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Consuming Blackberry as a Traditional Nutraceutical Resource from an Area with High Anthropogenic Impact

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Received: 31 January 2019; Accepted: 5 March 2019; Published: 11 March 2019

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Abstract: The most serious quality issue of natural resources for human consumption or medicinal purposes is the contamination with pollutants harmful to consumers. Common blackberry (*Rubus fruticosus* L.) is a sought-after nutraceutical and an important component in herbal medicine in many places around the globe. The present study aims to analyze the level of heavy metal bioaccumulation in blackberry organs, as well as its spatial distribution in two consecutive years immediately after the interruption of the extended activity of the industrial source of pollution. The research was conducted in one of the most polluted areas in Romania and Eastern Europe, within a 26 km radius of the source of pollution. The Pb, Cd, Cu, and Zn concentrations in the leaves, flowers, and unwashed blackberry fruits were analyzed spectrophotometrically through flame atomic absorption spectroscopy (FAAS). The results show that blackberry is an important bioaccumulator of these heavy metals—71% of the Pb concentration values and 100% of the Cd concentration values exceeded the World Health Organization thresholds by up to 29 and 15 times, respectively. Also, the leaves are the largest reservoirs of Pb and Zn (the median values: 51.4 mg/kg dry weight and 105.2 mg/kg d.w., respectively), and the flowers contained the largest quantities of Cd and Cu (2.54 mg/kg d.w. and 11.3 mg/kg d.w., respectively). The Pb concentrations decreased by a power function in relation to the distance from the source of pollution. The implications of these results on the safety of the use of blackberry are discussed. The urgent necessity for food education of the local population which consumes contaminated nutraceutical products is emphasized.

Keywords: heavy metal contamination; herbal medicine; historically polluted area; wild food; blackberry

1. Introduction

In spite of all the qualitative changes which human activity has experienced over time, the attraction for products provided directly and generously by nature has not diminished [1]. The diversity of uses which every natural resource offers is the most convincing evidence of its value. For instance, blackberry is simultaneously edible, medicinal, and melliferous, thus it can be classified as a highly interesting nutraceutical (Table 1).

Table 1. The spectrum of traditional uses of blackberry.

Resource		Utilization			Information Source
Part of Plant	**Species**	**Range**	**Product**	**Uses/Disease**	
Whole plant	*Rubus fruticosus*	Romanian folk medicine	tea, decoction	leukorrhea	[2]
	Rubus sp.	Native American folk medicine	extract from fruit, root, and leaves	hair and fabric dye	[3]
Aerial parts	*Rubus fruticosus*	Europe	various	hypoglycemia	[4]
Stem	*Rubus* sp.	Native American practices	rope	transport	[3]
Young shoots	*Rubus fruticosus*, *R. ulmifolius* Schott	Sardinian and Sicilian traditional medicine	decoction	menstrual pain	[5]
	Rubus fruticosus	Romanian folk medicine	decoction	bronchitis, diarrhea, dysentery	[2]
Leaves	*Rubus fruticosus*, *R. ulmifolius*	Sardinian and Sicilian traditional medicine	fresh leaves for chewing	strengthening spongy gums	[5]
	Rubus fruticosus	Central Italy folk medicine	maceration	cicatrizant for skin, fungal infections, skin abscesses	[6]
	Rubus villosus Aiton.	around the world	leaves for chewing	bleeding gums	[7]
	Rubus fruticosus	European folk medicine	mouthwash, decoction	strengthening spongy gums, mouth ulcers, sore throats, diarrhea, hemorrhoids	[8]
Fruits	*Rubus* sp.	around the world	jam, syrup, jelly, marmalade, cake stuffing, wine, liqueur, ice cream, in yoghurt, drink and chewing gum dye		[3,9]
	Rubus fruticosus	Romanian folk medicine	decoction in lard wine	tuberculosis leukorrhea	[2]
	Rubus fruticosus	Ancient Greeks	fresh fruit	gout	[3]
Fruits and leaves	*Rubus fruticosus*	Pakistani traditional medicine	various	skin diseases, itching, scabies, eczema	[10]
Roots	*Rubus villosus*	around the world	dried root tea used for edema, leaves and roots used for diarrhea, enteritis, chronic appendicitis, leukorrhea, expectorant properties		[7]

Since the time of Hippocrates [11], the belief that food has therapeutic properties has gradually consolidated [12] and has engaged a rich terminology, with interchangeable notions that have created confusion and controversies [13]. A nutraceutical is a hybrid concept better located at the boundary between food and drug [14]. It was introduced by DeFelice in 1989 and brought about a revolution in nutrition [15]. In contrast to other food-derived products claimed to have benefits on human health, nutraceuticals have a proven clinic efficiency in preventing and even treating certain pathological conditions [16]. At least in Europe, the lack of nutraceuticals identity and insufficient clinical evidence from in vivo experiments has kept down the formulation of shared regulatory framework for nutraceuticals [17] that would guarantee consumers the efficacy and safety of this pharma-food.

Despite increasing research on the properties of bioactive compounds [18], nutraceuticals, as rich substance mixtures [17], require: (1) a supplementary chemometric effort to identify the dietary markers which enable the quality control of the products [19] and (2) robust clinical evidence to support their use [20] and thus the transition from potential nutraceutical to established nutraceutical [15].

Taking into account the blackberry, the most important bioactive ingredients are: (1) the ellagitannins, which, besides their usual antidiarrheal and antidysenteric astringency, inhibit the growth of cancerous cells [3,21–23] and (2) the anthocyanins and other polyphenols, with their significant antiradical, antioxidant, and chemoprotective activities [8,21–35].

In many places around the world, especially in rural and tribal areas, exploiting natural, food, and medicinal resources is a survival issue, therefore a social factor [36] or, in any case, an alternative source of income. In 2005, 14,837 t of wild blackberries were harvested, in addition to 154,578 t of cultivated blackberries [37]. In Romania, 1 ha of forest land can yield up to 12.5 t of blackberries per year [9]. In our researched area, there are over 17,400 people who have access to contaminated natural products. With the cease of pollutant activity, 80% of employees were fired in 2009 and directed towards other fields. Consequently, the interest for the exploitation of the agricultural, medicinal, and nutritional potential of the area increased.

The large number of uses of vegetal products and their composition raises the often-times vital issue of product safety. The contamination with pollutants, either local, regional, or cross-border, endangers the health of consumers of such bio-products. Heavy metals, resulting from metallurgical activities by means of the refining and burning of fossil fuels or fertilizing agricultural soils, enter the food chain via the air, water, and soil, manifesting toxicity even in very small concentrations [38,39]. For instance, lead, cadmium, and zinc poisoning attacks the nervous system, causing a decrease in intellectual performance, as well as aggressiveness, delinquency, and narcomania in youths [40–45]. Blackberry is more prone to cadmium accumulation than other fruit [46].

Impact studies on historical pollution of the chemical composition of blackberry were carried out in Sudety Mountains SW, Poland [47]; Pirdop, Bulgaria [48]; Vladivostok, Russia [49]; Lori region, Armenia [50]; Berlin, Germany [46]; Middle Spis, Slovakia [51]; and Moldova Nouă, Romania [52]. The level of heavy metal contamination in leaves, fruits, and products derived from blackberries (blackberry leaf tea, blackberry wine) was also determined [53,54].

Our research aims to determine the recent pollution level through the concentration of certain heavy metals in blackberry vegetal material (*Rubus fruticosus* L.), and to characterize its spatial distribution in relation to the distance from the source of pollution and the site geomorphology. The investigations were carried out in one of the most polluted areas in Romania and Eastern Europe. The age and seriousness of the pollution in these areas prompted a variety of impact studies on the environment and the living organisms—revised by Smejkal [55] and Micu [56]. However, blackberry was not analyzed in these studies, in spite of its wide popularity among local and national consumers [9].

2. Materials and Methods

2.1. The History of the Pollution

The source of the pollution whose effects are analyzed in this article is the industrial park in the town Copşa Mică (46°06′59.10″ N and 24°13′15.43″ E), in the center of Romania. Until now, it produced large quantities of carbon black (for 58 years: 1935–1993), metallurgical and refined zinc, electrolytic lead, bismuth, antimony, iron, cadmium powder, sulphuric acid, sulfur dioxide, sulfates, sulfurs, carbon monoxide, nitrogen oxides, volatile arsenic compounds, and ammonia (for 70 years: 1939–2009).

The location of the industrial park on the wide valley of the Târnava river, which channels the local circulation of air mass, allowed the pollutants to distribute over large distances. The hydrographic fragmentation of the territory extended the pollution transversally to the secondary valleys. At the nearest weather station, according to the climatic data provided by the National Meteorological Agency [57], the mean annual temperature is 8.4 °C, the mean annual rainfall is 625.6 mm·year^{-1}, the annual wind frequency is 65.5%, and the speed of the wind with the highest frequency is 3.1 m·s^{-1}. The low amount of rainfall leads to the persistence of pollutants in the atmosphere and the high percentage of atmospheric calm allows air mass stagnation and pollutant deposition.

The plant material was collected in two consecutive years, starting with the year when the activity on the polluting industrial platform ceased. In the two years of sampling the mean temperatures were 9.4 and 9.1 °C and the rainfall levels were 648.4 and 782.3 mm·year^{-1}, respectively [57].

2.2. Sampling Design

The distribution of pollutants in blackberry organs was examined in nine sampling plots, eight of which were grouped in the first 8 km from the source of pollution (Figure 1), and one control plot, located 26 km from the industrial park in Copşa Mică (type of site D). The target was the study of pollution in various topoclimates. Each plot was identified geographically and geomorphologically, using Global Positioning System coordinates, the side aspect, the exposure to the circulation of polluted air (Table 2), and the distance to the main flue-gas stack for emissions—which is 250 m tall.

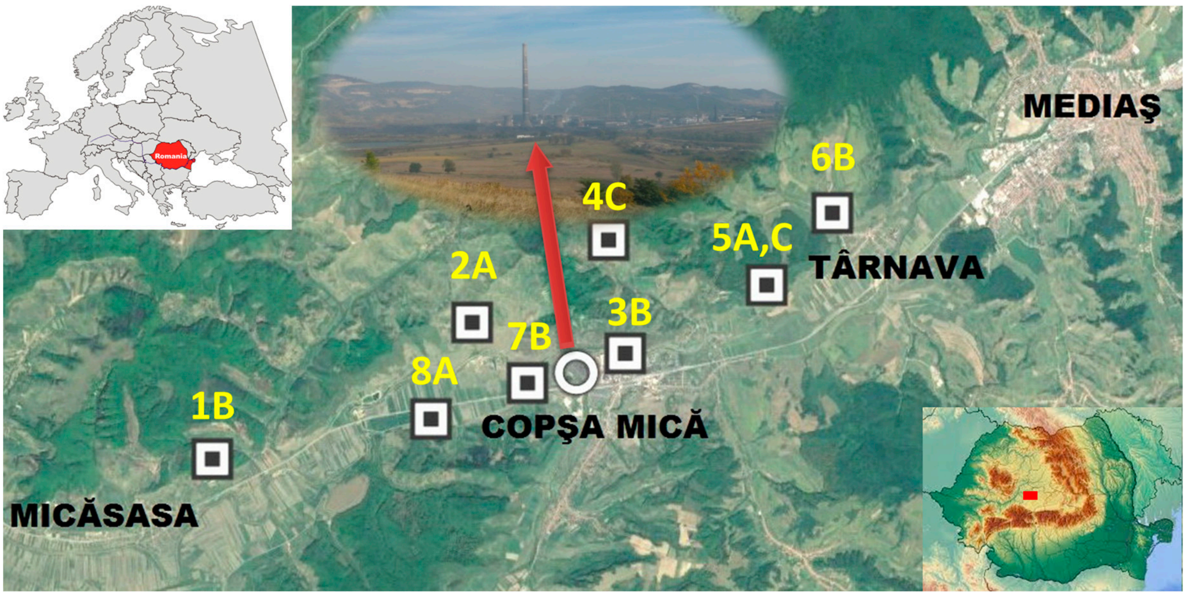

Figure 1. The sampling plots area: the circle marks the source of pollution; the squares mark the sample plots (numbered from 1 to 8 and identified with the type of site).

Table 2. Classification of sampled sites according to the location in relation to the source of pollution.

Type of Site	Site Description
A	Site located in the main valley (where the source of pollution is found) with frontal exposure to the source of pollution (slope facing the flue-gas stack).
B	Site located in the main valley (where the source of pollution is found) with tangential exposure to the source of pollution (slope not facing the flue-gas stack).
C	Site located in a secondary valley with frontal exposure to the local circulation of air mass.
D	Site located in a secondary valley, partially protected from the source of pollution.

The vegetal material samples were collected according to the regulations of the United Nations Economic Commission for Europe-International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests- [58]. At least five blackberry dominant bushes were chosen from every sampling plot. The material (20–30 g leaves, flowers/sampling plot and 100–200 g fruits/plot) was collected systematically from the four cardinal sides of the bush. Only healthy samples were considered [59], and great care was taken to avoid touching or contaminating them with the tools used. The flowers were collected no later than 2–3 days after bloom or in the budding stage, to prevent loss of pollen due to insect pollination. The leaves were collected in the second half of the growing season, but before the autumnal senescence, when the heavy metal concentration peaks [60]. The ripe fruits were harvested in the firm stage.

2.3. Processing the Material

The vegetal material was not washed, so as to identify the total pollutant concentrations in the state in which the resource is used [61]. For instance, the blackberry long-lived leaves are eaten by game, particularly by cervids [9], and blackberries are not washed before consumption. To avoid pollen removal, which bioconcentrates an important fraction of heavy metals, the flowers were not washed either.

The laboratory investigations followed Kelp's [62] recommendations. The samples were oven-dried to a constant mass at 60 °C, which did not affect the sanogenetic qualities of the product [9]. Mineralization was achieved after wet digestion [58], using the Berghof MWS-2 microwave oven. The mixture of 0.3–0.5 g dried plant powder, 2 mL concentrated HNO_3 (65% concentration, Merck extra pure), and 3 mL H_2O_2 (30% concentration, Merck, Darmstadt, Germany) were introduced in the microwave system (Berghof MWS-2, Eningen, Germany). Mineralization was carried out in three steps, at temperatures of 145, 180, and 100 °C (Table 3).

Table 3. The settings for mineralization of samples.

Temperature (°C)	145	180	100
Power (%)	75	90	40
Time (min)	5	10	10

After mineralization, samples were filtered through a 0.45 mm filter and brought to a volume of 50 mL in a volumetric flask with ultrapure water with a specific resistance of 18.2 MΩ/cm obtained from a Direct Q3UV Smart (Millipore SAS, Molsheim, France). The digested samples were analyzed by flame atomic absorption spectroscopy (FAAS) with ZEEnit 700 Atomic Absorption Spectrometer (Analytik Jena AG, Jena, Germany). Calibrating standard solutions of Cd, Cu, Pb, and Zn were prepared daily by the accurate dilution of the respective stock standard solutions (1000 mg/L). Ultrapure water with a specific resistance of 18.2 MΩ/cm obtained from a Direct Q3UV Smart (Millipore SAS, Molsheim, France) was used to prepare the standard solutions. For quality control purpose, blanks and triplicates samples (n = 3) were analyzed during the procedure. The variation coefficient was under 5%. The operation conditions were those recommended for each metal in the instrument's method (Table 4).

Table 4. Instrumental parameters for metal determination by flame atomic absorption spectroscopy (FAAS).

Standard Conditions	Element			
	Cd	Cu	Pb	Zn
Wavelength, λ (nm)	228.8	324.8	283.3	213.9
Slit width (nm)	1.2	1.2	1.2	0.5
Hollow-cathode lamp current (mA)	3	3	3	4
Background correction	Deuterium	Deuterium	Deuterium	Deuterium
Flame	C_2H_2/air	C_2H_2/air	C_2H_2/air	C_2H_2/air
Fuel flow (N L/h)	50	50	65	50

The sensitivity of the FAAS method was estimated using the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ (Table 5) were calculated based on the standard deviation of the response and the slope [63–66]. A total number of 171 spectrometric determinations were carried out.

Table 5. Limit of detection (LOD) and limit of quantification (LOQ) of the flame atomic absorption spectroscopy method.

Parameter	Element			
	Cd	Zn	Pb	Cu
Linear working range (mg/L)	0–1	0–1	0–1	0–3
Limit of detection (mg/L)	0.012	0.013	0.083	0.036
Limit of quantification (mg/L)	0.039	0.042	0.276	0.119

2.4. Data Processing

Data analysis was performed using Microsoft EXCEL 2007 and STATISTICA 8.0. The results were related to the World Health Organization [67] limits for heavy metals in products with ecosanogenetic qualities (Table 6).

Table 6. Tolerable limits for heavy metals in food supplements and herbal drugs.

Reference	Pb (mg/kg)	Cd (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
[68]	10.0	0.5	-	-
[67]	10.0	0.3	-	-
[62]	5	4	-	-
[69]	-	-	-	5 (berries and small fruits)
[62]	5	0,5	-	-

3. Results and Discussions

3.1. The Level of Heavy Metal Contamination in Blackberry

The concentrations of the studied microelements were found to be strongly scattered around the mean (high coefficients of variation—Table 7). Thus, the arithmetic mean was no longer relevant and was replaced with the median to express the central tendency. Most of the lead and cadmium concentration values greatly exceeded the toxicity thresholds (Table 7). Furthermore, these thresholds were exceeded in the control plot as well, which was believed to be unaffected by the influence of the pollution caused by the industrial park in Copşa Mică. As such, 40% of the measured lead concentrations, 100% of the cadmium concentrations, and 67% of the copper concentrations in the control plot exceeded the WHO permissible limit. This result is proof of the area expansion of heavy metal pollution. The other sampling plots are located up to 8 km from the source of pollution and have pollutant concentrations which exceeded the permissible limit for lead by up to 29.1 times, the permissible limit for cadmium by up to 14.9 times, and the permissible limit for copper by up to 38.8 times. Approximately a quarter of the values of lead concentration exceeded the permissible limit by at least 5 times. More than half of the values of cadmium concentration exceeded the permissible limit by at least 5 times.

Table 7. Statistics of heavy metal content in the blackberry samples from Copsa Mică area, Romania.

Metal	The Significance of the Differences between Individual Values *		Range	Arithmetic Mean	Median	Coefficient of Variation (%)	Relative Frequency (%) of Values Which Exceed the World Health Organization Threshold	The Significance of the Differences between Blackberry Organs ** (Kruskal–Wallis Test)	
	t	*p*						*H*	*p*
Pb (mg/kg dry weight)	4.64	<0.001	1.67–291.39	34.72	20.27	141.59	**70.5**	14.27	<0.001
Cd (mg/kg d.w.)	11.49	<0.001	0.32–4.46	1.86	1.61	57.08	**100.0**	8.18	0.02
Zn (mg/kg d.w.)	10.01	<0.001	10.91–193.54	76.03	70.29	65.49	-	23.05	<0.001
Cu (mg/kg d.w.)	7.84	<0.001	1.23–34.08	8.51	7.18	69.89	**83.3**	9.34	0.01

* All values of heavy metals concentrations either from leaves, either from fruits or flowers were merged; ** The differences refer to the concentrations of Pb, Cd, Zn and Cu grouped according to the three blackberry organs (leaves, fruits, flowers).

Based on the blackberry average yield in Romania [9], this means that a hectare of blackberry shrubs from the Copșa Mică area sequesters yearly through leaves, flowers, and fruits: 2.17 kg Pb, 0.17 kg Cd, 7.52 kg Zn, and 0.77 kg Cu.

The fact that these discovered values are greater than those highlighted in pollution literature is worrisome for the local consumers. Gasser et al. [70] processed the database of the German Medicines Manufacturers' Association and indicated that the following values of Cd and Pb concentrations range in blackberry leaves: <0.07–0.32 mg/kg dry weight and <0.4–2.8 mg/kg d.w., respectively. Shikhova [49] highlighted average concentrations of 15.07 mg/kg d.w. Pb in *Rubus sachalinensis* H. Lév. from the suburban forest phytocenosis in Vladivostok. After analyzing samples of *Rubus fruticosus* harvested from different sampling plots in Berlin, von Hoffen and Säumel [46] found average cadmium concentrations of 0.0081 mg/kg d.w., and lead concentrations of 0.0595 mg/kg d.w.

Investigations of heavy metal content in blackberry were also carried out in areas with historical pollution of mining or metallurgical origin. Micu et al. [52] identified average concentrations of 12 ppm Cu, 0.03 mg/kg d.w. Cd, and 19 mg/kg d.w. Pb in the blackberry leaves on the spoil heaps of Moldova Nouă (Romania). Wisłocka et al. [47] found in washed *Rubus idaeus* L. leaves grown on uranium mine dumps in the Sudety Mountains range heavy metal concentrations of 17.6–41.0 mg/kg d.w. for Pb, 0.40–1.60 mg/kg d.w. for Cd, 1.20–10.50 mg/kg d.w. for Cu, and 27–88 mg/kg d.w. for Zn, which were consistent with their concentrations in the soil. In the Middle Spis (Slovakia), which was affected by acid and heavy metal pollution for decades, Vollmannova et al. [51] found the following range of toxic metal concentrations: 0.30–1.19 mg/kg d.w. Pb, 0.18–0.42 mg/kg d.w. Cd, 5.50–6.50 mg/kg d.w. Cu, and 16.1–30.7 mg/kg d.w. Zn in dry blackberry leaves, as well as 0.03 mg/kg d.w. Pb, 0.03–0.05 mg/kg d.w. Cd, 0.48–0.99 mg/kg d.w. Cu, and 2.08–3.13 mg/kg d.w. Zn in fresh blackberries. Compared to our results, the investigations carried out by Teofilova et al. [48] in the area of the copper foundry in Pirdop (Bulgaria) reported higher average values of copper content (62.5 mg/kg d.w.), the same values for zinc (80 mg/kg d.w.), and lower values for lead (8.5 mg/kg d.w.) and cadmium (0.275 mg/kg d.w.) in blackberry fruits.

The large discrepancy with the literature data is partly due to the way our samples were prepared, i.e., without pre-washing. We intended to quantify the total bioaccumulation of heavy metals in the blackberry organs, as they are used directly by consumers (humans, cervids, bees) and thus the input to the trophic chain through the contaminants is more widely dispersed in the ecosystem structure. The data from the literature of other species reveal that, by washing, the heavy metal concentrations were reduced by 3.09–85.79% for Pb, 4.00–86.11% for Cd, 0.78–84.85% for Zn, and 0.76–86.41% for Cu, varying by species, organ harvested, culture system, sampling period, and degree and type of pollution [71–76]. We assume that even in the case of blackberry, as a species with hirsute organs, the deposition of heavy metals at least at the surface of the leaves is considerable. It has been shown that Pb and Cd concentrations are 10 times higher in hirsute plants than in those with a smooth surface [77].

The non-parametric Kruskal–Wallis test (Table 8) led to the stratification of the values of metal concentration according to the blackberry organs.

Table 8. The significance of the differences between the heavy metal concentration values by some nonbiological factors.

Independent Variable	Dependent Variable			
	Pb	**Cd**	**Zn**	**Cu**
	***p* from Kruskal–Wallis Test (0.05 is the Threshold Value for Statistical Significance) ***			
Distance from source of pollution	0.005	0.13	0.36	0.80
Altitude	0.01	0.31	0.27	0.07
Aspect	0.08	0.92	0.59	0.70
Exposure to air circulation	0.09	0.01	0.15	0.48
Year of sampling	0.26	0.41	0.89	-

* Kruskal-Wallis *p*-values for the effects of independent nonbiological factors on heavy metal concentration in blackberry organs sampled across air pollution gradients in Copșa Mică area, Romania.

The values in the content of lead, cadmium, zinc, and copper in fruits are noticeably different compared to those in flowers and leaves (Figure 2). Of the sampled blackberry organs, the fruits retained the smallest metal quantities, except for copper—the copper content had the highest variation in the fruits. The leaves contained 4.5 times more lead than the fruits. The flowers contained 3 times more cadmium and 3.8 times more zinc than the fruits. The flowers contained 2.2 times more copper than the leaves (Figure 2).

This means that the risk to consumers of such resources, quantified for a portion of 100 grams of fresh blackberries, with an average moisture content of 91.4% (own data), consists in the ingestion of 8.51 mg Pb, 0.74 mg Cd, 19.64 mg Zn, and 5.71 mg Cu.

Yedoyan and Yedoyan [50] found notable differences between blackberry organs which were polluted anthropogenically, especially in terms of lead and copper content. For instance, the root was found to be an important copper reservoir.

Concerning other species besides blackberry, from the Copșa Mică area, Alexa et al. [78] spectrometrically measured the heavy metal content. The comparisons emphasized the fact that trees are more important heavy metal bioaccumulators than blackberry. In June 2001, in full industrial season, up to 620 mg/kg d.w. lead and up to 8.5 mg/kg d.w. cadmium were found in locust leaves [78].

The net differentiation of blackberry organs in heavy metals storage is the consequence of their different and asynchronous lifespan, the morphological characteristics of their surface, and the exposure to the pollutant flow. Blackberry leaves—long-living, hirsute on both sides, and more exposed to atmospheric pollutants—are the largest reservoirs of heavy metals (Figure 2). The consistency and morphology of the floral tissue and the synchronization of the flowering stage with the rainiest months reduce the retention of heavy metals in flowers compared to leaves. Blackberries themselves, sheltered by leaves and slowly ripening, accumulate smaller amounts of heavy metals (Figure 2).

The unnoticeable differences of heavy metal concentrations between the two years of sampling (Table 8) suggest a long-lasting soil pollution and a strong sequestration of these pollutants in the blackberry organs, which goes beyond the rainfall increase in the second year.

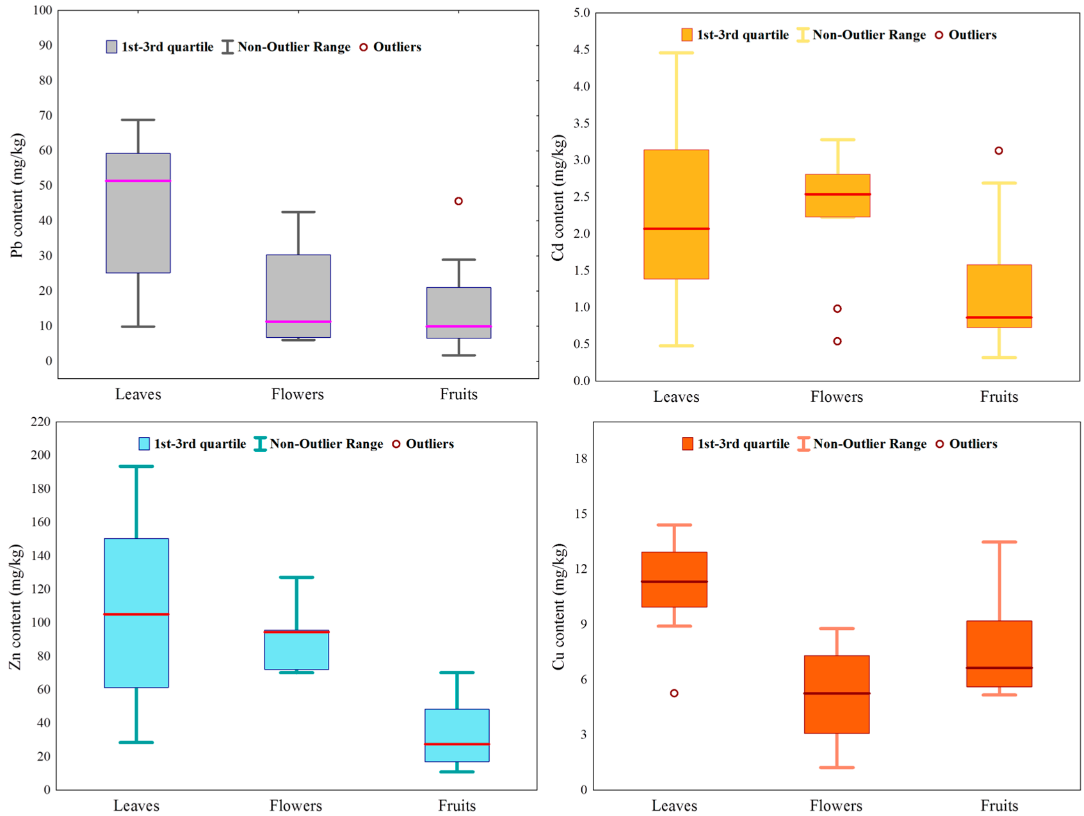

Figure 2. The stratification of heavy metal content according to the sampled blackberry organs.

3.2. The Spatial Variability of the Heavy Metal Content in Blackberry

The factors of influence on the metal content in the vegetal material were identified by using the non-parametric Kruskal–Wallis test, when the majority of dependent variables had non-Gaussian distributions. The results show that only the lead content is more sensitive to location change (Table 8). It decreases by a power function according to the distance from the source of pollution (Figure 3). In the first 3 km, the higher dispersion of lead concentrations and the more pronounced decay with the distance in the case of the leaves were noted. The matrix of the sign test (not listed) indicated that, in fact, the variation of lead content according to altitude is due only to the lowest altitude, which had the largest lead quantities. The zinc and copper concentrations were changeless from one sampling plot to another. The cadmium concentration depended on the exposure of the location to the local circulation of polluted air. The differences between the sampled years were not statistically significant (Table 8).

3.3. Safety in Herbal Medicine

In spite of the growing popularity of natural products, one must be realistic and admit that none can be completely free from various contaminants. The risk of contaminated nutraceuticals intake is much higher in the absence of specific legislation, as manufacturers are not compelled to oversee the nature, safety, and therapeutic and nutritional efficacy of these products [11]. Furthermore, the preference for nutraceuticals is fueled by consumers' false belief that the natural product is inevitably healthy and safe [19].

Food products consumed by people undoubtedly contain metals and metalloids [38,79]. Even if it comes up in a biotope where the anthropogenic pressure is low, the collected raw material may be contaminated due to certain non-hygienic harvesting techniques or poor storage and conditioning. Heavy metals can bioaccumulate in plants in concentrations which exceed the maximum limits permitted by the environmental regulations, where they can reach the human or animal organism directly or indirectly through the food chain. A lot of metals give rise to toxicity even with reduced concentrations.

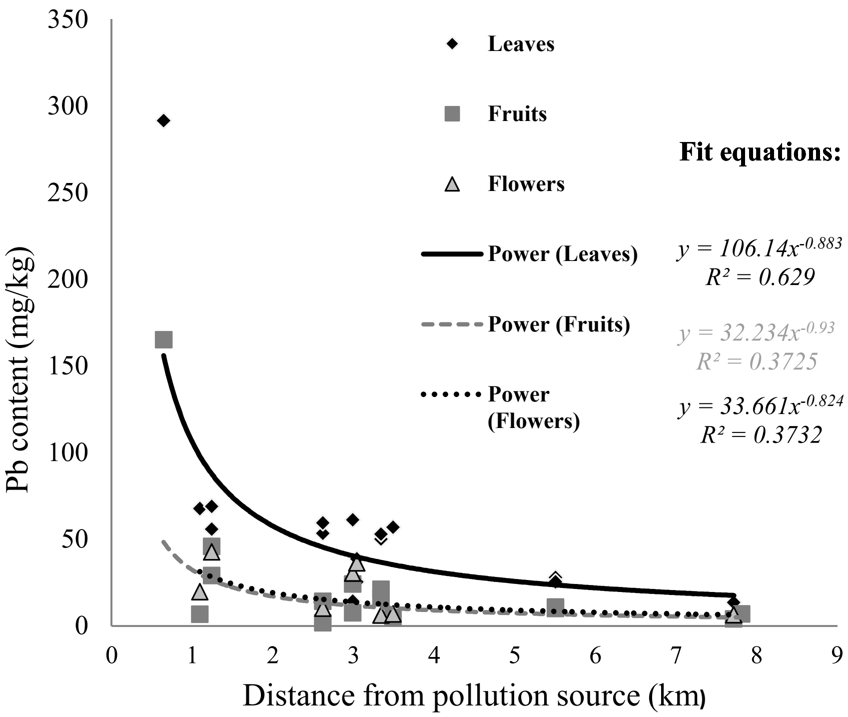

Figure 3. The variation of lead content in relation to the distance from the main source of pollution (control plot is excluded).

Important lead, cadmium, and zinc concentrations were found in consumer finished food or medicinal products, such as tea and blackberry wine [53,54]. Small quantities of these metals in *Rubus* species were found in Himalaya as well [34].

The smaller the quantities of non-essential heavy metals in traditional nutraceutical products, the lower will be the risk to the consumers health Small quantities of non-essential heavy metals in traditional nutraceutical products as their absence eliminates the risk of noxious effects on health [80]. Consequently, it is necessary to implement a qualitative assessment of wild resources consumed directly or used in ethnomedicine, before using or processing them, by determining the heavy metal content [81].

Resources, such as blackberry in Copşa Mică, are consumed by the local population in raw or processed forms. At the observed lead and cadmium concentrations (Table 7, Figure 2), the therapeutic value of the blackberry active ingredients decreases.

The International Agency for Research on Cancer classifies the anorganic compounds of Pb into group 2A—probably carcinogenic to humans. The symptoms of lead poisoning are abdominal pain, constipation, nausea, cramps, vomiting, anorexia, and weight loss [82]. Chronic exposure to high levels of Pb produces significant accumulations in the bones, as well as disorders of the central nervous system, hepatic and renal disorders, gout, and high blood pressure. Furthermore, it affects the optimal functions of the male and female reproductive system, with negative effects on pregnancy [83–88].

As a non-essential metal, Cd accumulates in the environment continuously, with one of its main sources being the atmospheric deposit. Chronic exposure to Cd causes kidney failure, increased risk of pre-diabetes and diabetes, high blood pressure, osteoporosis, and cancer [89–92]. In our researched area children represent the age range most exposed to the risk of contamination by eating blackberries. The poor education of some people maintains this risk. Hence, an acute need for food education for all social categories from the area is felt.

4. Conclusions

Blackberry is a popular nutraceutical, but unfortunately it is also an important heavy metal bioaccumulator. The extended industrial activity (which began in 1935) of metallurgical and chemical production in Copşa Mică led to the remnant contamination of blackberry with lead, cadmium, zinc, and copper. Shortly after the interruption of the pollution emission, the lead concentrations in blackberry were found to exceed the recommended threshold by up to 29 times in 71% of cases. Furthermore, all the cadmium concentrations exceeded the WHO threshold by up to 15 times, and 83% of the values of copper concentration exceeded the permissible limit by up to 39 times. The organs of blackberry store these elements differently—the flowers and leaves are the largest bioaccumulators. The lead bioaccumulation was found to have a definite spatial distribution. Conversely, the zinc and copper concentrations were changeless from one sampling plot to another. The results indicate a wide geographic expansion of pollution with these metals, within a radius of at least 26 km.

Author Contributions: Conceived and designed the experiment: G.G., F.D., and I.A.V. Performed the experiment: G.G. and T.M. Analyzed the data: F.D. and T.M. Interpreted the results: I.A.V., F.D., and G.G. Conceived the paper, wrote the first draft and edited the manuscript: F.D., M.M.V., and S.B. Supervised the manuscript: F.D.

Conflicts of Interest: The authors declare no conflict of interest.

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