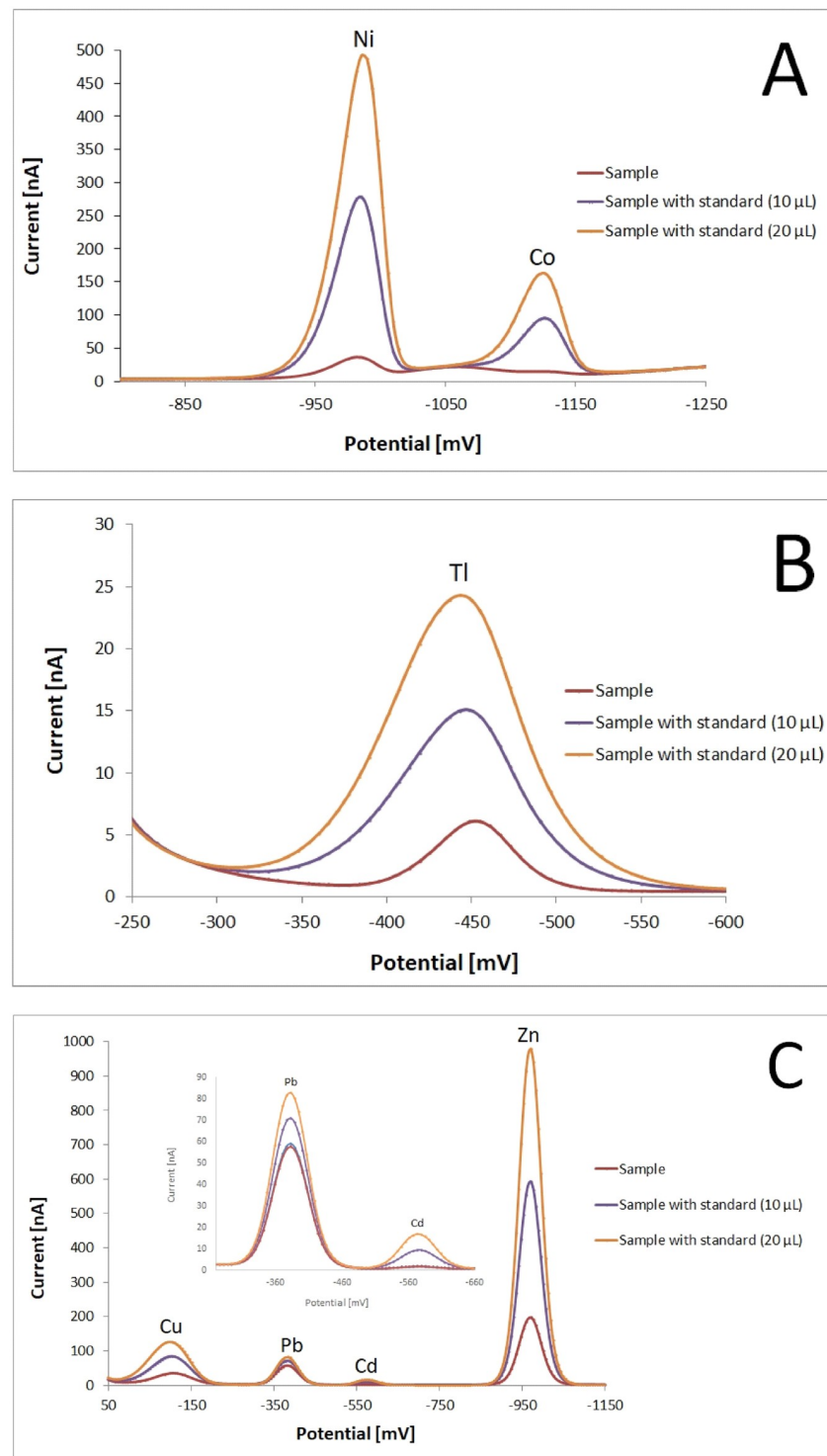


Fig 1. Heavy metal detection in *Taraxacum officinale* flowers collected in Warszawa 2. (A) Co and Ni determined by adsorptive stripping voltammetry (AdCSV) at hanging mercury dropping electrode (HMDE); (B) Tl determined by anodic stripping voltammetry (ASV) at hanging mercury dropping electrode (HMDE); (C) Zn, Cd, Pb and Cu determined by anodic stripping voltammetry (ASV) at hanging mercury dropping electrode (HMDE).

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Table 1. Concentration of heavy metals (mg/kg $\pm$ SD) in various parts of *Taraxacum officinale* and soil.

Metal	Rudenska (49.486555, 22.412092)				Warszawa 1 (52.173292, 21.153900)				Warszawa 2 (52.218959, 21.098110)			
	Flowers	Leaves	Roots	Soil	Flowers	Leaves	Roots	Soil	Flowers	Leaves	Roots	Soil
Cadmium (Cd)	0.81 $\pm$ 0.24 AB ab	1.37 $\pm$ 0.31 C ac	1.23 $\pm$ 0.28 E bd	0.65 $\pm$ 0.32 G cd	0.47 $\pm$ 0.13 A e	0.66 $\pm$ 0.19 C ef	0.46 $\pm$ 0.13 E f	0.53 $\pm$ 0.23 H	4.89 $\pm$ 0.14 B gh	0.31 $\pm$ 0.06 C gi	0.65 $\pm$ 0.16 E hij	0.34 $\pm$ 0.15 GH j
Cobalt (Co)	0.28 $\pm$ 0.02 a	0.35 $\pm$ 0.05 CD a	1.02 $\pm$ 0.28 EF a	5.76 $\pm$ 2.22 GH a	0.32 $\pm$ 0.21 e	0.53 $\pm$ 0.12 C e	0.69 $\pm$ 0.14 G e	1.44 $\pm$ 0.82 g	0.24 $\pm$ 0.07 g	0.48 $\pm$ 0.11 D g	0.63 $\pm$ 0.09 F g	1.03 $\pm$ 0.40 H g
Copper (Cu)	23.07 $\pm$ 3.04 AB a	15.00 $\pm$ 0.64 C a	16.50 $\pm$ 1.70 EF a	13.56 $\pm$ 0.21 G a	26.97 $\pm$ 4.12 A a	26.23 $\pm$ 1.34 C b	23.79 $\pm$ 2.24 E abc	38.14 $\pm$ 6.35 G abc	29.77 $\pm$ 4.47 B g	19.80 $\pm$ 1.12 C g	23.34 $\pm$ 2.08 F g	16.44 $\pm$ 0.43 G g
Lead (Pb)	33.04 $\pm$ 10.23 A a	53.86 $\pm$ 20.60 C ab	43.19 $\pm$ 6.95 E ac	21.40 $\pm$ 11.55 abc	31.63 $\pm$ 4.19 B ef	27.34 $\pm$ 4.21 C ef	20.62 $\pm$ 5.42 E e	20.46 $\pm$ 9.52 f	79.62 $\pm$ 14.29 AB g	77.34 $\pm$ 21.75 C h	80.15 $\pm$ 30.60 E i	14.70 $\pm$ 10.08 ghi
Nickel (Ni)	5.99 $\pm$ 0.79 A a	3.62 $\pm$ 0.35 C a	7.85 $\pm$ 1.27 E a	13.43 $\pm$ 4.54 GH a	3.85 $\pm$ 0.78 A e	11.69 $\pm$ 1.52 C e	14.57 $\pm$ 1.61 E e	6.07 $\pm$ 0.92 G e	4.89 $\pm$ 0.34 A g	6.75 $\pm$ 0.48 C g	10.93 $\pm$ 0.76 E g	3.54 $\pm$ 1.13 H g
Thallium (Tl)	0.64 $\pm$ 0.18 A a	1.06 $\pm$ 0.72 b	0.93 $\pm$ 0.23 E c	12.56 $\pm$ 0.79 G abc	0.65 $\pm$ 0.29 A e	0.82 $\pm$ 0.38 f	0.37 $\pm$ 0.06 EF ef	23.45 $\pm$ 12.47 ef	1.14 $\pm$ 0.35 A g	1.00 $\pm$ 0.39 h	0.93 $\pm$ 0.06 F i	7.51 $\pm$ 1.38 G ghi
Zinc (Zn)	35.04 $\pm$ 1.38 AB a	29.58 $\pm$ 3.19 C a	24.79 $\pm$ 1.51 E a	37.48 $\pm$ 2.90 G a	37.93 $\pm$ 3.85 A e	66.31 $\pm$ 5.53 C e	54.78 $\pm$ 5.66 E e	76.16 $\pm$ 7.00 G e	39.35 $\pm$ 2.99 B g	60.39 $\pm$ 8.23 C gh	60.59 $\pm$ 5.35 E gi	137.66 $\pm$ 4.58 G ghi

N = 4–21 independent measurements. Statistically significant differences (t-test, $p < 0.05$) are marked with the same letter. Capital letters: comparisons made between samples of flowers (A, B), leaves (C, D), roots (E, F), and soil (G, H) collected at various sites (Rudenska, Warszawa 1, and Warszawa 2, respectively). Lower case letters: comparisons made between metal concentration in various parts of plants and soil surrounding their roots, Rudenska (a, b, c, d), Warszawa 1 (e, f), and Warszawa 2 (g, h, i, j). For the sake of clarity of the table, no comparisons between individual metals have been marked.

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and Zn values were found in the soil samples. No regular trends were found in the measurements of Cu and Ni.

Some trends were observed regarding the concentrations of metals measured in individual plant parts and soil samples (S5 Table). No correlation of any kind was seen between the leaf and root contents. Regarding the material collected in Rudenka and Warszawa 1, positive correlations in Ni content were found between flowers and leaves ($r = 0.93$ and $r = 0.70$, respectively), while negative correlations in Co ($r = -0.89$ Rudenka), Cu ($r = -0.91$ Warszawa 1), Zn ($r = -0.84$ Warszawa 1), and Pb ($r = -0.80$ Warszawa 1) content were found between flowers and roots. Zn content in the soil negatively correlated with its content in the roots ($r = -0.75$ Rudenka, $r = -0.90$ Warszawa 1), leaves ($r = -0.95$ Warszawa 2) and flowers ($r = -0.89$ Warszawa 1), while Co content in soil positively correlated with root content ($r = 0.90$ Rudenka). Cu content in soil was negatively correlated with the levels in leaves ($r = -0.86$ Rudenka, $r = -0.87$ Warszawa 2), flowers ($r = -0.70$ Rudenka, $r = -0.82$ Warszawa 1) and roots ($r = -0.90$ Warszawa 2). Pb soil content was positively correlated with leaf content ($r = 0.92$ Rudenka, $r = 0.93$ Warszawa 2). Cd soil content was negatively correlated with its content in leaves ($r = -0.95$ Warszawa 1) and flowers ($r = -0.84$ Warszawa 2); however, it positively correlated in flowers collected in Rudenka ($r = 0.70$). Similarly, the soil Co content negatively correlated with the contents in leaves ($r = -0.98$ Warszawa 2) and flowers ($r = -0.84$ Rudenka), while soil Ni content positively correlated with leaf content ($r = 0.75$ Rudenka). Tl present in soil samples was positively correlated with root ($r = 0.70$ Rudenka), leaf ($r = 0.88$ Rudenka) and flower content ($r = 0.96$ Warszawa 1), but negatively with in leaf content at Warszawa 2 ($r = -0.82$).

Interestingly, the concentration of Cd in flowers was negatively correlated with the concentration of Pb in the leaves ($r = -0.83$ Rudenka, $r = -0.72$ Warszawa 1 and $r = -0.88$ Warszawa 2, respectively). However, no other relationships in metal concentrations were found.

The biological concentration factor (BCF) values, i.e. the metal concentration in the roots vs. its concentration in soil surrounding the roots, calculated for each metal, are presented in Table 2, together with the values of two metal translocation factors (TF) demonstrating the translocation of metals from dandelion roots to leaves (TF1) and from leaves to flowers (TF2). BCF values significantly exceeding 1.0, indicating metal uptake from the soil by the roots, were found in the case of Pb (BCF range 6.78–1.45) and Cd (BCF range 4.23–1.05) while BCF values of Cu and Ni were more variable (BCF ranges 1.38–0.68 and 3.31–0.65, respectively). Low BCF values were noted for Co (0.70–0.16), Tl (0.13–0.03) and Zn (0.74–0.47), indicating a lack of

Table 2. Cumulation of heavy metals absorbed from soil by *Taraxacum officinale* roots and translocation to leaves and flowers.

Metal	Rudenka (49.486555, 22.412092)			Warszawa 1 (52.173292, 21.153900)			Warszawa 2 (52.218959, 21.098110)		
	BCF (roots/soil)	TF1 (leaves/roots)	TF2 (flowers/leaves)	BCF (roots/soil)	TF1 (leaves/roots)	TF2 (flowers/leaves)	BCF (roots/soil)	TF1 (leaves/roots)	TF2 (flowers/leaves)
Cadmium (Cd)	4.23±1.39 A ab	1.09±0.521 C a	1.23±0.28 E b	1.05±0.44 AB	1.48±0.60 D c	0.81±0.37 F c	2.39±1.79 B f	0.52±0.17 CD ef	1.57±0.81 EF e
Cobalt (Co)	0.16±0.04 AB a	0.37±0.12 CD a	0.82±0.11 E a	0.64±0.52 A	0.82±0.18 C c	0.57±0.31 c	0.70±0.23 B	0.78±0.20 D	0.61±0.20 E
Copper (Cu)	1.25±0.07 A a	0.88±0.06 CD a	1.63±0.26 E a	0.68±0.11 AB cd	1.11±0.13 C c	1.03±0.16 EF d	1.38±0.11 B f	0.87±0.11 D ef	1.52±0.30 F e
Lead (Pb)	2.95±1.69 A ab	1.30±0.73 a	0.88±0.35 E b	1.45±0.81 A	1.36±0.35	1.18±0.23 E	6.78±5.15 A ef	1.22±0.42 e	1.11±0.25 f
Nickel (Ni)	0.65±0.19 AB b	0.47±0.03 C a	1.65±0.09 E ab	2.44±0.54 A c	0.80±0.11 C c	0.33±0.07 E c	3.31±0.78 B e	0.62±0.06 C e	0.74±0.06 E e
Thallium (Tl)	0.08±0.02 A a	1.58±0.83 a	1.15±1.45	0.03±0.02 A c	2.26±0.78 C c	0.91±0.53 c	0.13±0.01 A ef	1.20±0.15 C e	1.29±0.60 f
Zinc (Zn)	0.67±0.09 ab	1.18±0.15 C a	1.22±0.14 E b	0.74±0.09 A c	1.22±0.20 D c	0.51±0.09 E c	0.47±0.04 A e	1.02±0.15 CD e	0.66±0.08 E e

BCF—Biological Concentration Factor, concentration ratio between metal absorbed from soil and accumulated in roots; TF1—Translocation Factor 1, concentration ratio between metal transferred to leaves from roots, TF2—Translocation Factor 2, concentration ratio between metal transferred to flowers from leaves. Statistically significant differences are marked with the same letter. Capital letters: comparisons made between BCFs of samples collected at various sites (A, B), TFs1 (C, D) and TFs2 (E, F), respectively. Lower case letters: comparisons made between BCF, TF1 and TF2 calculated for the samples collected in Rudenka (a, b), Warszawa 1 (c, d) and Warszawa 2 (e, f), respectively. For the sake of clarity of the table, no comparisons between individual metals have been marked.

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accumulation. Pb accumulated in roots was translocated to the leaves (TF1 range 1.36–1.22), and to a lower extent, the flowers (TF2 range 1.18–0.88). Despite the lack of Tl accumulation in roots, the TF1 (2.26–1.20) and TF2 (1.29–0.91) values indicate that Tl is translocated into the leaves and flowers.

Soil pH

The soil collected in Rudenka was slightly alkaline (pH 7.10 ± 0.07), while the soil collected in Warszawa 1 was neutral (pH 7.01 ± 0.04) and the soil from the site Warszawa 2 was slightly acidic (pH 6.94 ± 0.02). The statistical significance of these data is presented in Table 3.

Lack of PAHs, PCBs, dioxins, pesticides and mycotoxin contaminants

The GC-MS analyses of the flowers, leaves, roots and soil samples taken from the three locations showed a complete absence of PCBs, dioxins and pesticides (Fig 2). Trace amounts of PAHs (below detection limit of 0.08 ng/ml) were found only in the soil sample collected in Rudenka, with their complete absence in other samples. None of the 65 tested mycotoxins (S1 Fig, S2 and S3 Tables) were detected in the tested samples.

Microbial contaminants

Cultures of the dandelion and soil samples showed the presence of numerous aerobic and anaerobic bacteria (Table 4). The lowest number of colony forming units (CFU) was found in the case of flowers collected in Rudenka (anaerobes 14 ± 9 CFU/mg, aerobes 21 ± 1 CFU/mg); in contrast, anaerobic and aerobic cultures of the leaves and roots of these plants yielded 21- to 38-times higher CFUs and cultures of the soil surrounding the roots yielded 19–27 times higher numbers. No such great differences in CFU number were found at the two remaining sites, where the differences were found to range from 0.4–1.2 times (anaerobes) and 0.3–1.9 times (aerobes).

As the Biomerieux Vitek-2 system used in these studies is designed to identify clinically-significant bacteria, not all colonies obtained by plating were identified. In total, 26 species of anaerobic bacteria and 69 species of aerobic bacteria were identified in the tested samples (Table 4). Images of four homogeneous cultures of aerobic bacteria and two cultures of anaerobic bacteria, responsible for serious systemic infections and skin diseases, isolated from dandelion and soil samples, are given in Fig 3.

In all tested samples, the number of identified species of aerobic bacteria outweighed the number of anaerobic species: 10–20 species versus 2–11, respectively (Table 4). In general, samples collected in the Warszawa sites contained a more species-diverse bacterial flora than those collected in Rudenka, with the exception of aerobes in leaves.

It should be emphasized that none of the anaerobic bacterial colonies obtained from the flowers, leaves and roots of the dandelion harvested at Rudenka were clinically significant: the anaerobic *Atopobium vaginae*, *Clostridium bifermentans*, *Clostridium sporogenes* and *Fusobacterium mortiferum* found in the Rudenka soil were not detected in any parts of the plants collected there. In contrast, *Clostridium bifermentans* and *Clostridium inoculum* present in the

Table 3. The acidity (mean pH $\pm$ SD) of the soil surrounding the *Taraxacum officinale* roots.

Soil acidity	Rudenka (49.486555, 22.412092)	Warszawa 1 (52.173292, 21.153900)	Warszawa 2 (52.218959, 21.098110)
pH	7.10 ± 0.07^A	7.01 ± 0.04^B	6.94 ± 0.02^{AB}

Statistically significant differences are marked with the same letter (t-test, $p < 0.05$).

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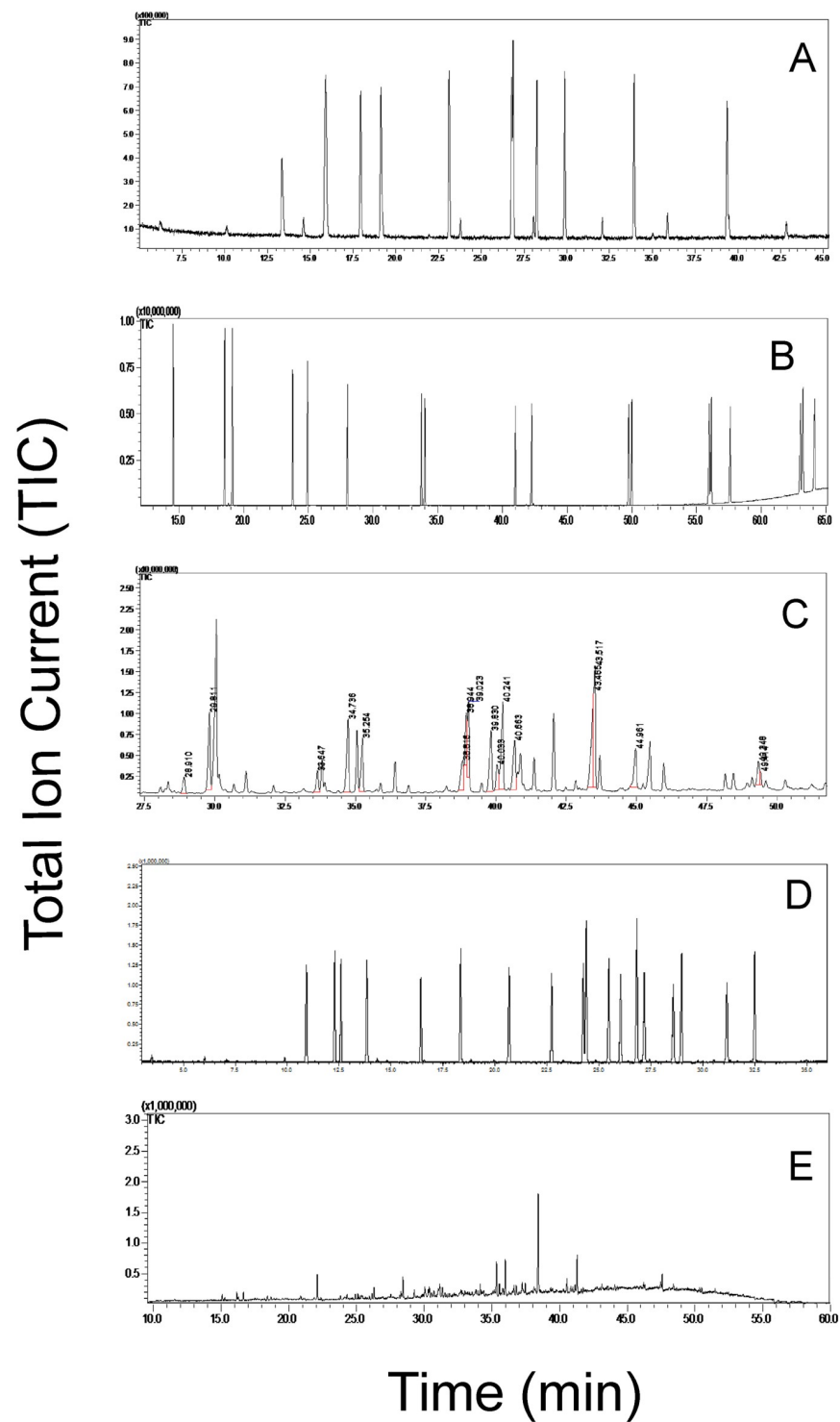


Fig 2. The total ion current (TIC) chromatograms of: (A) PAH standards, (B) PCB standards, (C) dioxins' standards, (D) pesticide standards, (E) soil sample collected in Rudenka. List of standards used, their retention times (RT) and limits of detection (LOD) is presented in [S1 Table](#).

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Table 4. Anaerobic and aerobic bacteria isolated from soil and various parts of *Taraxacum officinale* plants (CFU/mg of dried sample; mean $\pm$ SD).

	Rudenska (49.486555, 22.412092)	Warszawa 1 (52.173292, 21.153900)	Warszawa 2 (52.218959, 21.098110)
Flowers			
Anaerobes:			
CFU/mg	14 $\pm$ 9 ^{AB}	390 $\pm$ 98 ^A	385 $\pm$ 114 ^B
Species	Colonies not identified by Vitek-2 system	<i>Anaerococcus prevotii</i> , <i>Clostridium perfringens</i> and colonies not identified by Vitek-2 system	<i>Actinomyces odontolyticus</i> , <i>Anaerococcus prevotii</i> , <i>Clostridium</i> group, <i>Propionibacterium acnes</i>
Aerobes:			
CFU/mg	21 $\pm$ 1 ^{CD}	458 $\pm$ 154 ^C	348 $\pm$ 139 ^D
Species	<i>Aerococcus viridans</i> , <i>Aeromonas salmonicida</i> , <i>Alloiococcus otitidis</i> , <i>Francisella tularensis</i> , <i>Kocuria kristinae</i> , <i>Leuconostoc mesenteroides cremoris</i> , <i>Methylobacterium</i> spp., <i>Pasteurella pneumotropica</i> , <i>Pediococcus pentosaceus</i> , <i>Photobacterium damsela</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus lentus</i> , <i>Staphylococcus saprophyticus</i> , <i>Staphylococcus vitulinus</i> ,	<i>Acinetobacter lwoffii</i> , <i>Aeromonas salmonicida</i> , <i>Agrobacterium tumefaciens</i> , <i>Alloiococcus otitidis</i> , <i>Bacillus cereus</i> , <i>Bacillus megaterium</i> , <i>Cronobacter sakazakii</i> , <i>Enterococcus casseliflavus</i> , <i>Helcococcus kunzii</i> , <i>Leuconostoc mesenteroides cremoris</i> , <i>Lysinibacillus fusiformis</i> , <i>Pantoea</i> sp., <i>Pasteurella testudinis</i> , <i>Pseudomonas luteola</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus hominis</i> , <i>Staphylococcus vitulinus</i> , <i>Staphylococcus warneri</i>	<i>Acinetobacter lwoffii</i> , <i>Alicyclobacillus acidoterrestris</i> , <i>Bacillus cereus</i> , <i>Francisella tularensis</i> , <i>Pseudomonas luteola</i> , <i>Rahnella aquatilis</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus lentus</i> , <i>Staphylococcus xylosus</i>
Leaves			
Anaerobes:			
CFU/mg	299 $\pm$ 185	162 $\pm$ 76 ^E	385 $\pm$ 114 ^E
Species	Colonies not identified by Vitek-2 system	<i>Atopobium vaginae</i> , <i>Clostridium bifermentans</i> , <i>Fusobacterium varium</i> , <i>Veillonella</i> spp	Colonies not identified by Vitek-2 system
Aerobes:			
CFU/mg	396 $\pm$ 107 ^F	173 $\pm$ 25 ^{FG}	348 $\pm$ 139 ^G
Species	<i>Aerococcus viridans</i> , <i>Alloiococcus otitidis</i> , <i>Bacillus cereus</i> , <i>Bacillus pumilus</i> , <i>Gemella sanguinis</i> , <i>Kocuria rhizophila</i> , <i>Leuconostoc mesenteroides dextranicum</i> , <i>Pantoea</i> spp., <i>Pasteurella testudinis</i> , <i>Staphylococcus vitulinus</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus lugdunensis</i> , <i>Staphylococcus pseudintermedius</i> , <i>Staphylococcus sciuri</i>	<i>Acinetobacter lwoffii</i> , <i>Aeromonas salmonicida</i> , <i>Bacillus cereus</i> , <i>Gemella morbillorum</i> , <i>Methylobacterium</i> spp., <i>Pantoea</i> spp., <i>Pseudomonas oryzihabitans</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus lugdunensis</i> , <i>Staphylococcus pseudintermedius</i>	<i>Aerococcus viridans</i> , <i>Alloiococcus otitidis</i> , <i>Bacillus cereus</i> , <i>Bacillus megaterium</i> , <i>Kocuria rosea</i> , <i>Pantoea</i> spp., <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus lentus</i> , <i>Staphylococcus vitulinus</i>
Roots			
Anaerobes:			
CFU/mg	535 $\pm$ 124 ^{JK}	273 $\pm$ 65 ^{HJKL}	481 $\pm$ 80 ^{IL}
Species	Colonies not identified by Vitek-2 system	<i>Atopobium vaginae</i> , <i>Clostridium baratii</i> , <i>Clostridium bifermentans</i> , <i>Clostridium glycolicum</i> , <i>Clostridium innocuum</i> , <i>Clostridium paraputrificum</i> , <i>Clostridium perfringens</i> , <i>Clostridium sordelli</i>	<i>Atopobium vaginae</i> , <i>Clostridium baratii</i> , <i>Clostridium bifermentans</i> , <i>Clostridium paraputrificum</i> , <i>Clostridium perfringens</i> , <i>Clostridium tertium</i> , <i>Eggerthia cateniformis</i> , <i>Fusobacterium mortiferum</i> , <i>Fusobacterium necrophorum</i> , <i>Fusobacterium nucleatum</i> , <i>Parabacteroides distasonis</i>
Aerobes:			
CFU/mg	467 $\pm$ 55 ^{MN}	146 $\pm$ 20 ^{HM}	152 $\pm$ 12 ^{IN}
Species	<i>Alloiococcus otitidis</i> , <i>Bacillus cereus</i> , <i>Bacillus megaterium</i> , <i>Brevibacillus choshinensis</i> , <i>Francisella tularensis</i> , <i>Leuconostoc mesenteroides cremori</i> , <i>Ochrobactrum anthropi</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus pseudintermedius</i> , <i>Virgibacillus pantothenticus</i>	<i>Acinetobacter baumannii</i> , <i>Acinetobacter haemolyticus</i> , <i>Aeromonas sobria</i> , <i>Alloiococcus otitidis</i> , <i>Bacillus cereus</i> , <i>Enterococcus columbae</i> , <i>Escherichia coli</i> , <i>Francisella tularensis</i> , <i>Gardnella vaginalis</i> , <i>Leuconostoc mesenteroides cremoris</i> , <i>Methylobacterium</i> spp., <i>Neisseria cinerea</i> , <i>Oligella ureolytica</i> , <i>Pantoea</i> spp., <i>Pasteurella canis</i> , <i>Pseudomonas luteola</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus pseudintermedius</i>	<i>Aeromonas salmonicida</i> , <i>Aeromonas sobria</i> , <i>Alloiococcus otitidis</i> , <i>Bacillus cereus</i> , <i>Enterococcus columbae</i> , <i>Escherichia coli</i> , <i>Francisella tularensis</i> , <i>Leuconostoc mesenteroides cremoris</i> , <i>Moraxella</i> grup, <i>Oligella ureolytica</i> , <i>Pasteurella testudinis</i> , <i>Pseudomonas oryzihabitans</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus lugdenensis</i> , <i>Staphylococcus pseudintermedius</i>

(Continued)

Table 4. (Continued)

	Rudenska (49.486555, 22.412092)	Warszawa 1 (52.173292, 21.153900)	Warszawa 2 (52.218959, 21.098110)
Soil			
Anaerobes:			
CFU/mg	349 ± 115 ^O	489 ± 75 ^R	268 ± 39 ^{PR}
Species	<i>Atopobium vaginae</i> , <i>Clostridium bifermentas</i> , <i>Clostridium sporogenes</i> , <i>Fusobacterium mortiferum</i> and colonies not identified by Vitek-2 system	<i>Actinomyces odontolyticus</i> , <i>Bifidobacterium</i> spp., <i>Campylobacter ureolyticus</i> , <i>Clostridium bifermentas</i> , <i>Clostridium difficile</i> , <i>Clostridium innocuum</i> , <i>Clostridium subterminale</i> , <i>Fusobacterium mortiferum</i> , <i>Fusobacterium necrophorum</i> , <i>Fusobacterium varium</i> , <i>Propionibacterium acnes</i> , <i>Terrisporobacter glycolicus</i>	<i>Atopobium vaginae</i> , <i>Clostridium perfringens</i> and colonies not identified by Vitek-2 system
Aerobes:			
CFU/mg	563 ± 120 ^O	382 ± 140 ^S	677 ± 113 ^{PS}
Species	<i>Aeromonas salmonicida</i> , <i>Alloicoccus otitidis</i> , <i>Bacillus cereus</i> , <i>Bacillus farraginis</i> , <i>Bacillus megaterium</i> , <i>Escherichia coli</i> , <i>Gemella haemolysans</i> , <i>Leclercia adecarboxylata</i> , <i>Methylobacterium</i> spp., <i>Pasteurella pneumotropica</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus lugdunensis</i> , <i>Staphylococcus pseudintermedius</i> , <i>Staphylococcus vitulinus</i> , <i>Streptococcus anginosus</i>	<i>Acinetobacter lwoffii</i> , <i>Aeromonas veronii</i> , <i>Bacillus cereus</i> , <i>Brevundimonas diminuta</i> , <i>Cedecea lapagei</i> , <i>Chromobacterium violaceum</i> , <i>Enterococcus durans</i> , <i>Pantoea</i> spp., <i>Pseudomonas luteola</i> , <i>Pseudomonas oryzae</i> , <i>Sphingomonas paucimobilis</i> , <i>Sphingobacterium spiritivorum</i> , <i>Staphylococcus lugdunensis</i> , <i>Staphylococcus pseudintermedius</i> , <i>Vibrio vulnificus</i>	<i>Acinetobacter haemolyticus</i> , <i>Alloicoccus otitidis</i> , <i>Bacillus megaterium</i> , <i>Haemophilus parainfluenzae</i> , <i>Lactococcus garvieae</i> , <i>Pasteurella testudinis</i> , <i>Photobacterium damsela</i> , <i>Pseudomonas luteola</i> , <i>Sphingomonas paucimobilis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus lugdunensis</i>

CFU—colony forming units. Statistically significant differences are marked with the same letters (t-test, $p < 0.05$). Comparisons made between samples of flowers (A-D), leaves (E-G), roots (H-N), soil (O-S) collected at various sites (Rudenska, Warszawa 1, Warszawa 2).

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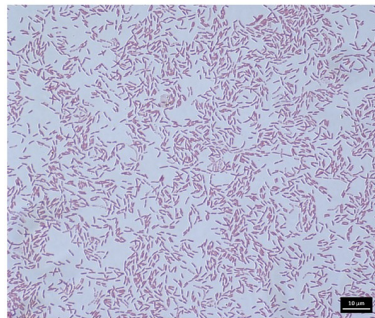
Warszawa 1 soil was also found in roots and leaves of dandelion collected there, and *Atopobium vaginae* and *Clostridium perfringens* present in the soil from Warszawa 2 were also present in the root and leaf samples of the dandelion harvested at this site.

The most common pathogenic aerobes were *Sphingomonas paucimobilis*, a rare infectious agent (detected in all samples), *Bacillus cereus*, producing toxins (absent only in Rudenska flowers and Warszawa 2 soil) and *Alloicoccus otitis* involved in middle ear infections (absent in Warszawa 2 flowers and Warszawa 1 leaves and soil) (Table 4). The presence of *Escherichia coli*, commonly found in the gut of humans and warm-blooded animals, was confirmed only in the Rudenska soil and Warszawa 1 roots, while *Staphylococcus aureus*, a major human pathogen, was detected in all Warszawa 2 samples and Warszawa 1 roots. Of the zoonotic pathogens, *Pasteurella pneumotropica* was found in Rudenska flowers and soil, *Pasteurella canis* only in Warszawa 1 roots, *Staphylococcus pseudintermedius* in all Rudenska and Warszawa samples except flowers and *Staphylococcus lentus* in Rudenska flowers and Warszawa 2 flowers and leaves. *Francisella tularensis*, present in all root samples, as well as in Rudenska and Warszawa 2 flowers, was absent in all soil and leaf samples.

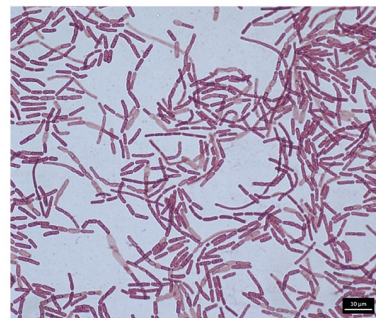
The septicemia agents *Vibrio vulnificus* and *Fusobacterium mortiferum* were detected in Warszawa 1 soil, while *Rahnella aquatilis* in Warszawa 2 flowers. Pneumonia-inducing *Clostridium baratii* was found in Warszawa 2 roots and gangrene-inducing *Clostridium perfringens* in Warszawa 1 roots. In addition, *Haemophilus parainfluenzae*, known to cause a variety of severe infections, was found in Warszawa 2 soil, while *Staphylococcus lugdunensis* an infective agent of various diseases, was detected in leaves and soil collected in the Rudenska and Warszawa 1 sites.

Aerobes:

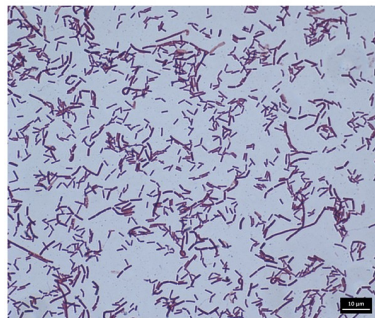
Aeromonas salmonicida



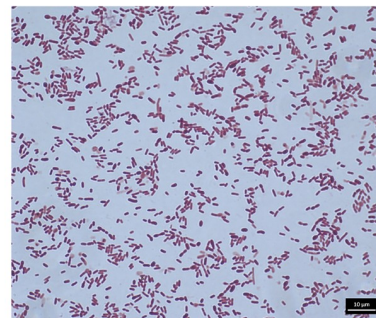
Bacillus megaterium



Pseudomonas luteola

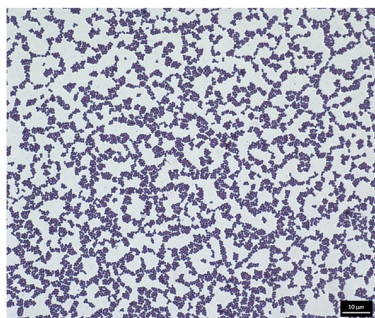


Sphingomonas paucimobilis



Anaerobes:

Anaerococcus prevotii



Propionibacterium acnes

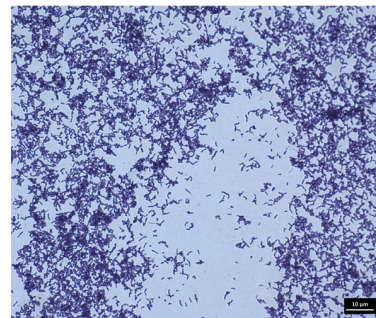


Fig 3. Examples of pathogenic bacteria detected in *Taraxacum officinale* and soil samples. Scale bars: 10 µm.

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Table 5 shows the result of mycological analysis of collected samples. The highest number of CFU was found in Warszawa 2 soil (9838 ± 1062 CFU/mg) and the lowest in Warszawa 2 flowers (212 ± 109 CFU/mg); however, the total number of detected fungal species, approximately 14, was significantly smaller than that of the bacteria. The macroscopic analysis of

Table 5. Fungi isolated from soil and various parts of *Taraxacum officinale* plants.

	Rudenka (49.486555, 22.412092)	Warszawa 1 (52.173292, 21.153900)	Warszawa 2 (52.218959, 21.098110)
Flowers:			
CFU/mg	740 ± 294	558 ± 270 ^D	212 ± 109 ^G
Identified species	<i>Aspergillus niger</i> , <i>Penicillium</i> sp.	<i>Alternaria alternata</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus niger</i> , <i>Cladosporium</i> sp., <i>Penicillium</i> sp.	<i>Alternaria alternata</i> , <i>Aspergillus niger</i> , <i>Penicillium</i> sp.
Leaves:			
CFU/mg	1573 ± 1492	1045 ± 648 ^E	281 ± 137 ^H
Identified species	<i>Alternaria alternata</i> , <i>Aspergillus fumigatus</i> , <i>Fusarium</i> sp., <i>Penicillium</i> sp.	<i>Alternaria alternata</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus</i> sp., <i>Cladosporium</i> sp., <i>Mucor</i> sp., <i>Penicillium</i> sp., <i>Rhizopus</i> sp.	<i>Alternaria alternata</i> , <i>Aspergillus</i> sp., <i>Mucor</i> sp., <i>Penicillium</i> sp.
Roots:			
CFU/mg	1692 ± 1687	1476 ± 1564 ^F	287 ± 87 ^I
Identified species	<i>Alternaria alternata</i> , <i>Fusarium</i> sp., <i>Paecilomyces</i> sp., <i>Penicillium</i> sp., <i>Trichoderma viride</i>	<i>Alternaria alternata</i> , <i>Aspergillus terreus</i> , <i>Chaetomium</i> sp., <i>Cladosporium</i> sp., <i>Fusarium</i> sp., <i>Mucor</i> sp., <i>Penicillium</i> sp.	<i>Aspergillus niger</i> , <i>Penicillium</i> sp., <i>Rhizomucor</i> sp.
Soil:			
CFU/mg	459 ± 226 ^{AB}	3872 ± 698 ^{ACDEF}	9838 ± 1062 ^{BCGHI}
Identified species	<i>Penicillium</i> sp., <i>Trichoderma viride</i>	<i>Mucor</i> sp., <i>Penicillium</i> sp.	<i>Mucor</i> sp.

CFU (colony forming units) are presented as mean CFU/mg of dried sample ± SD. Statistically significant differences are marked with the same letters (t-test, p range from p = 0.0494 to p < 0.0001).

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fungal colonies and microscopic images of fungal structures only allowed precise species identification in five cases (Fig 4). *Aspergillus niger* was identified in all flower samples and in Warszawa 2 roots, *Aspergillus fumigatus* in Warszawa 1 flowers and leaves as well as Rudenka leaves, while *Aspergillus terreus* was only recorded in Warszawa 1 roots. *Alternaria alternata* was found in all leaf samples, both Warszawa flowers, Rudenka and Warszawa 1 roots, while *Trichoderma viridae* was found only in Rudenka roots and soil. For the fungi belonging to genera *Penicillium*, *Mucor*, *Rhizomucor*, *Fusarium*, *Rhizopus*, *Cladosporium* and *Chaetomium* it was not possible to determine their species. *Penicillium* spp. was present in all flower, leaf and root samples, as well as in Rudenka and Warszawa 1 soil. *Mucor* spp. was found in Warszawa 1 leaves, roots and soil, as well as in Warszawa 2 leaves and soil. *Fusarium* spp. was detected in Rudenka leaves and roots, as well as in Warszawa 1 roots, and *Rhizopus* spp. in Warszawa 1 leaves. *Paecilomyces* spp. was found in Rudenka roots. *Rhizomucor* spp. was found Warszawa 2 roots, *Chaetomium* spp. in Warszawa 1 roots, and *Cladosporium* spp. in all dandelion parts in Warszawa 1 (Table 4).

Parasite contamination

All the collected samples were tested for the presence of parasite eggs and DNA. Neither helminth eggs nor DNA (tapeworms, nematodes, and flukes) were found in any of them (Fig 5A and 5B). In the Warszawa 2 site, the presence of the protozoan parasite *Giardia intestinalis* DNA was confirmed in dried leaves and roots (Fig 5C). The 489 bp partial sequence of triose-phosphate isomerase (tpi) gene obtained from dried dandelion leaves (GenBank accession number OM964640.1), was 99.8% similar to seven *Giardia intestinalis* (assemblage C) sequences obtained from dogs in Slovakia, Brazil, Japan and Poland (GenBank accession numbers: LC437516.1, KT728510.1, KT728509.1, KT728507.1, MZ160223.1, EU781006.1, OM964641.1).

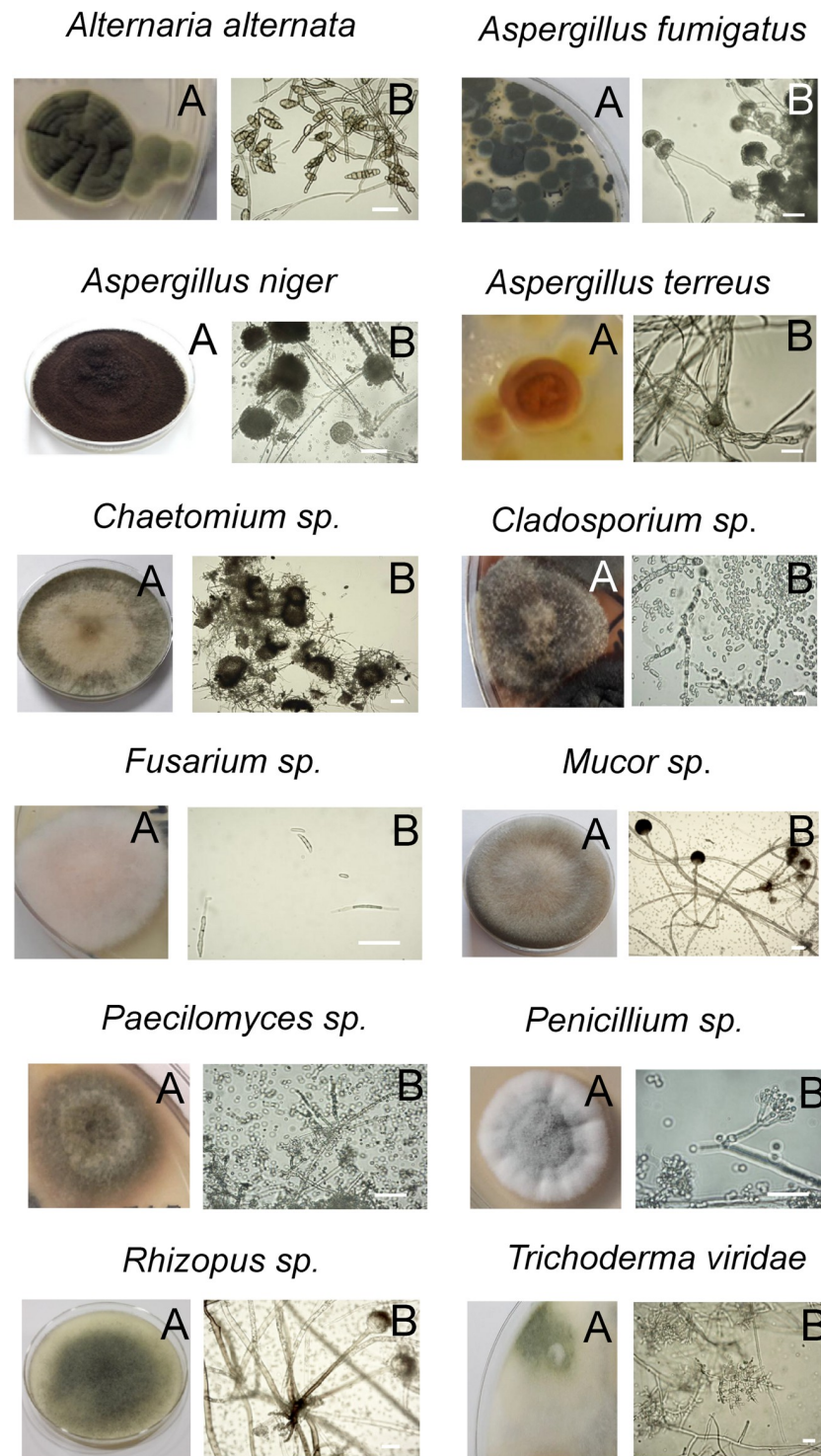


Fig 4. Fungi detected in *Taraxacum officinale* and soil samples. (A) macroscopic view of fungal colony, (B) microscopic image of the fungus. Scale bars: 20 μ m.

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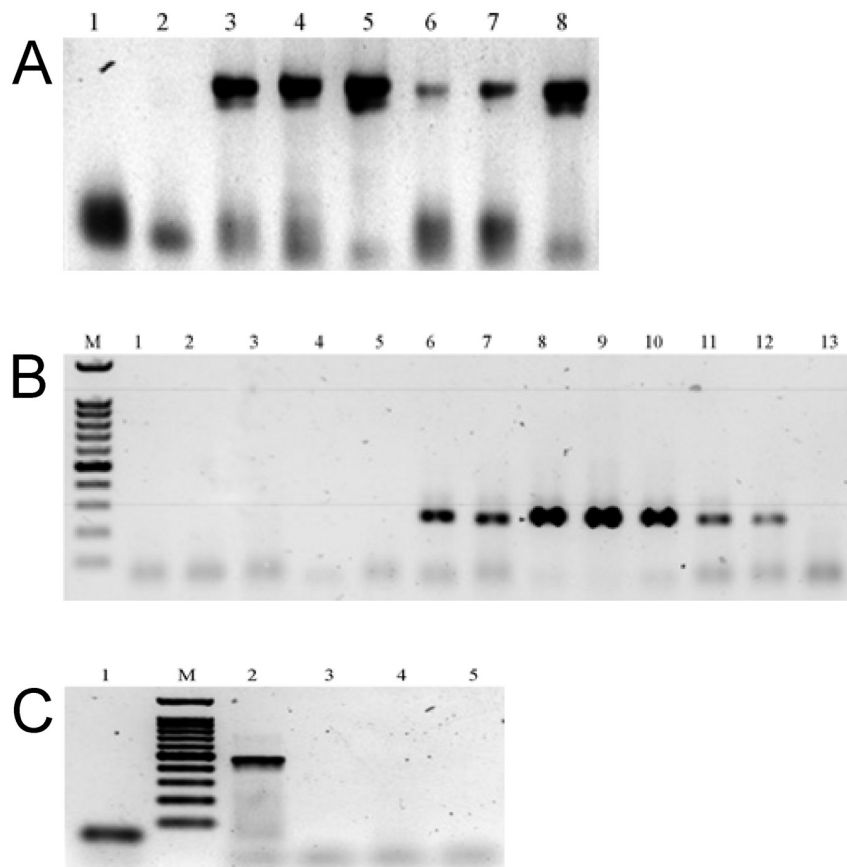


Fig 5. Detection of parasites' DNA in *Taraxacum officinale* samples. (A) Helminth Test, 1—H₂O, 2—dried dandelion leaves (Rudenka), 3—*Oxyuridae* sp. (eggs), 4—*Echinococcus* sp. (adult), 5—*Taenia* sp. (eggs), 6—*Trichuris* sp. (eggs), 7—*Fasciola hepatica* (adult), 8—*Toxocara cati* (adult); (B) Helminth Test, M—Marker DNA, 1- dried dandelion leaves (Warszawa 1), 2—dried dandelion leaves (Warszawa 2), 3—dried dandelion flowers (Warszawa 2), 4—*Echinococcus* sp. (adult), 5—*Toxocara cati* (adult), 6—*Echinococcus* sp. (adult), 7—*Toxocara cati* (adult), 8—*Dipylidium caninum* (adult), 9—*Fasciola hepatica* (adult), 10—*Taenia taeniaeformis* (eggs), 11—*Toxocara canis* (eggs), 12—*Oxyuridae* sp. (eggs), 13—H₂O; (C) Giardia Test, 1—H₂O, M—Marker DNA, 2—dried dandelion leaves (Warszawa 2), 3—dried dandelion flowers (Warszawa 2), 4—dried dandelion leaves (Rudenka), 5—dried dandelion flowers (Rudenka). DNA marker—100 bp DNA Ladder (Novazym).

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Discussion

In the present study, a comprehensive examination was made of a wide range of possible contaminants of dandelion collected from three different sites. One was a mountain meadow in a small village, forming part of the Natura 2000 European Ecological Network, close to the Polish-Ukrainian border. For over 30 years, this meadow has not been subjected to agrotechnical treatments, apart from mowing once a year, and has not been used for grazing livestock; it is commonly visited by wild animals such as deer, roe deer, hares and foxes, with some visits by bears and wolves. The other two sampling sites for testing were located in Warsaw, the Poland's capital city. Sampling site Warszawa 1 was situated in an area with dense single-family housing, without any industry but with heavy traffic, while Warszawa 2 was in an extensive recreation area on the Vistula River bank, also with heavy traffic and located near to the coal-fired heat and power plant providing heat for 25% of the facilities in Warsaw. At all of these sites, the dandelion leaves are collected by the local population for salads, flowers to prepare honey substitutes and roots for medication, and for feeding small pets kept by children, such as rabbits and guinea pigs.

All the collected plant and soil samples were tested for heavy metal content using the anodic stripping voltammetry (ASV) method at hanging mercury dropping electrode (HMDE); this method allows a simultaneous evaluation of several metals including Pb, Cu, Cd, Zn and Tl, even at very low levels of parts-per-million (ppm) or even parts-per-billion (ppb). In addition to being simple and selective, this approach is also much less expensive than spectroscopic techniques [44, 45]. ASV is a two-step measurement. In the first step, called *amalgamation*, the metal ions present in the test solution are deposited on the surface of the mercury electrode, while in the second step, all the deposited ions are anodically stripped. ASV probes the species really existing in solutions which is a great advantage over atomic spectroscopy, in which the species present in solution are transferred to a plasma where they are present as atoms [45]. Ni and Co were also assayed using adsorptive cathodic stripping voltammetry (AdCSV) at hanging mercury dropping electrode (HMDE). The AdCSV technique is based upon the adsorptive accumulation of the metal ion complex with a suitable ligand at the electrode. In both methods, the working electrode was a mercury electrode; it is easy to prepare, and offers sensitive and reproducible results, with fast and simple electrode kinetics [44, 46]. Presented in this work voltammetry measurements of Zn, Pb, Cd, Ni and Cu in *T. officinale* were similar to those obtained by other authors using spectroscopic methods [47–51]. However, samples demonstrated a much higher Cu content than that reported by Habh and co-workers [52].

According to Minister of Agriculture and Rural Development regulations, the concentrations of heavy metals polluting the light agricultural soils in organic farming in Poland must not exceed 50 mg/kg (Pb), 0.75 mg/kg (Cd), 30 mg/kg (Ni), 100 mg/kg (Zn) and 30 mg/kg (Cu) [53]. Data in Table 1 clearly show that the Pb, Cd and Ni concentrations were significantly below these limits in all soil samples; however, the Cu content in the Warszawa 1 soil sample (38.14 mg/kg) slightly exceeded the limit for light soils but was below the limit for medium heavy and heavy soils (50 and 75 mg/kg, respectively) [53]. Similarly the Zn content exceeded the levels allowed for the light soil in Warszawa 2 (137.66 mg/kg), but was below the limit in the other soil samples. The maximum permitted Zn content is 200 mg/kg in medium heavy soils and 300 mg/kg in heavy soils [53]. As the soil is medium heavy at both Warszawa sites and heavy in Rudenka, our findings confirm the purity of all soil sample collection sites particularly compared with soil and plants sampled in the industrial region of Silesia in Poland [48, 54].

The presence of slightly elevated Zn levels in soil and dandelion samples collected in Warsaw may reflect the impact of road traffic and power plant emissions [49, 55, 56]. Literature data indicate strong Zn absorption by plant roots and high mobility inside plant [57]; however, our data only confirm these observations to a certain extent, and only in terms of Zn translocation from roots to leaves (TF1). The BCF accumulation index was in each case significantly below the 1.0 value. In contrast, high BCF values were observed for Pb and Cd, 6.78 and 4.23 respectively; these indicate intensive uptake of these metals from the soil by dandelion roots. The absorbed metals were translocated to both leaves and flowers.

However, the process of Tl absorption from soil is unclear. Its very low BCF coefficients suggest no absorption, while TF1 and TF2 values exceeding 1.0 suggest active Tl transfer from the roots to the leaves and from the leaves to the flowers. Further research is needed to clarify this issue as the accumulation of the metals in plants and their distribution inside plant tissues might be affected by numerous environmental and physiological factors.

No chemical contaminants (PAHs, PCB, dioxins, pesticides) were found in any samples of dandelion flowers, leaves or roots collected at the three different sites, suggesting the dried plants are safe for consumption in this respect. It is not surprising that unidentifiable traces of PAHs were detected in the soil from Rudenka since this is located in the oil-bearing flysch Carpathians, with a geological structure typical of young fold mountains, in which tectonic

engagement results in good hydrocarbon traps [58, 59]. The Rudenka site is about 20 km from shafts and oil and gas deposits that are currently being exploited.

Unfortunately, the chemical purity of the samples was not accompanied by equal biological purity: a total of 95 pathogenic bacterial species (26 anaerobes and 69 aerobes) were identified. It should be noted that while no anaerobic pathogenic bacteria were found in the plant samples collected in Rudenka, they were present in the plants collected in Warsaw. The *Propionibacterium acnes* (present name *Cutibacterium acnes*) anaerobic bacteria, believed to play a significant role in the pathogenesis of acne vulgaris and prosthetic joint infections [60, 61], were identified only in Warszawa 1 soil and Warszawa 2 flowers, i.e. in places with high population density. In contrast, anaerobic *Atopobium vaginae*, an important component of the complex abnormal vaginal flora in bacterial vaginosis [62, 63], was present in samples from all tested places. In our samples, the most common aerobic bacterial species were *Sphingomonas paucimobilis*, a rare infectious agent which may cause both nosocomial and community-acquired infections, albeit rarely [64, 65], *Bacillus cereus*, producing the heat-stable toxin cereulide known to cause food-borne illness [66, 67], and *Alloiococcus otitidis*, a common bacterium of the human ear; however, while it has been found in a high percentage of middle ear effusions in children, it is still not clear whether this bacterium really is a pathogen [68].

Most of the tested samples demonstrated *Staphylococcus pseudintermedius*, a canine opportunistic pathogen, one of the important human pathogens of zoonotic origin known to cause wound and skin infections [69, 70] and *Francisella tularensis* a highly infectious coccobacillus transmitted to humans by a number of different routes including ingestion of contaminated food [71, 72]. In addition, they were found to harbor *Staphylococcus lentus*, an animal pathogen isolated from rodents, chickens, mammals, farm soil and water, known to cause endocarditis septic shock, urinary tract infection, wound infections, endophthalmitis, and pelvic inflammatory disease in humans [73]. In contrast, *Pasteurella pneumotropica* responsible for rodent pasteurellosis known as a causative agent in infected humans of endocarditis, pneumonia, meningitis, osteomyelitis, arthritis, septicemia and epidural abscess [74, 75], was detected only in Rudenka flowers and soil, i.e. in an area with a large number of voles. Similarly, *Pasteurella canis*, known as a part of the normal flora of healthy domestic animals but a major pathogen of infections caused by dog bites [76, 77], was found only in dandelion roots collected at the Warszawa 1 site, where there is a large population of dogs and cats. Most disturbingly, the “carnivorous” *Vibrio vulnificus*, causing wound infections and septicemia [78, 79], *Fusobacterium mortiferum* and *Rahnella aquatilis* causing bacteremia [80, 81] were also identified in the Warsaw samples.

It is difficult to account for the presence of *Pasteurella testudinis*, which is a pathogen of desert turtles [82, 83], in the samples at all three sites, i.e. in the mountains and in Warsaw, because no turtles are present in these sites. A similar problem applies to *Aeromonas salmonicida* a fish pathogen causing a disease known as furunculosis [84, 85], as there are no breeding ponds in the vicinity of these sites; however, one report concerning isolation of this bacterium from human blood is available [86].

Herbal plants have been used in traditional medicine for centuries due to their ability to synthesize numerous antibacterial and antifungal compounds, which probably evolved as defenses against infection and/or predation [87]. Dandelion has long been known to have antimicrobial potential, and it has had a place in traditional medicine worldwide for centuries, being prescribed for malaria, tuberculosis, cough, and various bacterial infections [88, 89].

Research on the antimicrobial properties of dandelion has gained particular relevance in the face of antibiotic resistance and various extracts from this plant have been tested on bacterial and fungal strains affecting humans, animals and plants, both to confirm its traditional usage and expand its known uses [1]. The list of pathogens tested so far includes only five

bacterial species found in our samples: *Bacillus cereus*, *Bacillus pumilus*, *Escherichia coli*, *Chromobacterium violaceum* and *Propionibacterium acnes*. The test results were clearly positive only in the case of the two latter species: the extracts from *T. officinale* were active against these pathogens [90–95].

The tests carried out so far on pathogenic fungi only clearly indicate that the dandelion extracts inhibit *Alternaria alternata* [96, 97], while the results concerning *Aspergillus niger* and *Aspergillus fumigatus* were inconclusive [1, 94, 98–101]. The sensitivity of the other fungal species present in our samples to dandelion extracts remains unknown.

Assuming that no trace of mycelia development was observed in the samples, it is possible that fungal spore germination was halted, perhaps by the antifungal substances present in dandelion plants. This inhibition of spore germination and mycelium growth might explain our failure to find any of the 65 mycotoxins tested, despite the presence of significant amounts of spores of mycotoxin-producing fungi in the samples. It is known that *Aspergillus niger* produces fumonisin B2 and ochratoxin A [102–104], *Aspergillus fumigatus* produces fumagillin and gliotoxin [105–107], *Aspergillus terreus* produces territrems A and B [108], while *Alternaria alternata* produces alternariol, alternariol-9-methyl ether and tenuazonic acid [109]. It has also been known that trichothecene mycotoxins produced by *Stachybotrys chartarum*, the etiologic agent of stachybotryotoxicosis, can accumulate to very high levels in the conidia [110, 111]; however, it remains unclear whether the spores of the fungi present in our samples contained mycotoxins, and if so, whether they were present in detectable amounts. Further research is needed to address this problem.

Although the presence of protozoan cysts, helminth eggs and invasive stages of intestinal parasites on fruits and vegetables and in the soil is mainly associated with tropical countries [112–115], their presence has also been recorded in Poland [30, 116]. Disease-causing intestinal parasites are traditionally detected by microscopic examination of stool samples which is still considered the “gold standard” for the diagnosis; however, owing to their greater sensitivity and accuracy, molecular techniques such as conventional PCR, RT-PCR, nested PCR, LAMP and other modern technologies are being increasingly introduced into diagnostics with some success [117–122].

Research presented here was performed using multiplex PCR tests. The analysis itself employed self-made primers based on partial 18S rRNA species-specific sequences deposited in GenBank; these allow the detection of DNA from tapeworms, nematodes, flukes and *Giardia* species reported in Poland. The 18S rRNA sequences are widely used for primer development as they are highly polymorphic and conserved, allowing species-level separation, and have proven effective for establishing phylogenetic relatedness among species [119]. In the material, only the DNA of *Giardia intestinalis* was found in samples of dandelion leaves and roots taken from the Warszawa 2 stand; this area is often visited by walkers with dogs.

Traditionally, when thinking on herbal plant contamination, everyone is more focused on chemical than biological contaminations, however the progressive deindustrialization of Europe has significantly reduced the threats from the accumulation of heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and dioxins [122]. Increasing acreages of organic farming also have reduced the risks from the use of pesticides [123]. This is confirmed by this research, which showed a very high chemical purity of the collected plant samples and the soil directly surrounding the roots of dandelion. On the other hand, the risks associated with biological contamination of herbs, especially in densely populated territories and with a large population of domestic animals, are frequently underestimated. The discovery of dangerous bacteria *Vibrio vulnificus*, *Fusobacterium mortiferum* and *Rahnella aquatilis* as well as the protozoan parasite *Giardia intestinalis* in tested samples is highly disturbing. These hazardous pathogens have been detected in the recreational area and in the vicinity of single-family

housing estates in Warsaw, the capital of Poland, a metropolis with a population of nearly two million.

Conclusions

A broad analysis of chemical and biological contaminations was performed on soil and dandelion samples using a wide range of analytical methods (GC-MS, LC-MS, voltammetry, microbiological and microscopic techniques, PCR). The findings provide the first comprehensive safety analysis of a herbal product. The presented analysis of the heavy metal content and the presence of other chemical pollutants (PAHs, PCBs, dioxins, pesticides, mycotoxins) indicate that all collected soil and dandelion samples were of high chemical purity. In turn, biological analyses showed the presence of 95 species of pathogenic bacteria, 14 species of pathogenic fungi and one protozoan parasite (*Giardia intestinalis*); this underlines the need for increased caution when collecting dandelion in densely populated areas with a large population of pets. Thorough washing of the harvested plants is necessary before using them for consumption, especially in the case of making salads from fresh dandelion leaves, which is becoming increasingly popular among people leading healthy and an environmentally friendly lifestyle. The use of heat treatment when preparing traditional infusions and flower preserves should significantly reduce any risk of infection.

Materials and methods

Plant and soil sample collection

Herbs were collected from two locations in Warsaw: Warszawa 1 (52.173292, 21.153900) situated in area of heavy traffic without any industry, Warszawa 2 (52.218959, 21.098110) in a recreation area with traffic near to the coal-fired Siekierki heat and power plant with a thermal power of 2068 MW and an electric power of 620 MW. A third set of samples was also taken from Rudenka (49.486555, 22.412092), part of the Natura 2000 European Ecological Network, located in a sparsely-populated region 30 km from the Polish-Ukrainian border. The sample site itself is a meadow which has lain fallow for over 30 years, during which time, it has not been subjected to any agrotechnical treatments nor used for breeding livestock. No permits were required for the collection of samples because the two collection sites in Warsaw (Warszawa 1 and Warszawa 2) are open to everyone without any restrictions and the third collection site in Rudenka is located on the private property of the corresponding author of this work (MIB). Whole *T. officinale* plants were harvested in May 2018 just after flowering began. The samples were divided into parts (flowers, leaves, roots) and air dried in a cool, shaded and airy room. The soil surrounding the dandelion roots was also collected and air dried.

Preparation of soil samples for pH determination

The soil samples were shredded in a mortar. Following this, 5 cm³ of the sample was placed in a 50 ml beaker and 25 ml of 1M KCl solution was added. The suspension was mixed thoroughly and left until the next day, while protected from evaporation. The next day, the suspension was mixed again and the pH measured using a pH meter.

Heavy metal determination

Heavy metal determination was performed in mineralized plant and soil samples using voltamperometry methods. Voltammetric measurements were performed using a MVA-22-894 Professional VA Automated Analyzer (Metrohm AG Ltd, Switzerland) with a three-electrode system consisting of a hanging mercury drop electrode (HMDE) as a working electrode, a

platinum (Pt) auxiliary electrode and an Ag/AgCl/ KCl (3 mol/l) reference electrode. Only ultrapure and Suprapur chemicals were used.

Plant and soil sample preparation. Accurately-weighed (circa 250 mg) air-dried *T. officinale* samples (flowers, leaves, roots) were shredded in a mortar, put into ceramic crucibles and mineralized in a muffle furnace according to the following time-temperature program: 60 min initial charring at 150°C, 120 min burning at 300°C, and 240 min combustion at 450°C. After cooling the oven, the crucibles were transferred to an exsiccator, 2 ml HNO₃ (65%) was added to each sample and allowed to evaporate slowly at 120°C. The samples were then subjected to 60 min calcination on a hot plate at 450°C. Following this, 1 ml HNO₃ (65%) was added to the residue, and the mixture was subjected to slow evaporation at 120°C and calcining (450°C, 60 min). These operations were repeated until a light gray, easily digestible ash was obtained. The solid was then dissolved in 2 ml HCl (10%), transferred to 100 ml flasks and made up to the mark with ultra-pure water.

Accurately-weighed (circa 1 g) air-dried soil samples were shredded in a mortar and put into ceramic crucibles for mineralization. Following this, 7.5 ml HCl (30%) was added, followed by 2.5 ml HNO₃ (65%). In parallel, two blank tests were prepared with only acids in the crucibles. The next day, the samples were mineralized for three hours, initially at 100°C then gradually increasing to 200°C. When 30% of the acid mixture evaporated, the crucibles were allowed to cool and then supplemented with a 3:1 mixture of HCl (30%) and HNO₃ (65%). The extracts were transferred to 50 ml volumetric flasks, made up to the mark with ultra-pure water, then filtered through PTFE syringe filters (0.45 µm, FilterBio) into 100 ml flasks and made up to the mark with ultra-pure water. Suprapur acids were from Merck. Ultra-pure water was obtained from a Milli-Q Pure Water System.

Determination of zinc, cadmium, lead, copper and thallium content by anodic stripping voltammetry (ASV) at hanging mercury dropping electrode (HMDE). ASV is a two-step measurement. In the first step, the metal ions present in the test solution are deposited on the surface of a mercury electrode (amalgamation) at a deposition potential range of -1.15 V to -0.8 V. In the second step, all the deposited ions are anodically stripped by scanning the potential range from -1.15 to +0.1 V. All the measurements were performed by standard addition, in which the sample was first taken into the polarographic vessel and then the current was measured. After the addition of 0.1 ml of standard, the procedure was repeated two times and the current was measured. After all the measurements, an extrapolation curve was plotted between current versus concentration, and this was used to indicate the amount of metals present in the sample solution.

The Zn, Cd, Pb and Cu levels were determined using KCl-acetate buffer (pH 4.6) with a scan rate of 59.5 mV/s and pulse amplitude 50mV by HMDE standard addition. The solution was stirred during pre-electrolysis at -1.15 V (vs. Ag/AgCl) for 90 seconds and the potential was scanned from -1.15 V to +0.1 V (vs. Ag/AgCl). The KCl-acetate buffer was prepared from 5.59 g of Suprapur KCl, 2.5 ml of Suprapur acetic acid (100%), 10 ml of ultrapure water to which 2.5 ml of Suprapur ammonia (25%) was added slowly (to prevent heat generation) and the 50 ml volumetric flask was made up to the mark with ultra-pure water. Standard solution consisted of zinc (4 mg/l), cadmium (0.5 mg/l), lead (0.5 mg/l), and copper (2.5 mg/l) standards (all from Chempur) and was prepared from stock solutions of 1 g/l. All other reagents were from Merck.

Measurements: 2 ml of sample, 8 ml ultra-pure water and 1 ml of KCl-acetate buffer (pH 4.6) were placed in a polarographic vessel and then the measurement was started under the given parameters. After the sample voltammogram was recorded, 0.1 ml of mixed standard (Zn, Cu, Cd and Pb) was added twice and then the voltammogram of the standard was recorded. Operating parameters: working electrode HMDE, stirrer speed 2000 cm⁻¹,

deposition potential -1.15 V, deposition time 90 s, pulse amplitude 0.05 V, start potential -1.15 V, end potential 0.05 V, voltage step 0.006 V, voltage step time 0.1 s, sweep rate 0.06 V/s, Zn peak potential—0.98 V, Cd peak potential—0.56 V, Pb peak potential—0.38 V, Cu peak potential—0.10 V.

Thallium content was determined using ethylenediaminetetraacetic acid (EDTA) solution. To a 25 ml volumetric flask, 0.93g of EDTA (Merck) was added and dissolved in ultra-pure water. A standard solution of thallium (0.5 mg/l) was prepared from a 1g/l stock solution (Inorganic Ventures).

Measurements: 5 ml of sample, 5 ml ultra-pure water, 0.2 ml EDTA solution and 1 ml of KCl-acetate buffer were placed in a polarographic vessel. After the sample voltammogram was recorded, 0.1 ml of thallium standard was added twice, and the voltammogram of the standard was recorded. Operating parameters: working electrode HMDE, stirrer speed 2000 cm^{-1} , deposition potential -0.8 V, deposition time 180 s, pulse amplitude 0.05 V, start potential -0.8 V, end potential -0.2 V, voltage step 0.006 V, voltage step time 0.1 s, sweep rate 0.06 V/s, Tl peak potential -0.45 V.

Determination of nickel and cobalt content by adsorptive cathodic stripping voltammetry (AdCSV) at hanging mercury dropping electrode (HMDE). The supporting electrolyte (ammonia buffer) was prepared by slowly adding 11.25 ml of Suprapur ammonia (25%) to 5.3 ml of Suprapur hydrochloric acid (30%) and 10 ml of ultra-pure water into a 50 ml volumetric flask. The level was made up to the 50ml mark with ultra-pure water. Dimethylglyoxime (DMG) solution was prepared by dissolving 0.76 g of DMG in 25 ml of ultra-pure water. The standard solution consisted of nickel (1 mg/l) and cobalt (0.5 mg/l), both from Chempur, prepared from stock solutions of 1g/l. All other reagents were from Merck from Merck.

Measurements: 0.5 ml of sample, 0.1 ml DMG solution, 9.5 ml ultra-pure water and 1 ml of ammonia buffer were added to a polarographic vessel and the measurement was started under the given parameters. After the sample voltammogram was recorded, 0.1 ml of mixed standard (Co and Ni) was added twice and then voltammogram of the standard was recorded.

The following operating parameters were used: working electrode HMDE, stirrer speed 2000 cm^{-1} , deposition potential -700 mV, deposition time 90 s, pulse amplitude 0.05 V, start potential -0.6 V, end potential -1.25 V, voltage step 0.004 V, voltage step time 0.3 s, sweep rate 0.013 V/s, Co peak potential—1.13 V, Ni peak potential—0.97 V.

Detection of PAHs, PBCs, dioxins and pesticides

Extraction of comminuted, air-dried soil and plant samples was performed three times (each 15 minutes) in an ultrasonic bath with a mixture of dichloromethane and acetone (9:1, v/v). The obtained extracts were pooled, dried over anhydrous sodium sulfate and then concentrated at 30°C in a rotary evaporator. About 1 mg of each extract was dissolved in 1 ml petroleum ether and uploaded to silica gel column (MN-Kieselgel 60, 1 cm x 6 cm); the PAHs and PCBs were separated into two fractions by elution with 8 and 4 ml of petroleum ether, respectively. The eluted PAH and PCB fractions were evaporated; about 1mg of each extract was dissolved in 100 μl of dichloromethane and analyzed by GC-MS.

Three calibration curves were made for each of 18 PAH, 12 PCB, 17 dioxin and 18 pesticide standards (S1 Table) to determine their retention times (RT), detection limits (LOD), precision and accuracy of the analyses by calculating the standard deviation, absolute and relative errors. Identification of individual compounds was made on the basis of characteristic mass spectra and NIST 11 library.

The analyses were carried out on a GCMS-QP2010 with a mass detector (Shimadzu). As a carrier gas, helium was used at a column head pressure of 65.2 kPa, with a DB-5 MS column

(Zebron, Phenomenex): thickness 0.25 μm , length 30 m, diameter 0.25 μm . The column oven temperature cycle started from 150°C; this temperature was held for three minutes then ramped to 310°C at 4°C/min; the final temperature was then held for 10 minutes. The ion source temperature was 200°C and the interface temperature was 310°C. Ionization 70 eV. The injection mode was split, and the split ratio was 10. Injection volume 1 μl . Quantitative determinations of the PAHs, PCBs, dioxins and pesticides in the plant and soil samples were made using the internal standard method. 2-methylanthracene was used as an internal standard for the determination of PAHs, and cholestane for the determination of other compounds. All standards, solvents and reagents were from Merck.

Detection of mycotoxins

Accurately weighed samples (c.a. 0.5 g each, [S2 Table](#)) were mixed with 5 ml acetonitrile/water mixture (3:1 volume ratio), shaken for 40 minutes on a shaker at 250 RPM and centrifuged for 30 minutes at 3000 x g. Supernatants were twice degreased with 4 ml of hexane (15 min shaking) and water was removed from the collected acetonitrile/water phases by the addition of 0.2 g NaCl. The collected solutions, evaporated at 70°C, were dissolved in 15 ml of chloroform/methanol mixture (9:1 volume ratio), sonicated and centrifuged (30 min, 3000 x g). The collected chloroform phases were dried under a nitrogen stream and re-dissolved in 2 ml of methanol or acetonitrile. They were then subjected to LC-MS analysis using LC-MS grade solvents from Merck. Prior to injection on the LC-MS system, the samples were filtered through a 4 mm, 0.2 μm polytetrafluoroethylene (PTFE) syringe filter (FilterBio).

Standards of 65 mycotoxins were purchased via the Axxora platform (<http://www.axxora.com>). The mycotoxins were separated by reverse-phase chromatography using a Shimadzu LC-MS 2020 system. The separation was achieved with a Kinetex EVO C18 column (2.6 μm particle size) preceded by a SecurityGuard™ ULTRA Holder pre-column, both supplied by Phenomenex. The applied mobile phase, ionization mode, characteristic ions and retention times of the mycotoxins are presented in [S3 Table](#), while selected-ion monitoring (SIM) chromatograms of mycotoxins' standards are shown in [S1 Fig](#). The following analysis parameters were used: injection volume 5 μl , flow 0.2 ml/min, detector voltage 4.5 kV, nebulizing gas flow 1.5 L/min, drying gas flow 15 L/min, scan speed 1500 u/sec, DL temperature 250 °C, heat block 250 °C, oven temperature 40 °C.

Microbiological analyses

Accurately-weighed samples (circa 1 g for bacteriological analysis and circa 3 g for mycological analysis, each in three independent replications) were flooded with 100 ml of sterile 0.85% NaCl, shaken for 20 minutes at 25°C, and then 250 μl portions of each extract were sterile plated onto Columbia Agar plates with 5% sheep blood (CASB) and Sabouraud Glucose Selective Agar plates (SGSA), both from Becton Dickinson. Half of the inoculated CASB plates were kept aerobically, while the second half was kept anaerobically using the anaerobic jar (Roth) and AnaeroGen™ sachets (Thermo Scientific). Each inoculation variant was performed four times. All CASB plates were incubated at 37°C for 24 hours. Following this, the number of colony forming units (CFU) was determined using a SCAN 500 automatic bacterial colony counter (Interscience). Individual bacterial colonies were picked separately from each CASB plate, sieved onto new sterile CASB plates and cultured in the same way as the plates from which they were taken. After 24 hours of incubation, the bacteria were Gram stained (Gram Stain Kit, Thermo Scientific) and examined under a microscope to distinguish gram-positive and gram-negative colonies and bacterial morphology; this was a prerequisite for strain identification using an automated Vitek-2 system (Biomérieux). The procedure was repeated until

complete homogeneity of the bacterial cultures was achieved. Homogeneous bacterial colonies were applied to correctly-selected Vitek-2 disposable cards (Biomérieux) according to the producer's instructions; this allowed the identification of over 350 clinically-relevant bacteria and yeast.

Inoculated SGSA plates were kept at 25°C for one to four weeks; following this, the cfus were counted. In order to obtain homogeneous fungal cultures, passages of the originally isolated colonies were performed several times. Fungi were identified macroscopically, based on the morphology of the grown colonies, and microscopically, based on preparations from isolated fungal colonies.

The Gram-stained bacteria and unstained fungi were subjected to phase-contrast microscope examination using a Axio Vert. A1 fluorescence inverted microscope (Zeiss) equipped with Axio Cam ICc 5 camera (Zeiss) and Zen lite 2012 software (Zeiss).

Parasitological analyses

The soil and dandelion samples were checked for the presence of parasite eggs by the flotation technique [124]. Due to the wide range of the parasites included in the study, i.e. comprising nematodes, tapeworms, flukes and protozoa, a multiplex PCR assay was used to detect them: the test was based on the amplification of a short (250 bp) 18S rDNA fragment shared by flukes, nematodes, tapeworms and protozoa (S4 Table).

DNA extraction and amplification. DNA was extracted individually from air dried dandelion and soil samples using a Stool DNA Purification Kit (EURx, Poland) according to the manufacturer's protocol. The dried samples were rehydrated with MiliQ water and then the DNA was isolated according to the manufacturer's protocol.

Helminth DNA (positive control samples) was isolated from adult worms (*Toxocara cati*, *Toxocara canis*, *Echinococcus* spp., *Dipylidium caninum*, *Taenia* spp., *Fasciola hepatica*) using a Blood and Tissue DNA isolation kit (Qiagen) according to the manufacturer's protocol. DNA from the eggs of *Trichuris vulpis* (fox), *Toxocara cati* (cat), *Toxocara canis* (dog) and *Oxyuridae* sp. (turtle) was isolated using a Stool DNA Purification Kit (EURx, Poland). All DNA amplifications were performed using the DNA Engine T100 Thermal Cycler (BioRad). In some cases, nuclease-free water was added to the PCR mix instead of the tested DNA as a negative control. The PCR products were then visualized on a 1.2% agarose gel (Promega) stained with SimplySafe (EURx). Visualization was performed using ChemiDoc, MP Lab software (Imagine, BioRad).

Helminth test. 18S rDNA sequences of *Dipylidium caninum*, *Hymenolepis nana*, *Taenia solium*, *Taenia saginata*, *Taenia taeniaeformis*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Fasciola hepatica*, *Fascioloides magna*, *Ascaris lumbricoides*, *Ascaris suum*, *Enterobius vermicularis*, *Toxocara canis*, *Toxocara cati*, *Trichuris trichiura*, *Trichuris vulpis* were used to design a six-primer set. Four 18S rDNA sequences of *Giardia intestinalis* were used to design PCR primers. Detailed data on the parasites and 18S rDNA sequences are presented in S4 Table. Partial regions (250bp) of 18S rDNA of all tested helminths were amplified using the following sets of primers: CF1 (5' GCGGGGRCGTTTGTATGGCTGC 3'), NF1 (5' ACGGGGRCATTTCGTATyGCTGC 3'), TtF1 (5' GCGGGGACGTTTGTATGGTTGCG 3'), FhF1 (5' ACGGGGGCATTTCGTATGGCGGT 3'), CNR1 (5' CAACCATACTTCCCCCGGAACCSAAA 3') and TR1 (5' CCATACTTCCCCCGGAGCCCAA 3'). PCR was performed in a TM 100 (BioRad) thermal cycler. The reactions were conducted in a 50 µl reaction mixture containing 5.0 µl of DNA template, 1 µl (1U) of Color Taq DNA Polymerase (EURx), 1 µl of dNTPs mix (10 mM), 0.5 µl of TR1, TtF1 and FhF1, 1 µl of CF1 and CNR1, 1.5 µl of NF1 primer (20 mM), 5 µl of 10 × Polymerase buffer (pH 8.6, 25 mM MgCl₂) and 33.0 µl of MiliQ water. A negative

control—nuclease-free water was added to the PCR mix instead of the tested DNA. DNA amplification was performed according to the following program: denaturation at 95°C for 1 min, followed by 34 cycles of denaturation at 95°C for 10 s, annealing at 58°C for 10 s and extension at 72°C for 20 s, with a final extension performed at 72°C for 3 min.

Giardia test. Partial regions of 18S rDNA were amplified using the following sets of primers: GF1 (5' TCCGGTCGATCCTGCCGGA 3') and G452R (5' GCTGCTGGCACCAGACCTTG 3'). PCR was performed in a TM 100 (BioRad) thermal cycler. The reactions were conducted in a 50 µl reaction mixture containing 5.0 µl template DNA, 1.0 µl (1U/ µl) of Color Taq DNA Polymerase (EURx), 1 µl of dNTPs mix (10 mM), 0.5 µl of GF1 and G452R primer (20 mM), 5 µl of 10 × Polymerase buffer (pH 8.6, 25 mM MgCl₂) and 37.0 µl of MiliQ water. A negative control—nuclease free water was added to the PCR mix instead of the tested DNA. DNA amplification was performed using the DNA Engine T100 Thermal Cycler (BioRad) according to the following program: denaturation at 95°C for 1 min, followed by 34 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 15 s and extension at 72°C for 30 s, with a final extension performed at 72°C for 3 min.

DNA sequencing. Giardia positive dandelion samples were used to amplify the triosephosphate isomerase (TPI) gene fragment using the tpi nested PCR protocol [125]. The selected PCR products were purified with QIAquick Gel Extraction Kit (Qiagen, Germany) and then were sequenced directly using ABI BigDye™ chemistry (Applied Biosystems, USA) on an ABI Prism 373xl or an ABI Prism 3100™ automated sequencer (Genomed, Warsaw, Poland). The results were compared with relevant sequences from the GenBank database.

Statistical analyses

The results of the voltamperometric measurements and the cfu determinations were subjected to statistical analysis. The normality of their distribution was checked using the Kolmogorov-Smirnov (K-S) test. As the distributions were normal, the Student's t-test was used to compare them. A significance level of 95% was assumed ($p < 0.05$). STATISTICA 6.1 software (StatSoft Polska) was used for all statistical testing.

Supporting information

S1 Fig. Selected-ion monitoring chromatograms of mycotoxins' standards.
(TIF)

S1 Table. List of standards used, their retention times (RT) and limits of detection (LOD).
(DOCX)

S2 Table. Extraction of mycotoxins from *Taraxacum officinale* and soil samples.
(DOCX)

S3 Table. Summary of LC-MS detection conditions of 65 mycotoxins.
(DOCX)

S4 Table. List of 18S rDNA sequences used to design PCR reaction primers (1–21) and parasites used as control samples (22–35).
(DOCX)

S5 Table. Correlations between the concentrations of metals measured in individual parts of plants and soil samples.
(XLSX)

S6 Table. Raw data: (A) concentration of heavy metals, (B) soil pH.
(XLSX)

S7 Table. Raw data: Microorganisms isolated from *Taraxacum officinale* and soil.
(XLSX)

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References

1. Valenzuela MEM, Peralta KD, Martínez L, Maggi RC. *Taraxacum* genus: potential antibacterial and antifungal activity. In: Builders PF, editor. *Herbal Medicine* [Internet]. London: IntechOpen. 2018. <https://www.intechopen.com/chapters/58339>.
2. Li Y, Mei J, Wang J, Liu H. Effects of dandelion (*Taraxacum* sp.) supplements on lactation performance, antioxidative activity, and plasma metabolome in primiparous dairy cows. *Animal Bioscience* 2023; 36(2):229–237. <https://doi.org/10.5713/ab.22.0061> PMID: 36108692
3. Mao J, Wang Y, Wang W, Duan T, Yin N, Guo T, et al. Effects of *Taraxacum mongolicum* Hand.-Mazz. (dandelion) on growth performance, expression of genes coding for tight junction protein and mucin, microbiota composition and short chain fatty acids in ileum of broiler chickens. *BMC Vet Res* 2022; 18:180. <https://doi.org/10.1186/s12917-022-03278-5> PMID: 35568942
4. Kania-Dobrowolska M.; Baraniak J. Dandelion (*Taraxacum officinale* L.) as a source of biologically active compounds supporting the therapy of co-existing diseases in metabolic syndrome. *Foods* 2022; 11: 2858. <https://doi.org/10.3390/foods11182858> PMID: 36140985
5. Li Y, Chen Y, Sun-Waterhouse D. The potential of dandelion in the fight against gastrointestinal diseases: A review. *J Ethnopharmacol.* 2022 Jul 15; 293:115272. <https://doi.org/10.1016/j.jep.2022.115272> PMID: 35405251
6. Xie Z, Finley JW. Herbs and Spices. In: *Principles of Food Chemistry*. Food Science Text Series. Springer, Cham. 2018.
7. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues 2007; pp.1–105). Geneva, Switzerland.
8. Holland J, Rose M, Petch S. Occurrence of polycyclic aromatic hydrocarbons in herbs, spices, supplements and tea. Report to Food Standards Agency. 2015. <https://www.food.gov.uk/sites/default/files/media/document/pahs-in-herbs-spices-supplements-and-tea-final-report.pdf>

9. Hwang HJ, Lee SH, Kim YY, Shin HS. Polycyclic aromatic hydrocarbon risk assessment and analytical methods using QuEChERS pretreatment for the evaluation of herbal medicine ingredients in Korea. *Foods* 2021; 10(9):2200. <https://doi.org/10.3390/foods10092200> PMID: 34574309
10. Nkansah MA, Hayford ST, Borquaye LS, Ephraim JH. Heavy metal contents of some medicinal herbs from Kumasi, Ghana. *Cogent Environmental Science* 2016; 2:1. <https://doi.org/10.1080/23311843.2016.1234660>
11. Rivera-Pérez A, Romero-González R, Garrido Frenich A. Persistent organic pollutants (PCBs and PCDD/Fs), PAHs, and plasticizers in spices, herbs, and tea—A review of chromatographic methods from the last decade (2010–2020). *Crit Rev Food Sci Nutr* 2021; 10:1–21. <https://doi.org/10.1080/10408398.2021.1883546> PMID: 33563047
12. Shaban NS, Abdou KA, Hassan NEHY. Impact of toxic heavy metals and pesticide residues in herbal products. *Beni-Suef Univ. J. Basic Appl. Sci* 2016; 5:102–106.
13. Tripathy V, Basak BB, Varghese TS, Saha A. Residues and contaminants in medicinal herbs—A review. *Phytochem Lett* 2015; 14:67–78.
14. Van Asselt ED, Banach JL, van der Fels-Klerx JJ. Prioritization of chemical hazards in spices and herbs for European monitoring programs. *Food Control* 2018; 83:7–17.
15. Kowalska G. Pesticide Residues in Some Polish Herbs. *Agriculture* 2020; 10:154; <https://doi.org/10.3390/agriculture10050154>
16. Aiko V, Mehta A. Prevalence of toxigenic fungi in common medicinal herbs and spices in India. *3 Bio-tech*. 2016; 159 <https://doi.org/10.1007/s13205-016-0476-9> PMID: 28330231
17. Altyn I, Twaruzek M. Mycotoxin Contamination Concerns of Herbs and Medicinal Plants. *Toxins* 2020; 12(3):182. <https://doi.org/10.3390/toxins12030182> PMID: 32183391
18. Kabak B, Dobson AD. Mycotoxins in spices and herbs—an update. *Crit Rev Food Sci Nutr* 2017; 57(1):18–34. <https://doi.org/10.1080/10408398.2013.772891> PMID: 26528824
19. Reinholds I, Pugajeva I, Bavrins K, Kuckovska G, Bartkevics V. Mycotoxins, pesticides and toxic metals in commercial spices and herbs. *Food Addit Contam Part B* 2017; 10:5–14. <https://doi.org/10.1080/19393210.2016.1210244> PMID: 27397646
20. de Sousa Lima CM, Fujishima MAT, de Paula Lima B, Mastroianni PC, de Sousa FFO, da Silva JO. Microbial contamination in herbal medicines: a serious health hazard to elderly consumers. *BMC Complement Med Ther* 2020; 20:17. <https://doi.org/10.1186/s12906-019-2723-1> PMID: 32020854
21. Mathot AG, Postollec F, Leguerinel I. Bacterial spores in spices and dried herbs: The risks for processed food. *Compr Rev Food Sci Food Saf* 2021; 20(1):840–862. <https://doi.org/10.1111/1541-4337.12690> PMID: 33325134
22. Jay-Russell MT. What is the risk from wild animals in food-borne pathogen contamination of plants? *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2013; 8, (40). <https://doi.org/10.1079/PAVSNNR20138040>
23. Kłapeć T, Cholewa A. Zagrożenia dla zdrowia związane ze stosowaniem nawozów organicznych i organiczno-mineralnych. *Med Og Nauk Zdr* 2012; 18(2):131–136.
24. Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=LEGISSUM:l28088>.
25. Zdybel J, Karamon J, Dąbrowska J, Różycki M, Bilska-Zajac E, Kłapeć T, et al. Parasitological contamination with eggs *Ascaris spp.*, *Trichuris spp.* and *Toxocara spp.* of dehydrated municipal sewage sludge in Poland. *Environmental Pollution* 2019; 248:621–626.
26. Jansen F, Dorny P, Gabriël S, Dermauw V, Vang Johansen M, Trevisan C. The survival and dispersal of *Taenia* eggs in the environment: what are the implications for transmission? A systematic review. *Parasites & Vectors* 2021; 14: 88. <https://doi.org/10.1186/s13071-021-04589-6> PMID: 33514421
27. O'Donnell CJ, Meyer KB, Jones JV, Benton T, Kaneshiro ES, Nichols JS, et al. Survival of parasite eggs upon storage in sludge. *Appl Environ Microbiol* 1984; 48(3): 618–625. <https://doi.org/10.1128/aem.48.3.618-625.1984> PMID: 6541889
28. Hodge JM, Coghill AE, Kim Y, Bender N, Smith-Warner SA, Gapstur S, et al. *Toxoplasma gondii* infection and the risk of adult glioma in two prospective studies. *Int J Cancer* 2021; 11. <https://doi.org/10.1002/ijc.33443> PMID: 33427315
29. Machicado C, Marcos LA. Carcinogenesis associated with parasites other than *Schistosoma*, *Opisthorchis* and *Clonorchis*: A systematic review. *Int. J. Cancer* 2016; 138(12):2915–2921. <https://doi.org/10.1002/ijc.30028> PMID: 26840624
30. Lass A, Szostakowska B, Myjak P, Korzeniewski K. Detection of *Echinococcus multilocularis* DNA in fruit, vegetable, and mushroom samples collected in the non-endemic territory of the Pomerania province and comparison of the results with data from rural areas of the neighbouring highly endemic

- Warmia-Masuria province, Poland. *Acta Parasitol* 2017; 62(2):459–465. <https://doi.org/10.1515/ap-2017-0053> PMID: 28426415
31. Bojnansky V, Fargasova A. Atlas of seeds and fruits of central and east-european flora: The Carpatian Mountains Region. Springer; 2007.
 32. Stewart-Wade S, Neumann S, Collins L, Boland G. The biology of Canadian weeds. 117. *Taraxacum officinale* G.H. Weber ex Wiggers. *Canadian Journal Plant Science* 2002; 82:825–853. <https://doi.org/10.4141/P01-010>
 33. Craymer L. Dandelions ruin your front yard but could be the future of the rubber industry. *Wall Street Journal* 2018. <https://www.wsj.com/articles/scientists-keep-trying-and-failing-to-grow-dandelions-1538503984>.
 34. Van Beilen JB, Poirier Y. Guayule and Russian dandelions as alternative sources of natural rubber. *Critical Reviews in Biotechnology* 2007; 27(4):217–31. <https://doi.org/10.1080/07388550701775927> PMID: 18085463
 35. Girard M, Chagnon M, Fournier V. Pollen diversity collected by honey bees in the vicinity of *Vaccinium* spp. crops and its importance for colony development. *Botany* 2012; 90(7):545–555. <https://doi.org/10.1139/b2012-049>
 36. Wirngo FE, Lambert MN, Jeppesen PB. The physiological effects of dandelion (*Taraxacum officinale*) in type 2 diabetes. *Rev Diabet Stud* 2016; 13(2–3):113–131. <https://doi.org/10.1900/RDS.2016.13.113> PMID: 28012278
 37. Rehman G, Hamayun M, Iqbal A, Khan SA, Khan H, Shehzad A, et al. Effect of methanolic extract of dandelion roots on cancer cell lines and AMP-activated protein kinase pathway. *Front. Pharmacol* 2017; 8:875. <https://doi.org/10.3389/fphar.2017.00875> PMID: 29234282
 38. Rashidi I, Rashtiani N, Pazhouhi M, Ghanbari A, Zhaleh M, Jalili C. Anti-invasion and anti-metastasis effects of dandelion (*Taraxacum officinale*) hydroalcoholic extract on glioblastoma multiforme cell line model. *WCRJ* 2022; 9: e2361. https://doi.org/10.32113/wcrj_20227_2361
 39. Puga TB, Schafer J, Tellis PL, Mosqueda AN, Agbedanu PN. Antimicrobial activity of *Taraxacum officinale* against *Enterobacter cloacae*. *Int J Herb Med* 2022; 10(2):44–46; <https://doi.org/10.22271/flora.2022.v10.i2a.813>
 40. Malawska M, Witkomirski B. Soil and plant contamination with heavy metals in the area of the old railway junction Tarnowskie Góry and near two main railway routes. *Roczn. PZH* 2000; 51(3):259–267.
 41. Malawska M, Witkomirski B. Accumulation rate of polychlorinated biphenyls (PCBs) in dandelion (*Taraxacum officinale*) in the conditions of soil contamination with oil derivatives. *Rocz Panstw Zakl Hig.* 2001; 52(4):295–311. PMID: 11878011.
 42. Muntean E, Muntean N. Polycyclic aromatic hydrocarbons in commercial herbal teas. MDPI in The 1st International Electronic Conference on Plant Science session Phytochemistry and Bioremediation. 2020.
 43. Santos L, Marín S, Sanchis V, Ramos AJ. Screening of mycotoxin multicontamination in medicinal and aromatic herbs sampled in Spain. *J. Sci. Food Agric* 2009; 89:1802–1807. <https://doi.org/10.1002/jsfa.3647>
 44. Kalvoda R, Kopanica M. Adsorptive stripping voltammetry in trace analysis. *Pure & Appl. Chem* 1989; 61(1): 97–112.
 45. Scholz F. Voltammetric techniques of analysis: the essentials. *ChemTexts* 2015; 1:17. <https://doi.org/10.1007/s40828-015-0016-y>
 46. Wang Y, Kaliyaray A, Kumar S, Compton RG. Optimising adsorptive stripping voltammetry: strategies and limitations. *ChemElectroChem* 2021; 8(12):2343–2352. <https://doi.org/10.1002/celec.202100679>
 47. Kicińska A. The application of *Taraxacum officinale* for monitoring of soil pollution in mountain areas—preliminary report. *Acta Environmentalica Universitatis Comenianae (Bratislava)* 2011; 19, Supplement:158–163.
 48. Królak E. Accumulation of Zn, Cu, Pb and Cd by Dandelion (*Taraxacum officinale* Web.) in Environments with Various Degrees of Metallic Contamination. *Polish Journal of Environmental Studies* 2003; 12(6):713–721.
 49. Królak E, Marciniuk J, Popijantus K, Wasilczuk P, Kasprzykowski Z. Environmental Factors Determining the Accumulation of Metals: Cu, Zn, Mn and Fe in Tissues of *Taraxacum* sp. sect. *Taraxacum*. *Bulletin of Environmental Contamination and Toxicology* 2018; 101:68–74.
 50. Ligocki M, Tarasewicz Z, Zygmunt A, Aniśko M. The common dandelion (*Taraxacum officinale*) as an indicator of anthropogenic toxic metal pollution of environment. *Acta Sci Pol Zootechnica* 2011; 10(4):73–82.

51. Adamczyk-Szabela D, Wolf WM. The impact of soil pH on heavy metals uptake and photosynthesis efficiency in *Melissa officinalis*, *Taraxacum officinale*, *Ocimum basilicum*. *Molecules* 2022; 27:4671. <https://doi.org/10.3390/molecules27154671> PMID: 35897849
52. Habh AMM, Idan RM, Khudhaier SR, Benayed SH, AL-Jaleel Khalil MA. *Taraxacum officinale* contamination of heavy metals (Iron, Copper). *Plant Archives* 2018; 18(1):652–654.
53. Minister of Agriculture and Rural Development. Decree on permissible concentrations of heavy metals polluting the soil. 2002. <https://www.prawo.pl/akty/dz-u-2002-37-344,16953518.html>.
54. Rostański A. Zawartość metali ciężkich w glebie i roślinach z otoczenia niektórych emiterów zanieczyszczeń na Górnym Śląsku. *Arch Ochr Środ* 1997; 23:181.
55. Giacomino A, Malandrino M, Colombo ML, Miaglia S, Maimone P, Blancato S, et al. Metal content in dandelion (*Taraxacum officinale*) leaves: influence of vehicular traffic and safety upon consumption as food. *J Chem* 2016;1–9. <https://doi.org/10.1155/2016/9842987>
56. Kajka K, Rutkowska B. Accumulation of selected heavy metals in soils and common dandelion (*Taraxacum officinale*) near a road with high traffic intensity. *Soil Science Annual* 2018; 69(1):11–16. <https://doi.org/10.2478/ssa-2018-0002>
57. Dudka S, Piotrowska M, Chłopecka A. Effect of elevated concentrations of Cd and Zn in soil on spring wheat yield and the metal content of the plants. *Water, Air Soil Pollut* 1994; 76:333–341.
58. Depowski S. Obszary gazonośne i roponośne Polski. *Przegląd Geologiczny* 1981; 29 (5):209–217.
59. Malata T. Development of Polish Flysch Carpathians revealed in outcrops and landscape. *Przegląd Geologiczny* 2008; 56 (8):688–691.
60. Mollerup S, Friis-Nielsen J, Vinner L, Hansen TA, Richter SR, Fridholm H, et al. *Propionibacterium acnes*: Disease-causing agent or common contaminant? Detection in diverse patient samples by next-generation sequencing. *Journal of Clinical Microbiology* 2016; 54(4), <https://doi.org/10.1128/JCM.02723-15> PMID: 26818667
61. Salar-Vidal L, Aguilera-Correa JJ, Brüggemann H, Achermann Y, Esteban J. Microbiological characterization of *Cutibacterium acnes* strains isolated from prosthetic joint infections. *Antibiotics* 2022; 11:1260. <https://doi.org/10.3390/antibiotics11091260> PMID: 36140039
62. Mendling W, Palmeira-de-Oliveira A, Biber S, Prasauskas V. An update on the role of *Atopobium vaginæ* in bacterial vaginosis: what to consider when choosing a treatment? A mini review. *Arch Gynecol Obstet* 2019; 300(1):1–6. <https://doi.org/10.1007/s00404-019-05142-8> PMID: 30953190
63. Mollin A, Katta M, Sobel JD, Akins RA. Association of key species of vaginal bacteria of recurrent bacterial vaginosis patients before and after oral metronidazole therapy with short- and long-term clinical outcomes. *PLoS One* 2022; 17(7):e0272012. <https://doi.org/10.1371/journal.pone.0272012> PMID: 35901180
64. Göker T, Aşık RZ, Yılmaz MB, Çelik İ, Tekiner A. *Sphingomonas paucimobilis*: A rare infectious agent found in cerebrospinal fluid. *J Korean Neurosurg Soc* 2017; 60(4):481–483. <https://doi.org/10.3340/jkns.2014.0102.004> PMID: 28689399
65. Birlutiu V, Dobritoiu SE, Ghibu AM, Birlutiu RM, Boicean LC. *Sphingomonas paucimobilis*—a rare cause of splenic abscesses: A case report. *Medicine*: 2022; 101 (1):p e28522. <https://doi.org/10.1097/MD.00000000000028522> PMID: 35029916
66. Dierick K, Van Coillie E, Swiecicka I, Meyfroidt G, Devlieger H, Meulemans A, et al. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *Journal of Clinical Microbiology* 2005; 43(8). <https://doi.org/10.1128/JCM.43.8.4277-4279.2005>
67. Meng L, Zhang R, Dong L, Hu H, Liu H, Zheng N, et al. Characterization and spoilage potential of *Bacillus cereus* isolated from farm environment and raw milk. *Front Microbiol*. 2022 Sep 14; 13:940611. <https://doi.org/10.3389/fmicb.2022.940611> PMID: 36177462
68. Lappan R, Jamieson SE, Peacock CS. Reviewing the pathogenic potential of the otitis-associated bacteria *Alloiococcus otitidis* and *Turicella otitidis*. *Front Cell Infect Microbiol* 2020; 14:10–51. <https://doi.org/10.3389/fcimb.2020.00051> PMID: 32117817
69. Bhooshan S, Negi V, Khatri PK. *Staphylococcus pseudintermedius*: an undocumented, emerging pathogen in humans. *GMS Hyg Infect Control* 2020; 15:Doc32. <https://doi.org/10.3205/dgkh000367> PMID: 33391967
70. Glajzner P, Szewczyk EM, Szemraj M. Pathogenic potential and antimicrobial resistance of *Staphylococcus pseudintermedius* isolated from human and animals. *Folia Microbiol* 2022; <https://doi.org/10.1007/s12223-022-01007-x> PMID: 36221001
71. Petersen JM, Mead PS, Schrieffer ME. *Francisella tularensis*: an arthropod-borne pathogen. *Vet Res* 2009; 40(2):7. <https://doi.org/10.1051/vetres:2008045> PMID: 18950590

72. Shibata K, Shimizu T, Nakahara M, Ito E, Legoux F, Fujii S, et al. The intracellular pathogen *Francisella tularensis* escapes from adaptive immunity by metabolic adaptation. *Life Sci Alliance*. 2022; 5(10):e202201441. <https://doi.org/10.26508/lsa.202201441> PMID: 35667686
73. Rivera M, Dominguez MD, Mendiola NR, Roso GR, Quereda C. *Staphylococcus lentus* peritonitis: a case report. *Perit Dial Int* 2014; 34(4):469–70. <https://doi.org/10.3747/pdi.2012.00303> PMID: 24991057
74. Sahagún-Ruiz A, Granados Martinez AP, Breda LC, Fraga TR, Castiblanco Valencia MM, Barbosa AS, et al. *Pasteurella pneumotropica* evades the human complement system by acquisition of the complement regulators factor H and C4BP. *PLoS One* 2014; 9(10):e111194. <https://doi.org/10.1371/journal.pone.0111194> PMID: 25347183
75. Alifragki A, Kontogianni A, Protopapa I, Baliou S, Ioannou P. Infective endocarditis by *Pasteurella* species: A systematic review. *J. Clin. Med.* 2022; 11:5037. <https://doi.org/10.3390/jcm11175037> PMID: 36078964
76. Kim B, Pai H, Lee KH, Lee Y. Identification of *Pasteurella canis* in a soft tissue Infection caused by a dog bite: the first report in Korea. *Ann Lab Med* 2016; 36(6):617–9. <https://doi.org/10.3343/alm.2016.36.6.617> PMID: 27578520
77. Maeda T, Yoshida H, Kim JM, Tsuyuki Y, Kurita G, Kim JS, et al. Draft genome sequence of blood-origin *Pasteurella canis* strain PA42, isolated from a dog in Japan. *Microbiol Resour Announc.* 2022; 11(7):e0026022. <https://doi.org/10.1128/mra.00260-22> PMID: 35638811
78. Kurpas M, Michalska M, Zakrzewski A, Zorena K. First report of the presence of *Vibrio vulnificus* in the Gulf of Gdansk. *International Maritime Health.* 2021; 72(4):247–251. <https://doi.org/10.5603/IMH.2021.0048>
79. Di W, Cui J, Yu H, Cui X, Sa H, Fu Z, et al. *Vibrio vulnificus* necrotizing fasciitis with sepsis presenting with pain in the lower legs in winter: a case report. *BMC Infect Dis.* 2022; 22(1):670. <https://doi.org/10.1186/s12879-022-07655-1> PMID: 35927648
80. Courcol RJ, Lee KW, Downes J, Wexler HM, Baron EJ, Finegold SM. In-vitro susceptibilities of *Bacteroides gracilis*, *Fusobacterium mortiferum* and *F. varium* to 17 antimicrobial agents. *Journal of Antimicrobial Chemotherapy* 1990; 26(1): 157–158. <https://doi.org/10.1093/jac/26.1.157> PMID: 2211439
81. Tash K. *Rahnella aquatilis* bacteremia from a suspected urinary source. *J Clin Microbiol* 2005; 43(5):2526–8. <https://doi.org/10.1128/JCM.43.5.2526-2528.2005>
82. Snipes KP, Biberstein EL. *Pasteurella testudinis* sp. nov.: a parasite of desert tortoises (*Gopherus agassizii*). *Int. J. Syst. Bacteriol* 1982; 32, 201–210.
83. Sandmeier FC, Leonard KL, Weitzman CL, Tracy CR. Potential facilitation between a commensal and a pathogenic microbe in a wildlife disease. *Ecohealth.* 2022; 19(3):427–438. <https://doi.org/10.1007/s10393-022-01603-w> PMID: 35752710
84. Wooster GA, Bowser PR. The aerobiological pathway of a fish pathogen: survival and dissemination of *Aeromonas salmonicida* in aerosols and its implications in fish health management. *Journal of the World Aquaculture Society* 1996; 27: 7–14. <https://doi.org/10.1111/j.1749-7345.1996.tb00588.x>
85. Girard SB, Paquet VE, Charette SJ. Improvements of virulence factor phenotypic tests for *Aeromonas salmonicida* subsp. *salmonicida*, a major fish pathogen. *J Fish Diseases* 2021; <https://doi.org/10.1111/jfd.13547> PMID: 34748248
86. Tewari R, Dudeja M, Nandy S, Das AK. Isolation of *Aeromonas salmonicida* from human blood sample: a case report. *J Clin Diagn Res.* 2014; 8(2):139–140. <https://doi.org/10.7860/JCDR/2014/6883.4032> PMID: 24701507
87. Modi C, Modi S, Dudhartra G, Kumar A, Awale M. Antibacterials: a review. *Journal Intercultural Ethnopharmacology* 2012; 1:52–61. <https://doi.org/10.5455/jice.20120207113423>
88. Caraballo A, Caraballo B, Rodriguez-Acosta A. Preliminary assessment of medicinal plants used as antimalarials in the southeastern Venezuelan Amazon. *Rev Soc Bras Med Trop* 2004; 37(2), 186–8. <https://doi.org/10.1590/s0037-86822004000200016> PMID: 15094909
89. Guarino C, De Simone L, Santoro S. Ethnobotanical study of the Sannio area, Campania, Southern Italy. *Ethnobotany Research and Applications* 2008; 6:255–317. <https://doi.org/10.17348/era.6.0.255-317>
90. Chan G, Kim H. Antimicrobial effects of extracts of *Taraxacum officinale* H. on acnes strains. *국제통합보완대체의학회지* [International Journal of Integrated CAM]. 2011; 7, 3–16.
91. Ghaima K, Hashim N, Ali S. Antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*). *Journal of Applied Pharmaceutical Science* 2013; 3:96–99.

92. Kenny O, Brunton N, Walsh D, Hewage C, McLoughlin P, Smyth T. Characterisation of antimicrobial extracts from dandelion root (*Taraxacum officinale*) using LC-SPE-NMR. *Phytotherapy Research* 2015; 29:526–532. <https://doi.org/10.1002/ptr.5276> PMID: 25644491
93. Tolmacheva A, Rogozhin E, Deryabin D. Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. *Acta Pharmaceutica* 2014; 64:173–186. <https://doi.org/10.2478/acph-2014-0019> PMID: 24914718
94. Woods-Panzaru S, Nelson D, McCollum G, Ballard L, Millar B, Maeda Y, et al. An examination of antibacterial and antifungal properties of constituents described in traditional Ulster cures and remedies. *Ulster Medical Journal* 2009; 78:13–15. PMID: 19252724
95. Li M, Zhang H, Hu X, Liu Y, Liu Y, Song M, et al. Isolation of a new polysaccharide from dandelion leaves and evaluation of its antioxidant, antibacterial, and anticancer activities. *Molecules* 2022; 27:7641. <https://doi.org/10.3390/molecules27217641> PMID: 36364468
96. Astafieva A, Rogozhin E, Andreev Y, Odintsova T, Kozlov S, Grishin E, et al. A novel cysteine-rich antifungal peptide ToAMP4 from *Taraxacum officinale* Wigg. flowers. *Plant Physiology and Biochemistry* 2013; 70: 93–99. <https://doi.org/10.1016/j.plaphy.2013.05.022> PMID: 23771034
97. Parveen S, Wani A, Ganie A, Pala S, Mir R. Antifungal activity of some plant extracts on some pathogenic fungi. *Archives of Phytopathology and Plant Protection* 2013; 47:279–284. <https://doi.org/10.1080/03235408.2013.808857>
98. Gatto MA, Ippolito A, Linsalata V, Cascarano NA, Nigro F, Vanadia S, et al. Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. *Postharvest Biology and Technology* 2011; 61(1):72–82. <https://doi.org/10.1016/j.postharvbio.2011.02.005>
99. Khan A, Qureshi R, Gillani S, Ullah F. Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad, Pakistan. *Journal of Medicinal Plants Research* 2011; 5:4665–4670.
100. Odintsova T, Rogozhin E, Sklyar I, Musolyamov A, Kuryavtsev A, Puhalsky V, et al. Antifungal activity of storage 2S albumins from seeds of the invasive weed dandelion *Taraxacum officinale* Wigg. *Protein Pept Lett* 2010; 17(4):522–9. <https://doi.org/10.2174/092986610790963591> PMID: 19594427
101. Ragasa C, Apuada M, Rideout J. Terpenoids from *Taraxacum officinale*. *NRCP Research Journal* 2009; 10:17–26.
102. Freire L, Guerreiro TM, Pia AKR, Lima EO, Oliveira DN, Melo CFOR, et al. A quantitative study on growth variability and production of ochratoxin A and its derivatives by *A. carbonarius* and *A. niger* in grape-based medium. *Sci Rep* 2018; 8:14573. <https://doi.org/10.1038/s41598-018-32907-z> PMID: 30275502
103. Pfliegler WP, István P, Zoltán G, Tünde P. The Aspergilli and their mycotoxins: metabolic interactions with plants and the soil biota. *Frontiers in Microbiology* 2020; 10:2921. <https://doi.org/10.3389/fmicb.2019.02921> eCollection 2019 PMID: 32117074
104. Soares C, Calado T, Venancio A. Mycotoxin production by *Aspergillus niger* aggregate strains isolated from harvested maize in three Portuguese regions. *Rev Iberoam Micol* 2013; 30(1):9–13. <https://doi.org/10.1016/j.riam.2012.05.002> PMID: 22634155
105. Gayathri L, Akbarsha M, Ruckmani K. In vitro study on aspects of molecular mechanisms underlying invasive aspergillosis caused by gliotoxin and fumagillin, alone and in combination. *Sci Rep* 2020; 10:14473. <https://doi.org/10.1038/s41598-020-71367-2> PMID: 32879392
106. Guruceaga X, Perez-Cuesta U, Abad-Díaz de Cerio A, Gonzalez O, Alonso RM, Hernando FL, et al. Fumagillin, a mycotoxin of *Aspergillus fumigatus*: biosynthesis, biological activities, detection, and applications. *Toxins (Basel)* 2019; 12(1):7. <https://doi.org/10.3390/toxins12010007> PMID: 31861936
107. Navale V, Vamkudoth KR, Ajmera S, Dhuri V. Aspergillus derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology Reports* 2021; 8:1008–1030. <https://doi.org/10.1016/j.toxrep.2021.04.013> PMID: 34408970
108. Ling KH, Yang CK, Peng FT. Territrems, tremorgenic mycotoxins of *Aspergillus terreus*. *Appl Environ Microbiol* 1979; 37(3):355–357.
109. Meena M, Swapnil P, Upadhyay RS. Isolation, characterization and toxicological potential of *Alternaria*-mycotoxins (TeA, AOH and AME) in different *Alternaria* species from various regions of India. *Sci Rep* 2017; 7:8777. <https://doi.org/10.1038/s41598-017-09138-9> PMID: 28821789
110. Brasel TL, Martin JM, Carriker CG, Wilson SC, Straus DC. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. *Appl Environ Microbiol* 2005; 71(11), 7376–88
111. Sorenson WG, Frazer DG, Jarvis BB, Simpson J, Robinson VA. Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*. *Appl Environ Microbiol* 1987; 53(6):1370–1375.

112. Alemu G, Nega M, Alemu M. Parasitic contamination of fruits and vegetables collected from local markets of Bahir Dar City, Northwest Ethiopia. *Res Rep Trop Med*. 2020; 11:17–25. <https://doi.org/10.2147/RRTM.S244737> PMID: 32280295
113. M'rad S, Chaabane-Banaoues R, Lahmar I, Ourmima H, Mezhoud H, Babba H, et al. Parasitological contamination of vegetables sold in tunisian retail markets with helminth eggs and protozoan cysts. *J Food Prot* 2020; 3(7): 1104–1109. <https://doi.org/10.4315/JFP-19-559> PMID: 32074629
114. Li J, Wang Z, Karim MR, Zhang L. Detection of human intestinal protozoan parasites in vegetables and fruits: a review. *Parasites & Vectors* 2020; 13:380. <https://doi.org/10.1186/s13071-020-04255-3> PMID: 32727529
115. Loganathan R, Agoes R, Arya I. Vegetables contamination by parasitic helminth eggs in Malaysia and Indonesia. *Althea Medical Journal*. 2016; 3:190–194. <https://doi.org/10.15850/amj.v3n2.796>
116. Kłapeć T, Borecka A. Contamination of vegetables, fruits and soil with geohelminths eggs on organic farms in Poland. *Ann Agric Environ Med* 2012; 19(3):421–425. PMID: 23020033
117. Hartmeyer GN, Hoegh SV, Skov MN, Dessau RB, Kemp M. Selecting PCR for the diagnosis of intestinal parasitosis: choice of targets, evaluation of in-house assays, and comparison with commercial kits. *Journal of Parasitology Research* 2017:6205257. <https://doi.org/10.1155/2017/6205257> PMID: 29181192
118. Czech T, Baran A, Wiecek J. Content of heavy metals in soil and plants from an area Borzęcin municipality (Małopolska Province). *Ecological Engineering & Environmental Technology* 2014; 37:89–98.
119. Köller T, Hahn A, Altangerel E, Verweij JJ, Landt O, Kann S, et al. Comparison of commercial and in-house real-time PCR platforms for 15 parasites and microsporidia in human stool samples without a gold standard. *Acta Tropica* 2020; 207:105516. <https://doi.org/10.1016/j.actatropica.2020.105516> PMID: 32371221
120. Parija SC, Poddar A. Molecular diagnosis of infectious parasites in the post-COVID-19 era. *Trop Parasitol* 2021; 11(1):3–10. https://doi.org/10.4103/tp.tp_12_21 PMID: 34195053
121. Sow D, Parola P, Sylla K, Ndiaye M, Delaunay P, Halfon P, et al. Performance of real-time polymerase chain reaction assays for the detection of 20 gastrointestinal parasites in clinical samples from Senegal. *Am J Trop Med Hyg* 2017; 97(1):173–182. <https://doi.org/10.4269/ajtmh.16-0781> PMID: 28719290
122. Elsig A. The management of soil pollution in Europe (Twentieth-Twenty-first Century). In: *Encyclopédie d'histoire numérique de l'Europe* [online], ISSN 2677-6588, published on 17/03/22, consulted on 26/04/2022. Permalink: <https://ehne.fr/en/node/14121>.
123. Benbrook C, Kegley S, Baker B. Organic farming lessens reliance on pesticides and promotes public health by lowering dietary risks. *Agronomy* 2021; 11(7):1266. <https://doi.org/10.3390/agronomy11071266>
124. Alvarado-Villalobos MA, Cringoli G, Maurelli MP, Cambou A, Rinaldi L, Barbachano-Guerrero A, et al. Flotation techniques (FLOTAC and mini-FLOTAC) for detecting gastrointestinal parasites in howler monkeys. *Parasites & Vectors* 2017; 10:586. <https://doi.org/10.1186/s13071-017-2532-7> PMID: 29169387
125. Sulaiman IM, Fayer R, Bern C, Gilmen RH, Trout JM, Schantz PM, et al. Triosephosphate isomerase gene characterization and potential zoonotic transmissions of *Giardia duodenalis*. *Emerg Infect Dis* 2003; 9(11):1444–52. <https://doi.org/10.3201/eid0911.030084> PMID: 14718089