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Evaluation of Selected Heavy Metal Contaminants as Well as Nitrates and Nitrites in the Microgreens of Nigella (*Nigella sativa* L.), Safflower (*Carthamus tinctorius* L.), and Camelina (*Camelina sativa* L.) at Different Stages of Vegetation

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Abstract: Microgreens are a new, rapidly growing group of foodstuffs. The decorative function of these is often accompanied by their use in traditional dishes. As microgreens are eaten at very early stages, when the development of the epidermis is at its minimum, the bioavailability of minerals will be found to be higher in microgreens then in mature vegetables. So, microgreens can be an excellent functional food, especially for mineral-deficient populations, although they can also be a source of contaminants such as heavy metals or nitrates and nitrites. The purpose of this study was to measure the levels of selected heavy metals (i.e., cadmium, arsenic, lead, chromium, aluminium, zinc, copper, cobalt, molybdenum, manganese, vanadium, boron, antimony, thallium, titanium and strontium), as well as nitrates and nitrites, in microgreens at various stage of vegetation, using uncommon oilseed plants like nigella—*Nigella sativa* L., safflower—*Carthamus tinctorius* L., and camelina—*Camelina sativa* L. The examined microgreens of rare oilseed plants may be a source of contaminants and nitrates. The mineral profile of these plants is mainly determined by their genotype. Microgreens' cultivation involves compliance with safety standards and replicable conditions to guarantee that the highest nutritional value is reached at the lowest possible contaminant level.

Keywords: microgreens; young shoots; safflower; camelina; nigella; heavy metal contaminants; nitrates; nitrites; food safety

Citation: Kapusta-Duch, J.; Smoleń, S.; Jędrszczyk, E.; Leszczyńska, T.; Borczak, B.; Kusznierewicz, B. Evaluation of Selected Heavy Metal Contaminants as Well as Nitrates and Nitrites in the Microgreens of Nigella (*Nigella sativa* L.), Safflower (*Carthamus tinctorius* L.), and Camelina (*Camelina sativa* L.) at Different Stages of Vegetation. *Appl. Sci.* **2024**, *14*, 4298. https://doi.org/10.3390/app14104298

Academic Editors: Chiara Portesi and Andrea Mario Giovannozzi

Received: 6 April 2024
Revised: 11 May 2024
Accepted: 15 May 2024
Published: 19 May 2024

1. Introduction

Food has a fundamental impact on human health and, therefore, on life expectancy. Increasing scientific interest in the ways to ensure the health and safety of manufactured food significantly influences consumer awareness. Thus, consumers are looking for wholesome food, the manufacture of which should be controlled from start to finish, i.e., food that has no negative impact on either human health or the environment. The health quality of food involves the safety of the product and its nutritional value, including its dietary and calorific value [1].

Food safety and quality therefore depend on a number of factors, both chemical and biological. The existence of chemicals that are either hazardous or necessary for the healthy functioning of the human body in food items is one of these aspects [2].

Chemical elements are everywhere in the environment and their origin can be both natural (the weathering of rocks) and anthropogenic (industry, transport, agriculture). They are released into the air, soil and natural waters and, as a consequence, then become an integral part of the food chain, of which humans are the last element. Due to increasing environmental pollution, food safety issues are gaining more and more importance [3]. Chemical elements can be classified into two groups based on their effects on the human body, i.e., toxic or those that are necessary for life. The primary criterion determining the toxic or beneficial effect of a chemical element on the human body is the dose; even in the case of essential micronutrients and macronutrients, too high a dose can induce toxic effects. The bioavailability of these chemical elements depends, among other things, on the form in which they are supplied to the body [3,4].

Excessive levels of nitrites and, indirectly, nitrates in food, can pose a risk to human health. Increases in nitrate content have been observed in plants at early stages of development, with insufficient sunlight, acidic soil pH, low moisture, a lack of nutrients such as magnesium or molybdenum, and when herbicides are applied [5]. Vegetables such as parsley, lettuce, spinach, radishes, beetroot, celery and leek have the highest nitrate accumulation capacity [6]. The toxic effects of nitrites include the induction of methaemoglobinaemia (cyanosis). The oxidation of the Fe^{2+} ion of haemoglobin to the Fe^{3+} ion is caused by the nitrite ion formed by the reduction of nitrates [6,7]. Nitrites are able to form nitrosamines, stable compounds with strong toxic, mutagenic, teratogenic and carcinogenic effects [8]. On the other hand, there are clinical studies on the positive effects of nitric oxide on cardiovascular disease [9]. The content of nitrates and nitrites in vegetable raw materials cannot be an indicator of their actual intake. Both vegetable pre-treatment (washing and peeling) and culinary processing can affect the content of these compounds [10,11].

Cadmium, lead and arsenic are the most hazardous heavy metals found in food. These metals mostly contaminate root vegetables, leafy vegetables (absorbing toxic substances from industrial dust or engine exhausts), cereals, fruit and food products whose contamination results not only from technology but also from substances added to them during their production. Heavy metal contamination is more and more prevalent in water as well, as evident in certain fish species. Studies have shown that they are most often contaminated with lead and arsenic compounds [12].

Microgreens have a number of beneficial aspects including a fast production cycle, space efficiency and a low production cost [13]. Despite the risk of infection that occurs with all products consumed raw, there is no question that the abundance of nutrients and bioactive components so beneficial to the human body makes sprouts and microgreens a product that is of great value and noteworthy [14]. In the existing literature, there is a lack of information regarding the nutritional and non-nutritive value of the microgreens of rare oilseed plants (such as nigella, camelina and safflower). The seeds of these plants are better explored. They contain many compounds with a wide range of pro-health effects. Since microgreens are consumed in the earliest stages of epidermis development, their mineral content will be higher than that of mature vegetables because of their better bioavailability. Therefore, microgreens possess the potential to serve as an exceptional functional food, particularly for populations worldwide that are deficient in minerals, although they can also be a source of contaminants such as heavy metals or nitrites [15].

The aim of this study was to measure the levels of selected heavy metals (i.e., cadmium, arsenic, lead, chromium, aluminium, zinc, copper, cobalt, molybdenum, manganese, vanadium, boron, antimony, thallium, titanium and strontium), as well as nitrates and nitrites, in the microgreens, at various stages of vegetation, of uncommon oilseed plants like nigella—*Nigella sativa* L., safflower—*Carthamus tinctorius* L., and camelina—*Camelina sativa* L. Plants were harvested twice: the first harvest with the cotyledons and the first true leaf and second harvest with 1–2 true leaves.

Microgreens are a new source of functional foods with great potential for the sustainable diversification of global food systems, the promotion of human health and the facilitation of access to fresh plants (microgreens) for ever-increasing populations, especially urban ones. Hence, there is a need to broaden our knowledge on their impact on health, including the presence of absolute or relatively harmful heavy metals, and monitor their levels.

So, an important reason for starting this research is, on the one hand, the high potential nutritional value of the young shoots of rare oilseed plants and, on the other, the risks associated with the amount of toxic contaminants they contain and the lack of available literature on the subject. A remarkable result of this study will be the knowledge, gained for the first time, on the content of nitrates and nitrites, as well as selected heavy metals, present in the microgreens of safflower (*Carthamus tinctorius* L.), nigella (*Nigella sativa* L.) and camelina (*Camelina sativa* L.) in various plant vegetation stages, including their so far unexplored immature stages.

2. Materials and Methods

2.1. Plant Material

The cultivation of microgreens of three rare oilseed plants—nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.)—was performed in the greenhouse of the Faculty of Biotechnology and Horticulture at the Agricultural University of Krakow. The seeds originated from the horticultural industry. Seeds were sown into seed boxes filled with TS 2 peat substrate, which consisted of PG Mix fertiliser and surfactant (pH = 6). The fertiliser (at a density of 2 g/L) was composed of 16% phosphorus (P_2O_5); 14% nitrogen (of which: 5.5% N-NO_3 and 8.5% N-NH_4); 0.8% magnesium (MgO); 19% sulphur (SO_3); 0.03% boron (B); 0.12% copper (Cu); 0.09% iron (Fe); 0.16% manganese (Mn); 0.20% molybdenum (Mo); 18% potassium (K_2O); and 0.04% zinc (Zn). The temperature conditions in the greenhouse during their growth were controlled: the day temperature oscillated around 21 °C, and the night temperature was 18 °C. Plants were watered daily to keep the moisture content of the substrate at the level of household water. Tap water was used, with the following parameters (Table 1).

Table 1. Selected quality indicators of the tap water used.

General hardness	**mg/dm^3**	**301**
Sodium	**mg/dm^3**	**21**
Ammonium ion	**mg/dm^3**	**0.021**
Magnesium	**mg/dm^3**	**11.6**
Calcium	**mg/dm^3**	**101**
Fluorides	**mg/dm^3**	**0.08**
Chlorides	**mg/dm^3**	**44.3**
Nitrites	**mg/dm^3**	**<0.01**
Nitrates	**mg/dm^3**	**16.3**
Sulphates (A)	**mg/dm^3**	**48**
General iron (A)	**mg/dm^3**	**<0.01**
Manganese	**mg/dm^3**	**<0.005**
Total chromium	**mg/dm^3**	**<0.001**
Cadmium	**mg/dm^3**	**<0.001**
Copper	**mg/dm^3**	**<0.01**
Nickel	**mg/dm^3**	**<0.001**

Plants were observed daily and, apart from watering, did not require any maintenance. Microgreens were harvested twice; at the first harvest, the plants had cotyledons and their first true leaf, and at the second harvest the plants had 1–2 true leaves. According to the BBCH (Biologische Bundesanstalt, Bundessortenamt, und Chemische Industrie) scale [16], the first harvest was performed at BBCH 11, and the second one at BBCH 12. Camelina and safflower developed similarly and reached the BBCH 11 stage at the same time, i.e., 11 days after sowing. Nigella grew more slowly, reaching its development stages about 12 days later, which means 24 days after sowing. The second harvest was performed at BBCH 12, it was 18 days after sowing for safflower and camelina, and 30 days for nigella. The Supplementary Materials include photographs of the microgreens.

During harvest, the above-ground part of the microgreens was cut with sterile scissors. The raw material was rinsed, drained using filter paper and frozen at −22 °C and finally freeze-dried (Christ Alpha 1–4, Osterode am Harz, Germany). The material obtained was then milled (Knifetec 1095 Sample Mill, Tecator, Höganäs, Sweden) using special titanium-nib-coated knives for grinding without heavy metal contamination. Finally, a homogeneous sample was obtained with small-in-diameter particles. Afterwards, the freeze-dried material was examined for selected heavy metals, as well as its nitrate and nitrite content. Analyses were conducted in triplicate.

2.2. Analytical Methods

Air-dried samples were examined for their micronutrients and trace element contents. The 0.5 g samples were transferred into 55 mL TFM vessels and mineralised in 10 mL 65% super-pure HNO3 (Merck no. 100443.2500) using the Mars 5 Xpress (CEM, Matthews, NC, USA) microwave digestion system. The mineralisation procedure was as follows: 15 min to achieve a temperature of 200 °C and 20 min for maintaining the temperature. Afterwards, the samples were cooled and quantitatively transferred to 25 mL graduated flasks with redistilled water. The amounts of zinc, manganese, boron, aluminium and copper were determined using a high-dispersion inductively coupled plasma optical emission spectrometer (ICP-OES, Prodigy Teledyne Leeman Labs, Hudson, NH, USA) [17]. The analysis of the quantities of cadmium, arsenic, lead, chromium, cobalt, molybdenum, antimony, thallium, strontium and titanium was performed by inductively coupled plasma mass spectrometry (ICP-MS/MS) with the use of a triple quadruple spectrometer (iCAP TQ ICP-MS ThermoFisher Scientific, Bremen, Germany). The internal standard used was a tellurium solution introduced into the spectrometer via an online system together with the sample at a concentration 40 μg Te·dm^{-3} [18]. The correctness of the analysis for the ICP-OES and ICP-MS/MS techniques was verified during the analysis by analyses of the control samples. The ICP-OES and ICP-MS/MS were calibrated using Merck's ICP multi-element standard no. VI and XVI.

The contents of the nitrite and nitrate ions present were determined after the homogenization of 5 g samples in 100 cm^3 of 2% acetic acid (puriss. p.a., Avantor Performance Materials) using an AQ2 discrete analyzer (Seal Analytical, Mequon, WI, USA), in accordance with the analytical procedures of the apparatus' manufacturer. The correctness of the analysis using the discrete analysis method was verified by measuring control samples [19].

2.3. Statistical Analysis

Analyses were conducted with at least three replications. The results obtained were given as means ± standard deviations (SD). A two-way analysis of variance and Duncan's test at $\alpha \leq 0.05$ were applied to compare significant differences between mean values. Plant species and harvest times were taken as two factors. All evaluations were performed using Statistica software v. 13.1 PL (Dell Inc., Tulsa, OK, USA).

3. Results

3.1. Selected Heavy Metal Contents

The amount of Cd (cadmium) in the microgreens ranged from 0.011 to 0.119 mg per 100 g d.m. (Figure 1). The highest statistically significant ($p \leq 0.05$) amount of Cd was determined in the microgreens of safflower from the second harvest, while the lowest was in those from the first harvest, compared to the other plants analysed. The microgreens of safflower from the second harvest contained 12 times more of this toxic element than the microgreens of nigella from the first harvest. The content of As (arsenic) in the analysed microgreens of uncommon oilseed plants ranged from 0.004 to 0.018 mg per 100 g dry matter (d.m.) (Figure 1). The highest statistically significant ($p \leq 0.05$) amount of As was determined in the microgreens of nigella from both the first and second harvests, compared to the remaining plants analysed. When compared to the microgreens of safflower from the first harvest, the As content in the microgreens of nigella from the second harvest was 4.5 times higher. The content of Pb (lead) in the examined microgreens ranged from 0.006 to 0.083 mg per 100 g d.m., and the microgreens of safflower from the first harvest contained 13 times less Pb than the microgreens of nigella from the second harvest (Figure 1).

The microgreens of safflower from the first harvest contained the smallest statistically significant ($p \leq 0.05$) content of Cr (chromium) (0.011 mg/100 g d.m.); the highest amount (3.5 times more) of Cr was found in the microgreens of nigella from the second harvest (0.041 mg/100 g d.m.) in comparison to the other plants tested (Figure 2). The amount of Al (aluminium) in the microgreens fluctuated between 4.62 and 17.16 mg per 100 g d.m. (Figure 2). The highest statistically significant ($p \leq 0.05$) amounts of Al were in the microgreens of camelina from the first harvest and the microgreens of nigella from the second harvest (11.03 mg), whereas the lowest amounts were in the microgreens of safflower (about 3.5 times less than the microgreens of camelina from the first harvest), compared to the remaining examined plants.

The contents of Zn (zinc), Cu (copper) and Mn (manganese) in the microgreens of rare oilseed plants were, respectively, 9.28–16.51; 1.01–3.10 and 4.84–14.22 mg/100 g d.m., and these were statistically significantly different ($p \leq 0.05$) (Figures 2–4). The lowest statistically significant ($p \leq 0.05$) amounts of Zn, Cu and Mn were determined in the microgreens of nigella from both the first and second harvests, compared to the other microgreens (Figures 2–4). As for Cu, there was 68% less of this element in the microgreens of nigella than in the microgreens of safflower from the first harvest (Figure 3).

The Zn content in the microgreens of camelina from the first harvest was about 1.78 times higher than that in the microgreens of safflower (Figure 2). In contrast, in the microgreens of safflower from the second harvest, the Mn content was about 66% higher than that in the microgreens of nigella from the second harvest (Figure 4). These differences were generally statistically significant ($p \leq 0.05$).

The amounts of Co (cobalt), Mo (molybdenum), B (boron) and V (vanadium) were in the range, respectively, of 0.002–0.005, 0.15–1.25, 2.80–6.43 and 0.003–0.019 mg per 100 g d.m (Figures 3 and 4). As for the content of Co, its highest, statistically significant ($p \leq 0.05$) levels were found in the microgreens of nigella from the second harvest and in the microgreens of safflower from the second harvest, compared to the remaining microgreens examined (Figure 3). In comparison to the other analysed microgreens, camelina's microgreens from the first harvest had the highest statistically significant ($p \leq 0.05$) amounts Mo, while the lowest amounts were recorded in the microgreens of safflower from the first harvest (Figure 3). B, in turn, was determined in the lowest amounts in the microgreens of safflower from the first harvest (2.80 mg/100 g d.m.), whereas its highest amounts were noted in the microgreens of camelina from the second harvest (6.43 mg), as well as in the microgreens of nigella from the first (5.97 mg) and second harvest (4.96 mg), compared to the remaining microgreens (Figure 4).

In addition, the microgreens of safflower also had the lowest, statistically significant ($p \leq 0.05$) amounts of Sb (antimony) and V compared to the other young plants (Figures 4 and 5). The contents of Sr (strontium), Sb, Ti (titanium) and Tl (thallium) were

3.75–10.24, 0.0009–0.006, 1.17–2.94 and 0.0008–0.024 mg per 100 g d.m., respectively (Figure 5). The highest statistically significant ($p \leq 0.05$) amounts of Tl, Sr and Ti were noted in the microgreens of camelina, and they averaged 0.023, 8.75 and 2.6 mg per 100 g d.m., respectively, compared to the remaining plants (Figure 5).

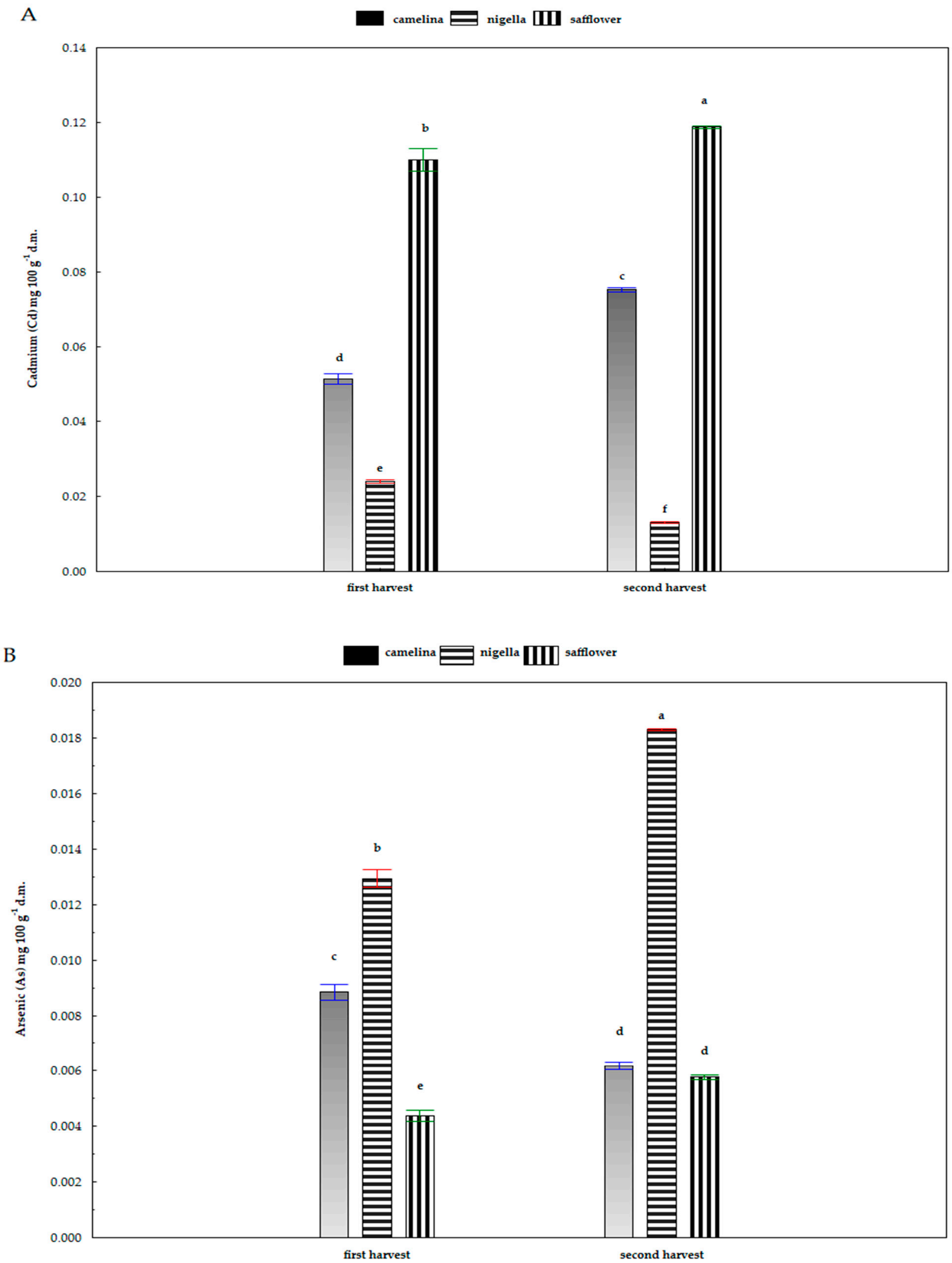


Figure 1. *Cont.*

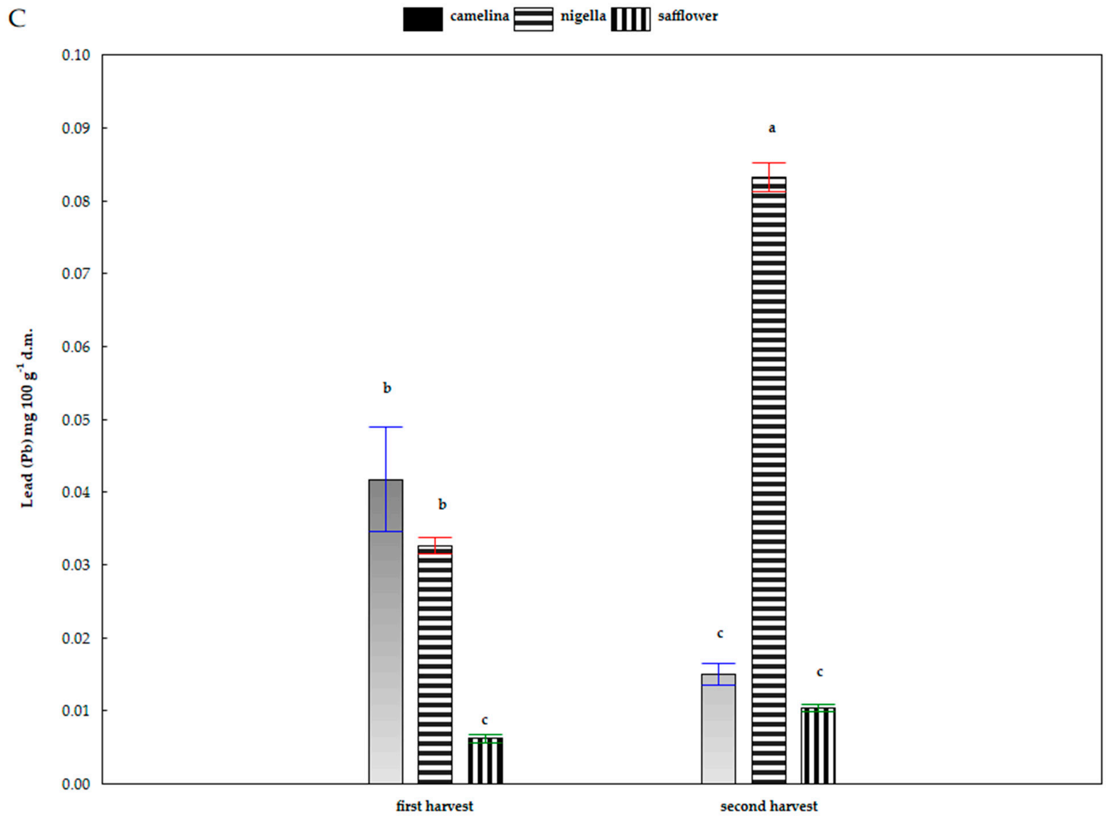


Figure 1. Cadmium (**A**), arsenic (**B**) and lead (**C**) contents in the young shoots of nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.) at different stages of vegetation (mg 100 g^{-1} dry matter—d.m.). Different letters (a–f) above the bars indicate statistically significant difference at $p \leq 0.05$.

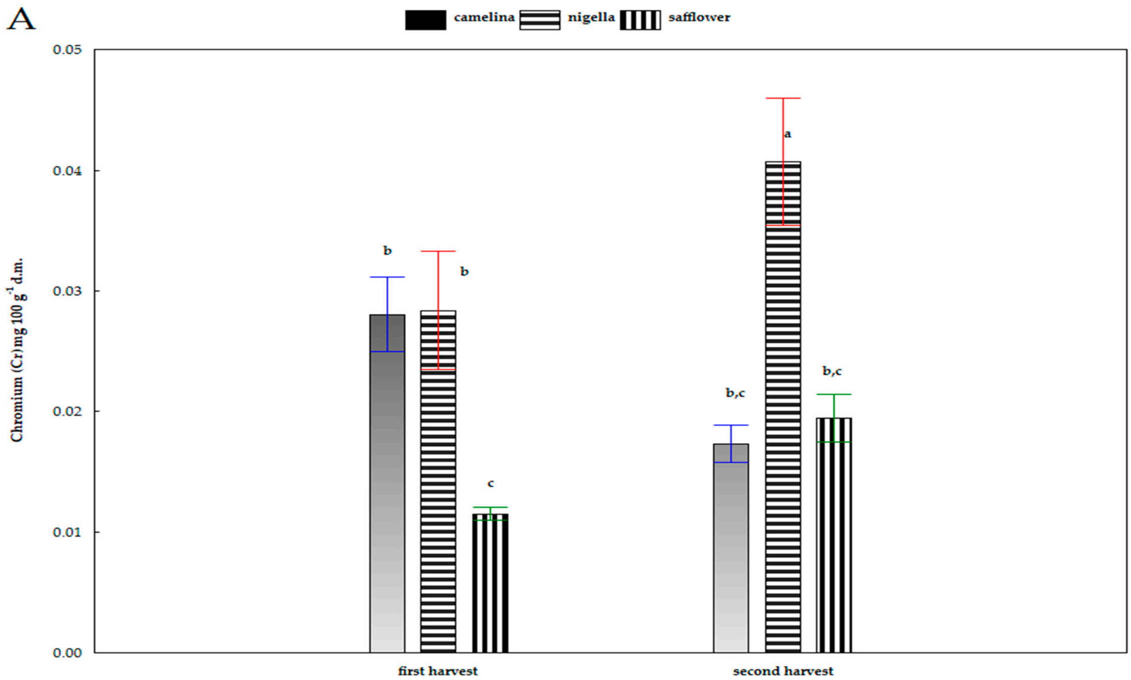
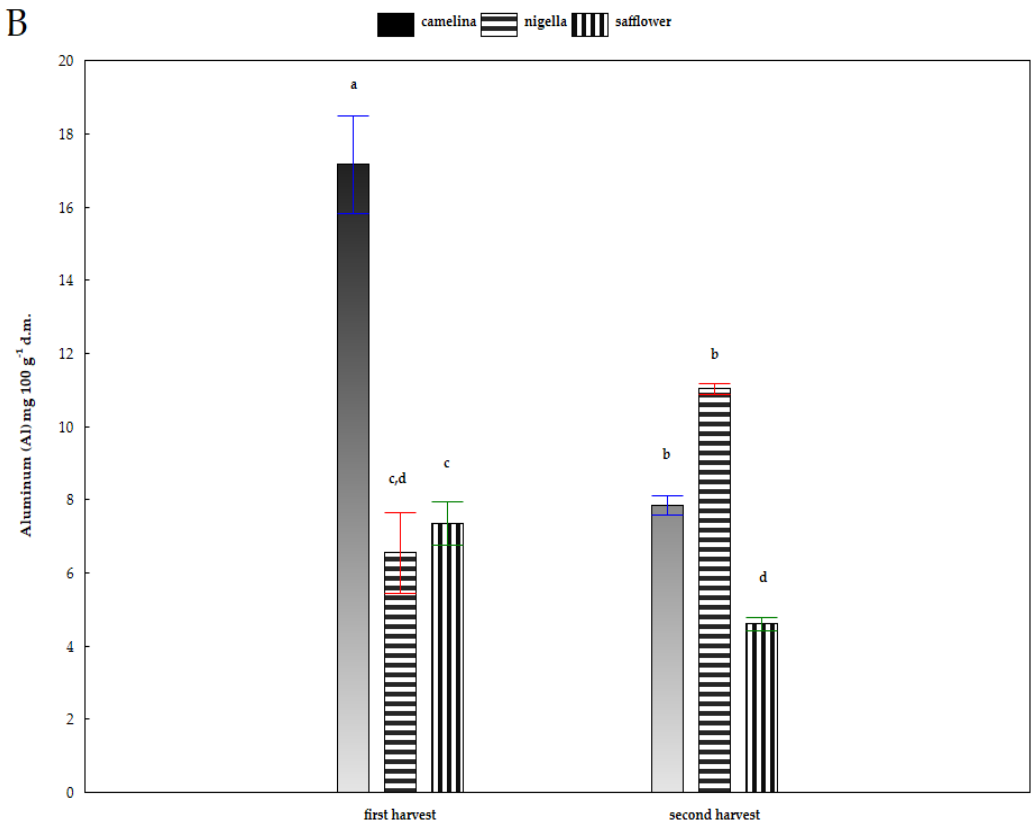


Figure 2. *Cont.*



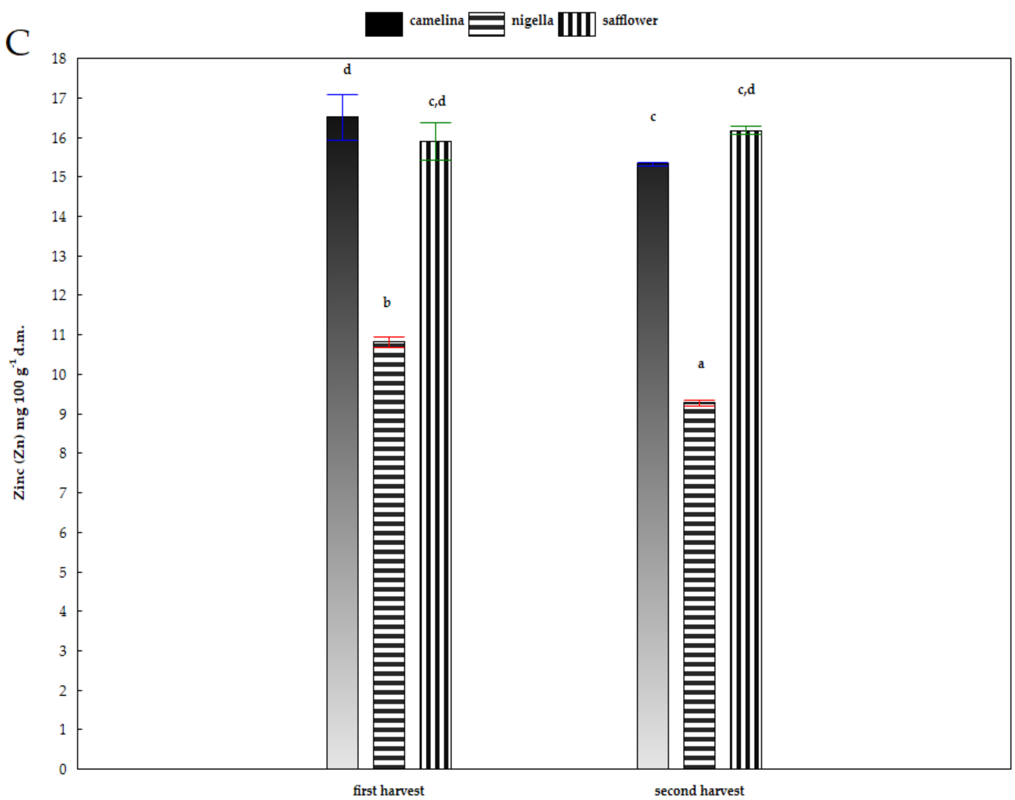
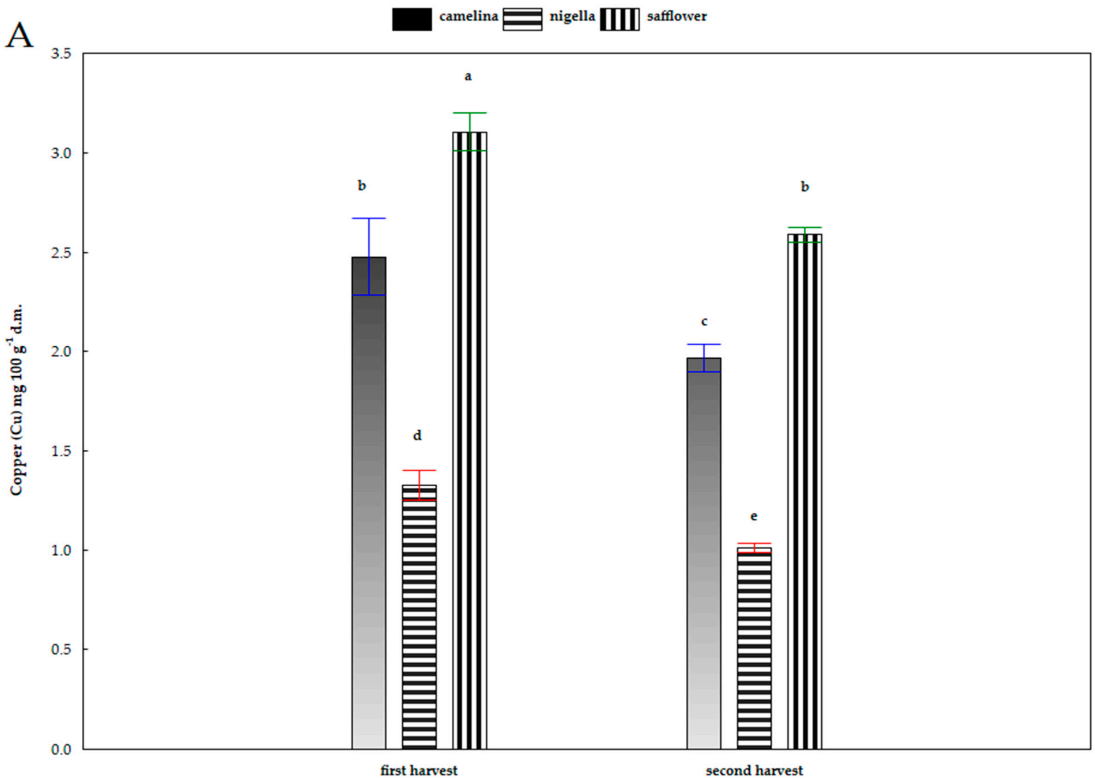
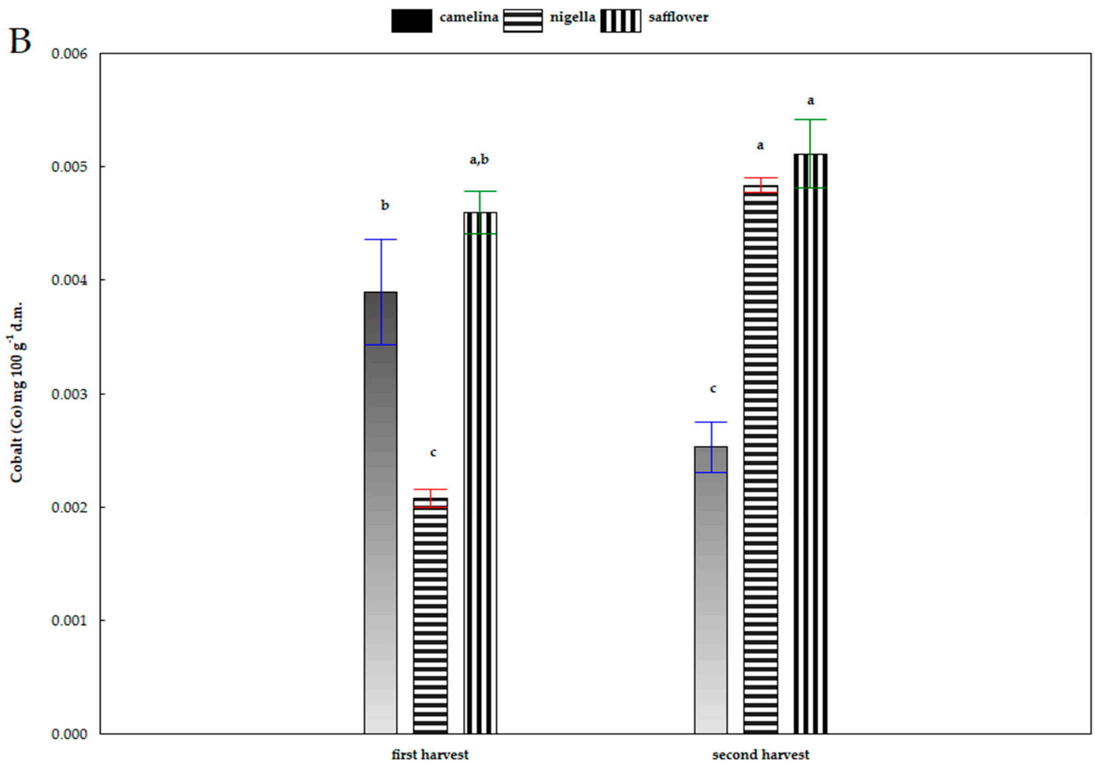


Figure 2. Chromium (**A**), aluminium (**B**) and zinc (**C**) contents in the young shoots of nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.) at different stages of vegetation (mg 100 g^{-1} dry matter—d.m.). Different letters (a–d) above the bars indicate statistically significant difference at $p \leq 0.05$.





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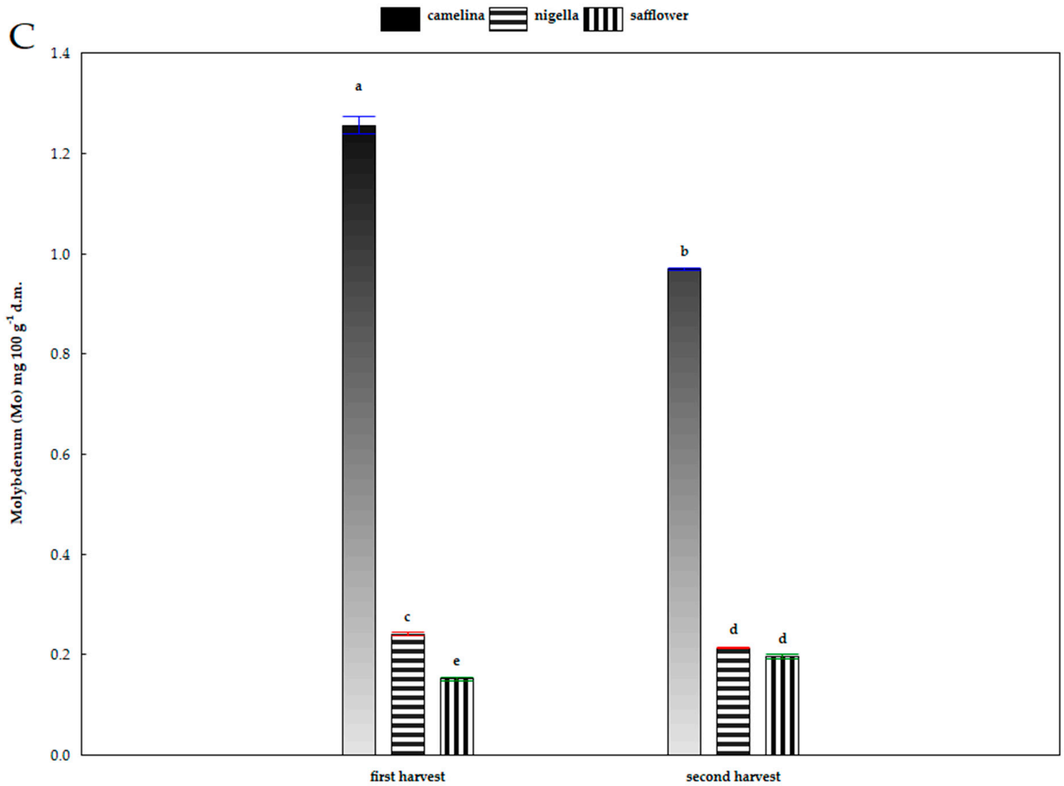
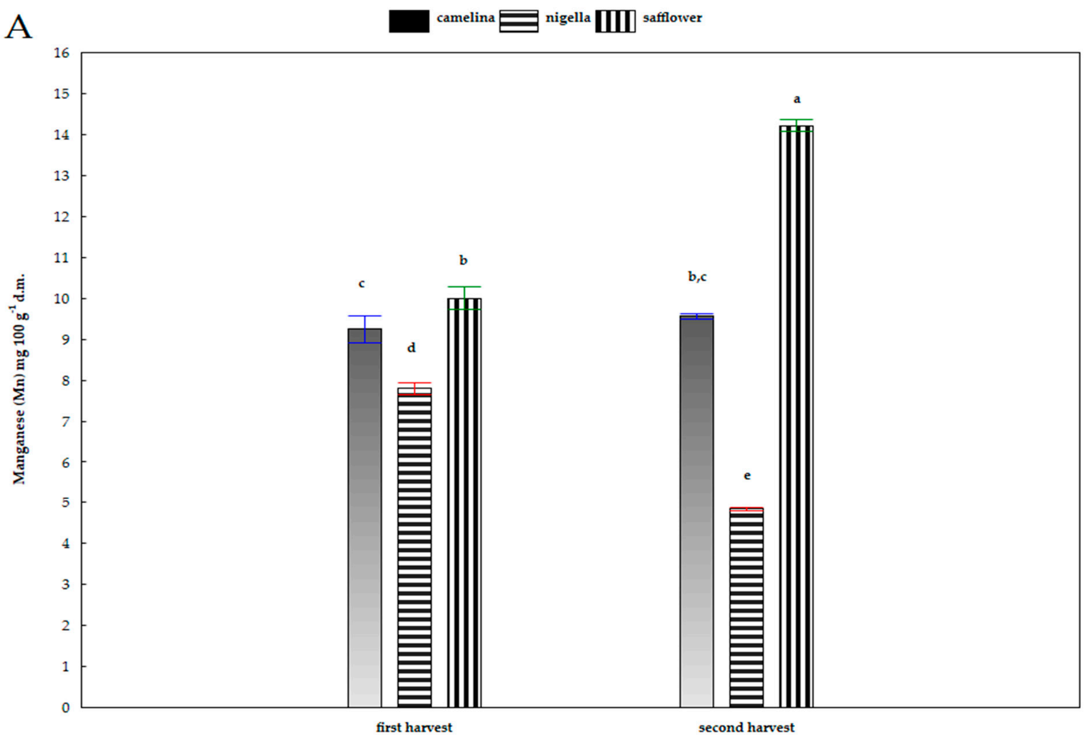


Figure 3. Copper (**A**), cobalt (**B**) and molybdenum (**C**) contents in the young shoots of nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.) at different stages of vegetation (mg 100 g^{-1} dry matter—d.m.). Different letters (a–e) above the bars indicate statistically significant difference at $p \leq 0.05$.



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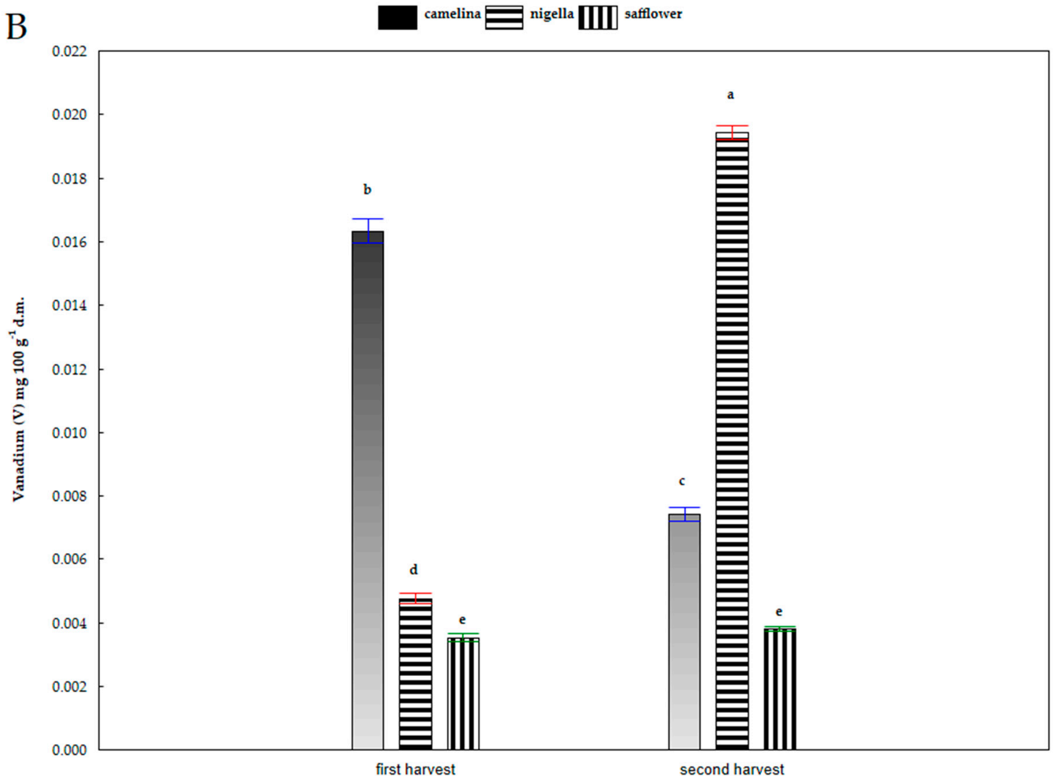


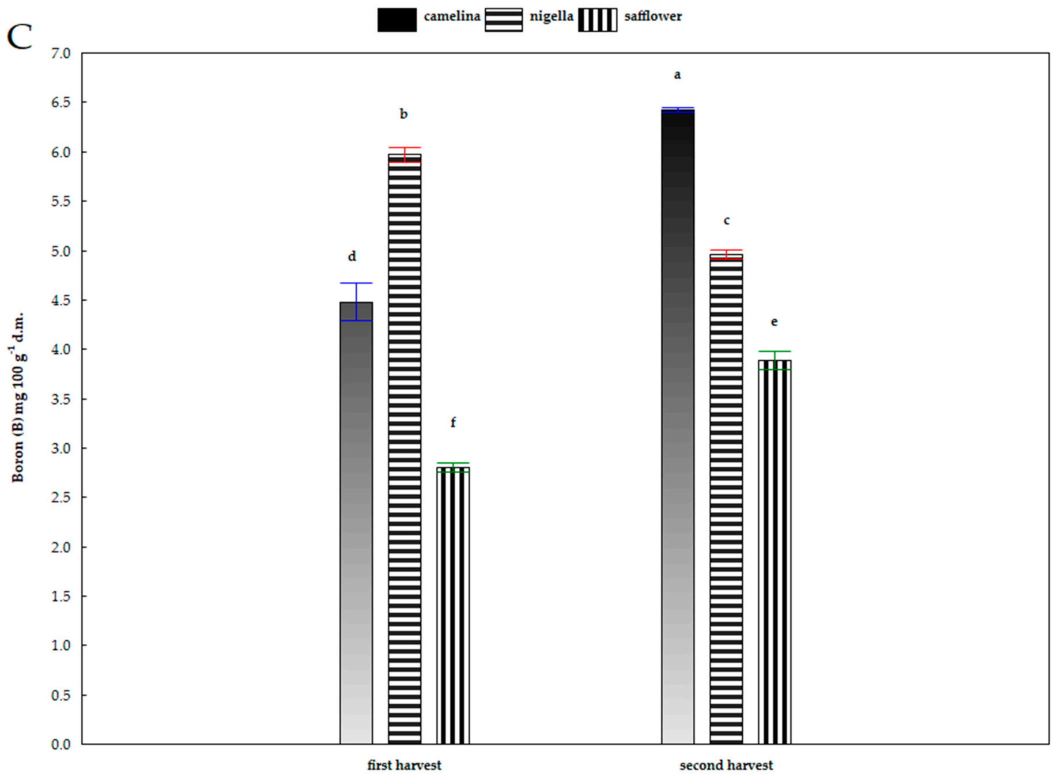
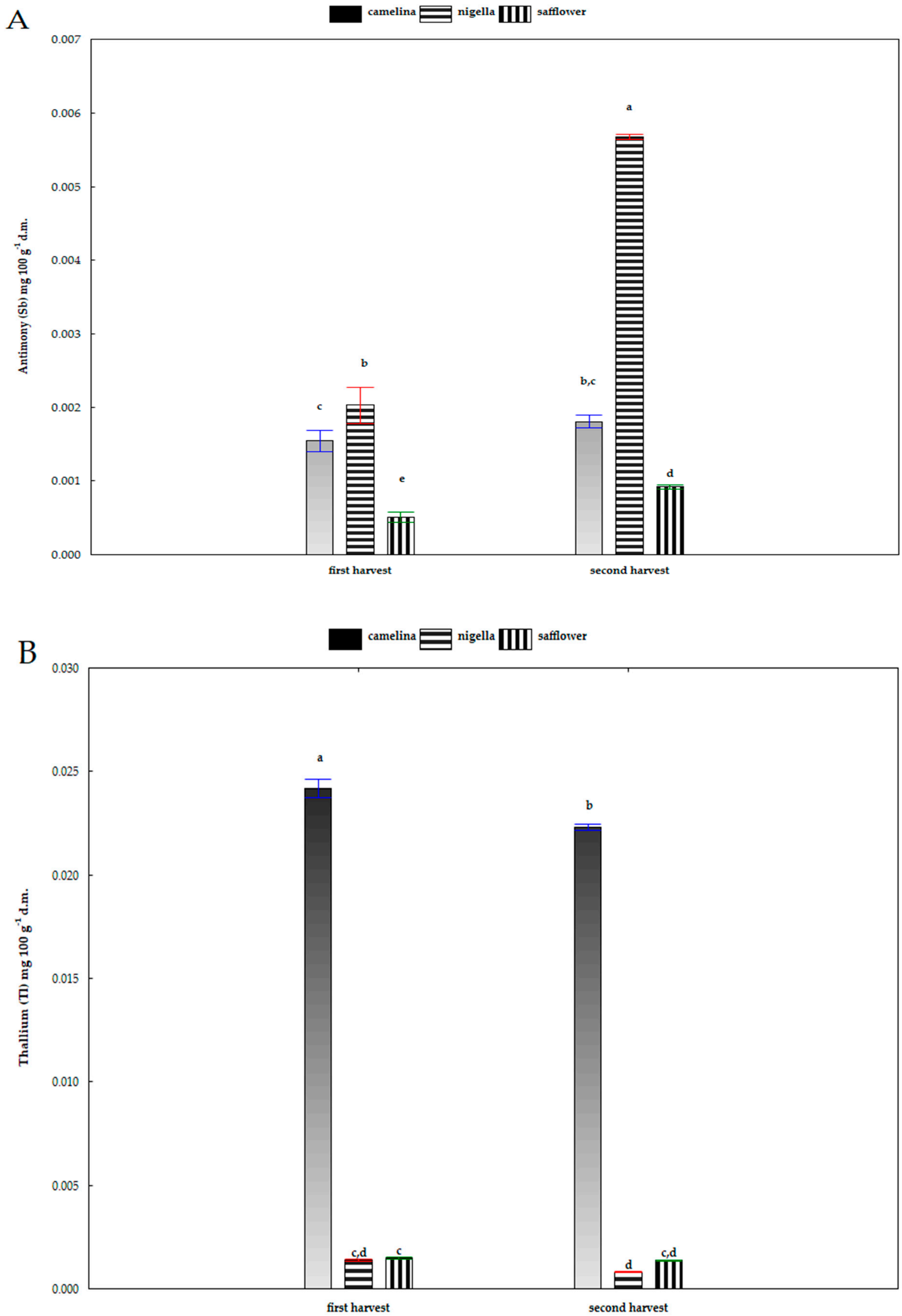


Figure 4. Manganese (**A**), vanadium (**B**) and boron (**C**) contents in the young shoots of nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.) at different stages of vegetation (mg 100 g^{-1} dry matter—d.m.). Different letters (a–f) above the bars indicate statistically significant difference at $p \leq 0.05$.



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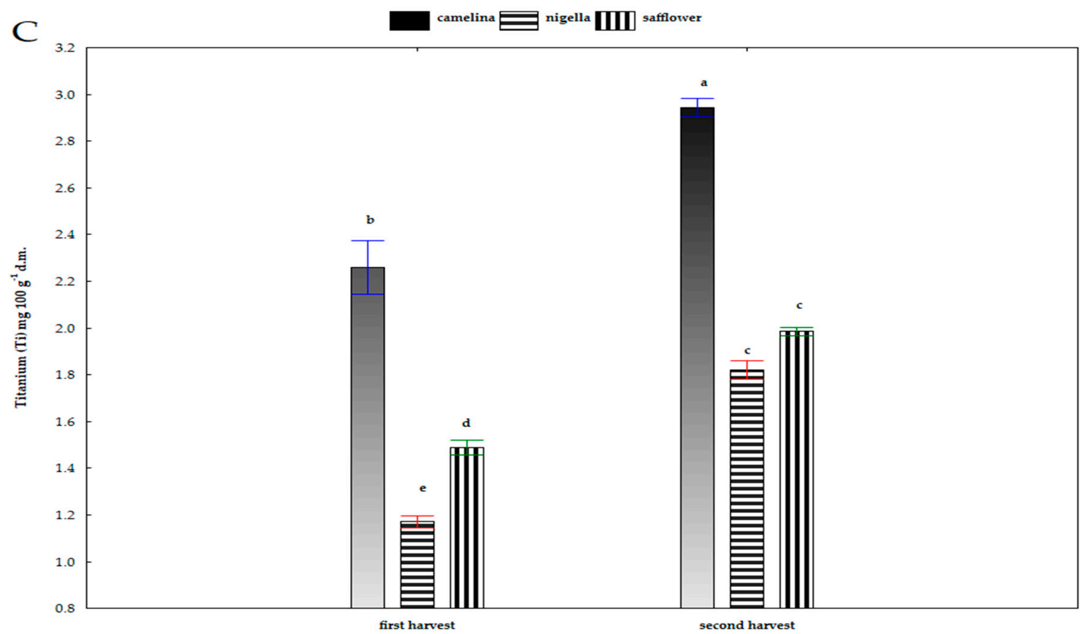

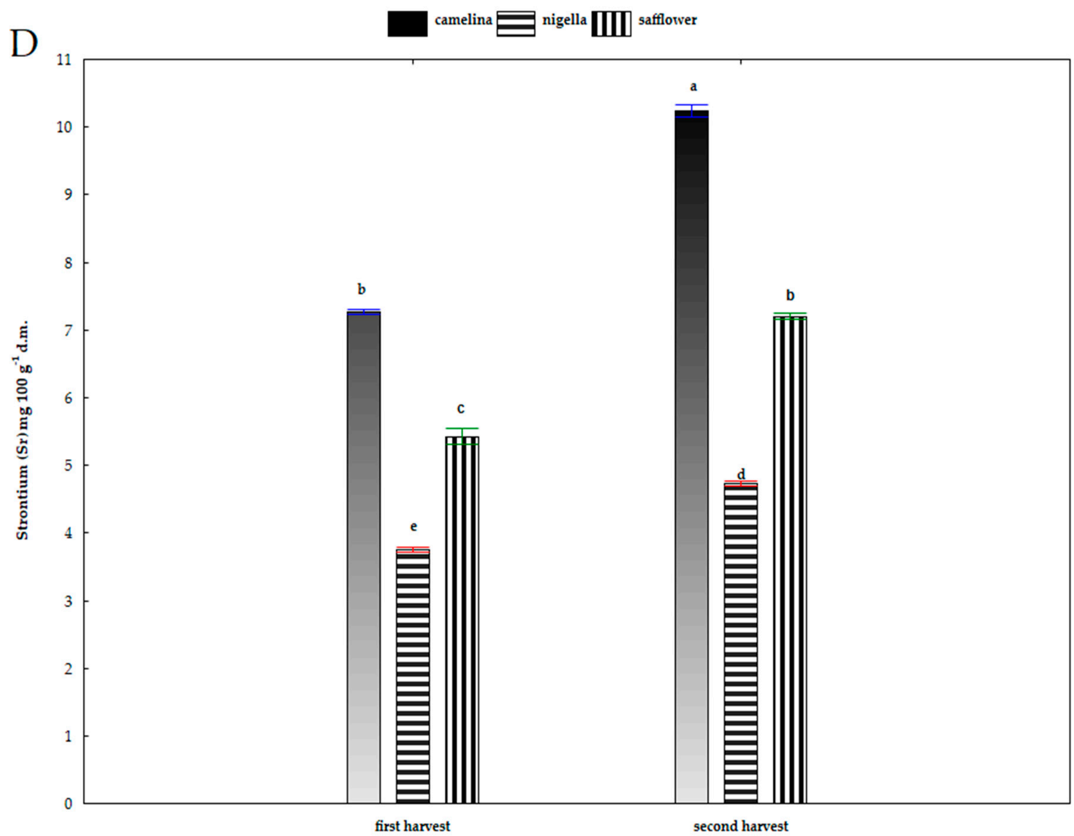

Figure 5. Antimony (**A**), thalium (**B**), titanium (**C**) and strontium (**D**) contents in the young shoots of nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.) at different stages of vegetation (mg 100 g^{-1} dry matter—d.m.). Different letters above the bars indicate statistically significant difference at $p \leq 0.05$.

3.2. Nitrate and Nitrite Content

Our statistical analysis showed that the microgreens of rare oilseed plants differed significantly ($p \leq 0.05$) in their nitrate contents, with the results fluctuating between 2712 and 11,985 mg per 100 g d.m. (Table 2). The highest statistically significant ($p \leq 0.05$)

nitrate contents were found in the microgreens of camelina from the first and second harvest and were, respectively, 10,883 and 11,985 mg per 100 g d.m., compared to the other microgreens analysed. In contrast, the lowest statistically significant ($p \leq 0.05$) nitrate contents were determined for the microgreens of nigella from the first and second harvest, which were 5159 and 2712 mg per 100 g d.m., respectively, in comparison to the remaining oilseed plants. In the case of the microgreens of safflower from the first and second harvests, these contents were, respectively, 9752 and 10,240 mg (average 9996 mg) and were statistically significantly ($p \leq 0.05$) higher, by 154%, than those of the microgreens of nigella (3935 mg), and statistically significantly lower ($p \leq 0.05$), by 12.6% on average, than those of the microgreens of camelina (11434 mg). There was no evidence of nitrites in the analyses performed.

Table 2. The nitrate (NO_3^-) and nitrite (NO_2^-) contents in the young shoots of nigella (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.) and camelina (*Camelina sativa* L.) at different stages of vegetation (mg 100 g^{-1} dry matter—d.m.).

Compounds	Young Shoots of Nigella (*Nigella sativa* L.)		Young Shoots of Safflower (*Carthamus tinctorius* L.)		Young Shoots of Camelina (*Camelina sativa* L.)	
	First Harvest *	Second Harvest **	First Harvest *	Second Harvest **	First Harvest *	Second Harvest **
Nitrates	5159 ± 196 ^{e}	2712 ± 122 ^{f}	9752 ± 303 ^{d}	10240 ± 245 ^{c}	10883 ± 615 ^{b}	11985 ± 264 ^{a}
Nitrites	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*

The results obtained from the analysis of three individual samples ($n \geq 3$) are shown as mean ± SD. The values within rows containing distinct letters are significantly different (*Duncan* test at $p \leq 0.05$). * Plants had cotyledons and their first proper leaf. ** Plants had 1–2 proper leaves each. *nd*—not detected.

4. Discussion

4.1. Selected Heavy Metals

The results of the analyses were presented per dry weight of the vegetable. However, the discussion also included references to the results converted to fresh vegetable weight. Conversions to the fresh weight of the vegetables were carried out on the basis of the results of their basic composition, including dry weight, presented in an earlier publication by Kapusta-Duch et al. [20], which is complementary to the present study. In plants, the tendency to uptake and accumulate arsenic is closely related to the similarity of this element to phosphorus. Inorganic forms of arsenic are usually taken up and accumulated in the root part of the plant, while organic forms, on the other hand, can be absorbed via leaves or tree bark [21]. The International Agency for Research on Cancer (IARC) has documented and then classified inorganic arsenic compounds as a human carcinogen (as a Group 1 carcinogen) [22]. A series of benchmark dose lower confidence limits ($BMDL_{01}$) were established by the Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA). The limits ranged from 0.3 to 8 µg/kg of body weight daily for the increased frequency of lung, skin and bladder cancers, as well as skin lesions [23,24]. The benchmark dose lower confidence limits elaborated by the Joint Expert Committee (FAO and WHO) on Food Additives (JECFA) varies from 2 to 7 µg/kg of body weight daily for increased incidences of lung and bladder cancers, as well as skin damage [24,25]. Lenzi et al. [26] determined, among others, the chromium, cobalt, aluminium, arsenic, cadmium and lead, as well as nitrates, concentrations in the microgreens of wild leafy species (*Sinapis arvensis* L., *Sanguisorba minor Scop.*, and *Taraxacum officinale Weber*) grown in a controlled environment and using a hydroponic system. As for the arsenic, cadmium and lead contents, the authors determined these to be 0.0013, 0.0004 and 0.0122 mg per 100 g fresh matter, respectively [26]. In this study, after converting the results from dry to fresh matter, the amounts of arsenic were markedly lower and averaged 0.0001 mg for the young safflower and camelina shoots and 0.0009 mg per 100 g of fresh matter for the young nigella shoots. There are a lot of microgreens that are being grown, but there is not a lot of research on the young shoots of uncommon oilseeds like nigella, safflower or camelina. With regard to cadmium, its contents averaged 0.0032 (young safflower shoots) and 0.0011 (young camelina and nigella shoots), while for lead this was 0.0002 (young safflower shoots), 0.0005 (young camelina shoots) and 0.004 (young nigella shoots), and these were higher for cadmium and lower for lead compared to the values obtained by Lenzi et al. [26]. Cadmium exhibits very strong toxicity; the ingestion of even small doses of this element can result in serious health effects. The higher risk with cadmium is due to the fact that this element has a toxic effect both when ingested, via the gastrointestinal tract, and also when inhaled, via the respiratory tract. Moreover, cadmium can also accumulate in the lungs [27]. It is assumed that the toxic effect of cadmium is mainly due to the Cd^{2+} ion to which cadmium is converted after the intake of cadmium compounds into the body. The biological function of cadmium in plants has not yet been recognised; however, it has been confirmed that this element is taken up from soil and water and can be accumulated in the root and transported to other parts of the plant. As in animal organisms, the cadmium in plants is bound by organic ligands. These are, i.a., phytochelatins, proteins and amino acids [28–30]. The exact mechanism of cadmium binding depends on the individual plant species. Cadmium can also be absorbed via plant leave leaves. All of the above-mentioned mechanisms of cadmium's uptake and translocation in plants can lead to the accumulation of this element in edible parts of the plant [28–30]. Minor elements have been rarely measured in microgreens. Xiao et al. [31] analysed the cadmium and lead content in 30 vegetable microgreens belonging to the *Brassicaceae* family and found that these elements were below the detection threshold. Additionally, Paradiso et al. [32] observed that lead was under the detection limit in some genotypes of microgreens belonging to the *Asteraceae* or *Brassicaceae* families. Allegretta et al. [33] determined the content of 13 elements, including cadmium, manganese, copper, zinc and strontium in microgreens of the *Asteraceae* and *Brassica* families. Two measurement techniques were used, i.e., ICP-AES and X-ray fluorescence spectroscopy (XRF), which differed in sample preparation. As far as the absolute level of harmful metals such as cadmium, is concerned, the highest amounts of it, amounting to 0.135 mg in 100 g of d.m., were determined in lettuce of the '*Trocadero*' variety. In the present work, all the results obtained for the cadmium contents in the examined young shoots were lower than those reported by Allegretta et al. [33]; the highest cadmium content was in young safflower shoots from the second harvest (0.119 mg in 100 g d.m.). Cadmium, being a contaminant, may cause potential health problems, mainly due to its toxicity to the kidney [34]. The European Food Safety Authority (EFSA) established a tolerable weekly intake (TWI) of 2.5 g/kg b.w. for cadmium based on dietary cadmium exposure, urinary cadmium and urinary beta-2-microglobulin, which is a biomarker associated with effects on renal tubules [24,34,35]. It has been found that the half-life of cadmium in the human kidney is up to 15 years. JECFA therefore removed their PTWI of 7 μg/kg of body weight and instead set the provisional tolerable monthly intake (PTMI) at 25 μg/kg of body weight [24,36]. Lead is not easily absorbed by plants due to the fact that the element is usually strongly bound to the soil via organic matter. Therefore, its transport in the food chain may be limited. As the lead mobility in plants is low, the highest amounts of the element may accumulate in root plants [29]. In the years 2010 and 2011, two institutions (EFSA and JECFA) withdrew their previous Provisional Tolerable Weekly Intake (PTWI) for lead [24,36,37]. A $BMDL_{01}$ of 0.5 μg Pb/kg of body weight per day (for developmental toxicity) and $BMDL_{01}$ of 0.63 and 1.50 μg Pb/kg of body weight per day (for kidney and cardiovascular effects, respectively) were established by the EFSA panel for contaminants in the food chain [24,37]. In the present study, these limits were not exceeded for arsenic, cadmium and lead levels, assuming a potential consumption of young shoots of 20 g per day.

As for aluminium, it has been found that it is the third most common element in the Earth's crust. Obviously, acid rain can elevate the aluminium concentration in the soil to toxic levels. As a result, it can then be detected in plants, food and groundwater sources, particularly in drinking water [38,39]. Aluminium generally occurs as oxides and aluminosilicates, which are non-toxic to plants, but as the soil pH decreases (pH $\leq$ 5), aluminium undergoes transformation into toxic Al^{3+}, which can be easily absorbed by plants [39,40].

Industrial food packaging, for example beverage boxes, baking trays, etc., are responsible for as much as 20% of aluminium use [38]. The main sources of food contamination are also industrial products and processed foods [41]. However, the main adverse effect of aluminium, as a toxic element, is its accumulation in the lungs, liver, kidneys, thyroid gland and brain. This element, with long-term exposure, can also be a strong neurotoxin, leading to impaired mental development and, when incorporated into infant bones, may result in weakened bone structure [38,42]. The exposure of humans to aluminium has been recognized as a potential cause of neurodevelopmental and neurodegenerative diseases (e.g., multiple sclerosis or Alzheimer's diseases) [38,40,43]. The PTWI (provisional tolerable weekly intake) value for all aluminium compounds in food (including food additives) of 2 mg kg^{-1} of body weight was set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [38,44,45]. On the other hand, the TWI (Tolerable Weekly Intake) for aluminium in all food sources has been established by the European Food Safety Authority (EFSA) at 1 mg kg^{-1} of body weight [38,42]. In a study by Lenzi et al. [26], the aluminium content of three species of wild-growing microgreens was determined at 4.73 mg per 100 g fresh weight of the vegetable, which was many times higher than the values obtained in the present work, which were averaged 0.01 (young safflower shoots) and 0.03 mg (young camelina and nigella shoots) after converting from 100 g dry matter to 100 g fresh matter.

Antimony occurs in soil mainly in its inorganic form, in its fifth and third oxidation states; some scientists have also reported the presence of methylated antimony compounds in surface soil layers. Regardless of the form of its occurrence, antimony is hardly extractable from soil [21,46,47]. Only inorganic and methylated forms of this element have been identified in plant samples. As antimony is used in the production of plastics, an obvious direction for the study of the speciation of this element would seem to be food products for which polymers are used as packaging [48,49]. There are no data in the available literature on the content of elements such as antimony, titanium and thallium in microgreens.

Until now, it has not been confirmed that chromium plays any biological role in plant physiology. An excess of this element, on the other hand, can provoke biochemical and morphophysiological processes in plants. The element is potentially harmful to plants, but it can be absorbed from the soil by forming complexes with aspartic acid, oxalic acid or citric acid [29,50]. Chromium toxicity strongly depends on the degree of its oxidation. Cr(III) is considered a micronutrient essential for normal lipid, glucose and protein metabolism in mammals. Cr(VI), in turn, exhibits strong mutagenic and genotoxic effects and is therefore classified by the IARC as a Group 1 human carcinogen, whereas Cr(III) is classified as a Group 3 carcinogen [22]. Lenzi et al. [26] determined, i.a., the chromium and cobalt contents of three microgreens species to be 0.1923 mg and 0.011 mg per 100 g fresh vegetable matter, respectively. These values were many times higher than those obtained in this work, which were, for chromium, at an average of 0.0004 mg (young safflower and camelina shoots) and 0.0021 mg (young nigella shoots), while for cobalt they were at an average of 0.0001 mg (young safflower and camelina shoots) and 0.0002 mg (young nigella shoots) after their conversion from 100 g dry to 100 g fresh matter. As for manganese contents, Allegretta et al. [33] found that, for three microgreens species, the results determined by ICP-AES were in the range of 6.4–20.2 mg in 100 g of dry matter, which agrees with most of the results obtained in this study. Copper, an essential micronutrient for plants, plays a key role in a number of biological processes such as photosynthesis and hydrocarbon metabolism. Plants absorb this element from the soil in the form of Cu^{2+}. As copper is transported to aboveground parts, it can be metabolised to organic forms and, as is the case with zinc, these can be proteins, peptides, amino acids, carotenoids, alcohols, phenolic compounds, flavonols, glycosides and stilbenoids [21,51,52]. As with zinc, the copper content in foods depends on the type of food and its origin. According to Allegretta et al. [33], the copper content in various microgreens varieties of chicory, lettuce and brassica species ranged widely, from 0.43 to 1.8 mg per 100 g d.m. In the present study, a considerable number of the results obtained exceeded 1.8 mg per 100 g d.m.; the highest result, 3.10 mg in 100 g d.m., was found for safflower from the first harvest. Zinc occurs in all the tissue types of various living organisms. This element is essential for the proper functioning of plants, animals and humans. Zinc is a constituent of over 200 enzymes. In food products, its content varies both due to the type of food product, but also due to its origin [53]. Plants can absorb zinc from both the soil and water. Its bioavailability to plants depends on the speciation form of this element, but also on environmental conditions such as soil pH, organic and inorganic matter content and moisture content [21,51,52]. Allegretta et al. [33] determined the zinc and strontium contents in three microgreens species. and the results ranged from 5.4–9.7 and 5.5–8.5 mg per 100 g d.m., respectively. All the results obtained for the zinc content in the microgreens of rare oilseed plants, except for the nigella microgreens from the second harvest, were higher in the present study. Concerning the amounts of strontium in the examined microgreens, only the results of its content in the microgreens of safflower (from the second harvest; 7.20 mg/100 g d.m.) and microgreens of camelina (from the first harvest; 7.27 mg) are consistent with the findings presented by Allegretta et al. [33]. In turn, Yadav et al. [15] estimated the contents of, i.a., copper, zinc and manganese in nine species of summer leafy microgreens and compared them to the content of these aforementioned elements in the mature vegetables. The authors expressed these contents as mg per kilogram of fresh vegetable weight. They observed considerable differences between microgreen species. Copper, manganese and zinc were found in the smallest amount in the microgreens of the poi species, while the highest amounts were noted in the microgreens of bottle gourd (copper), radish (manganese) and amaranthus cv. *Katwa* (zinc). The contents of the minerals in kale cultivars (*Brassica oleracea*) at the microgreen, young leaf and adult stages were determined by Waterland et al. [54]. According to the authors [54], microgreens contained significantly more zinc and copper than their relative adult vegetable. Butkutė et al. [55], in turn, examined the mineral contents in seeds, seed sprouts and the microgreen stage of the different legumes. Compared to the raw seeds and sprouts of these small legumes, microgreens possessed between 0.6 and 3.2 times more zinc [55]. Tavan et al. [56] investigated the content of microelements such as, for example, boron, copper, manganese, zinc and molybdenum in kale microgreens subjected to different processes of biofortification with selenium. With regard to the untreated control, these amounts, expressed as mg per 100 g d.m., were 2.53 (boron), 0.797 (cooper), 9.767 (manganese), 8.967 (zinc) and 0.090 (molybdenum). These results generally corresponded to or were slightly lower than our results. According to Pant et al. [57], who examined the levels of, i.a., copper, manganese and zinc in four Brassica plant species, the average contents of these elements were slightly lower (0.75–1.04 mg, for copper), higher (20.65 mg, for zinc) or similar (5.35 mg/100 g d.m., for manganese) to the results obtained in this work. Vitres-Portales et al. [58], in turn, determined the contents of, among others, boron, manganese, copper, zinc and molybdenum in the microgreens of three species (kale, kohlrabi and wheat). The highest concentrations were found for kale (manganese and zinc) and wheat (copper and molybdenum) compared to kohlrabi. As for boron, the values were 5.2, 5.6 and 5.8 mg per 100 g d.m. and were closest to our results obtained for the young shoots of nigella from the first harvest. The contents of manganese determined by Viltres-Portales et al. [58] at the levels of 4.3, 3.8 and 5.28 mg in 100 g d.m. were the most similar to its content in young shoots of nigella from our second harvest (4.84 mg). With regard to the contents of copper, zinc and molybdenum in the young shoots of kale, kohlrabi and wheat, these were 1.3, 6.0, 0.063; 1.1, 5.6, 0.067; and 1.32, 3.6, 0.136 mg in 100 g d.m., respectively, which agree with the results for the copper content in young shoots of nigella and the molybdenum content in young shoots of safflower from the first harvest.

4.2. Nitrates and Nitrites

Kyriacou et al. [59] investigated a selection of the health quality parameters of 13 microgreens, including their nitrate levels. The nitrate contents in microgreens varied markedly between species. The highest mean nitrate contents were in mustard, jute and kohlrabi (4488, 5164 and 5386 mg kg^{-1} of fresh matter—f.m.—respectively). Low nitrate accumulation (<1000 mg kg^{-1} f.m.) was found in Swiss chard and moderate accumulation

(1000–2600 mg kg^{-1} f.m.) was determined in radish and purple basil, while high accumulation (2600–4000 mg kg^{-1} f.m.) was recorded in coriander, green basil, komatsuna, cress, tatsoi, mibuna and pak choi. According to Di Gioia et al. [60], eleven of the seventeen microgreen species had very high nitrate concentrations. High concentrations were noted in borage, kale, beetroot, broccoli and radish, while low concentrations were noted in sunflower. These results are consistent with those reported by Di Gioia et al. [61,62].

The acceptable daily intake (ADI) for nitrate ions is 3.7 mg/kg of body weight, while for nitrite ions it is 0.06 mg/kg of body weight, according to experts from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) [63]. The Commission Regulation (EU) No 1258/2011 of 2 December 2011 pertaining to the maximum permissible level of nitrates in foodstuffs establishes such a value solely for selected vegetables, such as fresh and iceberg lettuce, rocket and fresh and frozen spinach [64]. The European Commission decided, in April 2023, to lower the limit values for nitrates and nitrites in food. The European Food Safety Authority (EFSA) conducted a thorough, scientific assessment prior to making this decision. The European Food Safety Authority (EFSA) set an acceptable daily intake (ADI) of 0.07 mg nitrite ion/kg of body weight per day and kept the daily allowance for nitrate ions at 3.7 mg per kilogram of body weight [65].

The high differentiation in nitrate accumulations between microgreen species can depend on the intensity of their fertilisation, climatic conditions, the content of some macro- and micronutrients in the soil, the action of some herbicides, the fungal infestation of plants or the way the product is stored. Differences in the levels of nitrates in various parts of vegetable crops are related to both their translocation and the intensity of their metabolism, and also to genetic factors [59,61].

5. Conclusions

The examined microgreens of rare oilseed plants may be a source of contaminants and nitrates. The mineral profiles of these plants are mainly determined by genotype. Microgreen cultivation involves compliance with safety standards and replicable conditions to guarantee that the highest nutritional value is reached at the lowest possible contaminant level.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app14104298/s1, pictures of microgreens of *Nigella sativa* L., *Carthamus tinctorius* L. and *Camelina sativa* L. from the first and the second harvest.

Author Contributions: Conceptualization, T.L., J.K.-D. and B.K.; Methodology, T.L., J.K.-D., S.S. and E.J.; Investigation, E.J., J.K.-D., S.S. and B.B.; Writing—Original Draft Preparation, J.K.-D.; Writing—Review and Editing, J.K.-D., B.B., S.S. and B.K.; Supervision, T.L. and B.K.; Funding Acquisition, J.K.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre (Poland), grant number 2022/06/X/NZ9/00311.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in article.

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

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