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ORIGINAL PAPER

EFFECT OF FLAT COVERS ON THE CONCENTRATIONS OF MICRONUTRIENTS, HEAVY METALS AND NITRATES IN ARUGULA LEAVES*

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Abstract

Arugula is a common name that refers to several leafy vegetable species of the family *Brassicaceae*. *Diplotaxis tenuifolia* (L.) D.C. and *Eruca sativa* Mill. have gained the greatest economic importance. The aim of this study was to determine the effect of plant covering with perforated polyethylene (PE) film and non-woven polypropylene (PP) fabric on the concentrations of micronutrients, heavy metals and nitrates in arugula leaves. A two-factorial experiment was conducted in the Experimental Garden of the University of Warmia and Mazury in Olsztyn. The experimental factors were two plant species: *D. tenuifolia* and *E. sativa*, and two types of plant cover: perforated PE film with 100 openings per m^2 and non-woven PP fabric with surface density of 17 g m^{-2}. In the control treatment, plants were grown without any covers. Each year, seeds were sown in mid-April. Immediately after seeding, the plots were covered with PE film or non-woven PP fabric. The covers were removed after approximately five weeks. Mineral content was determined in dry plant material collected at first harvest. The content of cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), chromium (Cr), zinc (Zn) and manganese (Mn) was determined by atomic absorption spectrometry (AAS), and Zn:Cu and Mn:Zn weight ratios were calculated. The concentrations of all analyzed heavy metals (including micronutrients) in arugula leaves were affected by both plant species and cover type. The leaves of *E. sativa* had a higher content of Cd, Pb, Ni, Cu, Cr, Zn and Mn. The use of covers contributed to a decrease in the accumulation levels of micronutrients and heavy metals in the edible parts of arugula plants. A widened Zn:Cu ratio was noted in all treatments, whereas Mn:Zn ratios were regarded as too narrow, relative to the normal ranges. The concentrations of nitrates (V) were higher in the leaves of *D. tenuifolia*. Plants of both species covered with non-woven PP fabric accumulated the highest levels of nitrates(V).

Key words: *D. tenuifolia, E. sativa,* perforated PE film, non-woven PP fabric.

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INTRODUCTION

Recent years have witnessed changes in the structure of vegetable consumption in Poland, in favor of species with high nutritional value. Eating habits change gradually due to the increased awareness regarding the health benefits of a diverse diet. Leafy vegetables are consumed mostly raw, to prevent the loss of valuable nutrients during thermal processing. Edible vegetable leaves are characterized by an attractive appearance, high nutritional value and a desirable flavor (Bieniek-Majda 2015, Jąder, Wawrzyniak 2015).

Arugula is a common name that refers to several leafy vegetable species of the family *Brassicaceae*, known for their pungent aroma and somewhat bitter taste. *Diplotaxis tenuifolia* (L.) D.C. (wall-rocket) and *Eruca sativa* Mill. (salad rocket) have gained the greatest economic importance. *Diplotaxis tenuifolia* is a perennial plant with narrow, deeply incised lobed or toothed leaves and edible, yellow flowers. *Eruca sativa* is an annual plant with spade-shaped, slightly indented leaves arranged in a rosette, and edible, white flowers with purple veins. Although the above species differ with respect to biological characteristics and structure, their uses and applications, properties and cultivation methods are identical. Arugula has been grown since the Roman times. Once forgotten, it has been recently rediscovered. Today, arugula is grown as a leafy vegetable and a spice plant mainly in the Mediterranean region, in Central Asia and in the USA. Arugula leaves can be eaten raw as a salad, and they can be added to sandwiches and cottage cheese. Due to its specific and strong flavor, arugula can also be used as a component of mixed salads. Arugula leaves can be stewed, cooked and fried, and they can be added to pizza and spaghetti (Morales, Janick 2002, Hall et al. 2015). Arugula is a rich source of bioactive compounds, and it is highly valued for its medicinal and therapeutic properties – stimulating, anti-scurvy, diuretic and improving digestion (Melchini et al. 2009, Cavaiuolo, Ferrante 2014, Tripodi et al. 2017).

Despite their high nutritional value, flavor quality and health-promoting properties, leafy vegetables (in particular those with a short growing season) tend to accumulate excessive concentrations of nitrates(V) and heavy metals. Heavy metals are elements with a density higher than 4.5 g cm^{-3}, which voluntarily lose their electrons to form cations. A common characteristic of all heavy metals is that they exert toxic effects on plants, animals and humans when their concentrations exceed the tolerable limits. This applies also to heavy metals that are considered to be nutritionally essential nutrients in trace or small amounts. In contrast, cadmium (Cd), mercury (Hg) and lead (Pb) are highly poisonous. They have no known vital or beneficial effects and pose serious health risks. In humans and animals, they disrupt protein synthesis and interfere with ATP production, thus contributing to the development of many diseases, including cancer (Ociepa-Kubicka, Ociepa 2012, Rai et al. 2019).

Vegetables are an abundant source of health-promoting compounds, including vitamins, minerals and secondary metabolites. However, vegetables (in particular leafy vegetables) can accumulate large amounts of nitrates. Vegetables account for 80% to 85% of total dietary nitrate intake. According to FAO/WHO experts, the acceptable daily intake of nitrates is up to 0.07 mg kg^{-1} body weight (ANJANA et al. 2007, VAN VELZEN et al. 2008, BIAN et al. 2020). The toxicity of nitrates(V) is low and they do not pose a direct threat to consumer health. However, nitrates(V) can be reduced to nitrates(III) under inadequate storage conditions, and the latter compounds are harmful to humans. As a result of nitrate(III) poisoning, blood pressure drops and hemoglobin is oxidized to methemoglobin, which is unable to transport oxygen to tissues (SANTAMARIA et al. 2001, SONG et al. 2015).

The onset on the vegetable growing season in spring is determined by weather conditions, in particular temperature. The local microclimate can be modified to accelerate the natural ripening process. For instance, plants can be covered with perforated polyethylene (PE) film and non-woven polypropylene (PP) fabric. According to SIWEK and LIBIK (2005), MAJKOWSKA-GADOMSKA (2010) and FRANCKE (2011), plastic covers have a beneficial influence on root zone temperature. Higher temperature stimulates plant growth, root system development and mineral uptake (SZULC, KRUCZEK 2008, OLLE, BENDER 2010).

The aim of this study was to determine the effect of plant covering with perforated PE film and non-woven PP fabric on the concentrations of selected micronutrients, heavy metals and nitrates(V) in arugula leaves.

MATERIALS AND METHODS

A two-factorial field experiment was conducted in the Garden of the Agricultural Research and Experiment Station of the University of Warmia and Mazury in Olsztyn (20°29′E, 53°45′N; 125 m a.s.l.). The experiment had a split-plot design with three replications in each year. The experimental factors were as follows:

– plant species: *Diplotaxis tenuifolia* (L.) D.C. (wall-rocket) – Enza Zaden and *Eruca sativa* Mill. (salad rocket) – Hortag Seed;

– type of plant cover: perforated PE film with 100 openings per m^2 and non-woven PP fabric with surface density of 17 g m^{-2}; in the control treatment, plants were grown without any covers.

All plants were grown on typical black earth soil of quality class IIIb and cereal-fodder strong complex in the Polish soil valuation system. Each year, the mineral composition of soil was determined before establishing the experiment. Throughout the study, the average content of soil minerals (mg dm^{-3}) was as follows: N-NO_3 – 32, phosphorus (P) – 121, potassium (K) – 97, calcium (Ca) – 2340, magnesium (Mg) – 166, chlorine (Cl) – 11, Cd – 0.07, Pb – 0.15; pH in H_2O – 7.49, soil salinity – 0.39 g NaCl dm^{-3}.

Chemical analyses of soil were performed by standard methods. $N\text{-}NO_3$ content determined by the colorimetric method with the use of phenyldisulfonic acid (UV-1201V spectrophotometer, Shimadzu Corporation Kyoto, Japan). Phosphorus (P) was determined by the vanadium molybdate yellow colorimetric method (UV-1201V spectrophotometer, Shimadzu Corporation Kyoto, Japan), calcium (Ca) and potassium (K) was determined by atomic emission spectrometry (AES) (BWB Technologies UK Ltd. Flame Photometers). Magnesium (Mg) was extracted with 0.01 M $CaCl_2$ and determined by atomic absorption spectrophotometry (AAS) (AAS1N, Carl Zeiss Jena, Germany). Salinity was determined by the conductometric method, Conductivity Meter N5773 (Teleko Wrocław, Poland), chloride content (Cl) and pH in H_2O using the potentiometric method. The concentrations of the analyzed micronutrients and heavy metals were determined by atomic absorption spectrometry (AAS).

Based on an analysis of soil nutrient availability and the nutrient requirements of arugula, 90 kg N ha^{-1} (ammonium nitrate) and only 50 kg K ha^{-1} (60% KCl) – due to relatively high K abundance in soil – were applied before seeding. Phosphate fertilizers were not used. The recommended tillage practices were applied.

Each year, seeds were sown in mid-April. Plot area was 1.0 m^2, and row spacing was 20 cm. Immediately after seeding, the plots were covered with PE film or non-woven PP fabric. The covers were removed after approximately five weeks. During the growing season, weeds were removed manually, soil was loosened and plants were watered according to needs. Leaves were harvested gradually, one to three times from each treatment.

Chemical analyses of leaves were performed on dry plant material collected at first harvest, in three replications. Averaged leaf samples from each treatment were dried to constant mass at a temperature of 65°C in the dryer Binder ED400 (Binder GmbH, Tuttlingen, Germany) and then ground in a laboratory knife mill Grindomix GM300 (Retsch, Germany). The levels of nitrates(V) in the edible parts of arugula plants were determined colorimetrically with the use of salicylic acid.

The concentrations of selected micronutrients and heavy metals in arugula leaves were determined after wet digestion in a mixture of acids: HNO_3+$HClO_4$+HCl on a CEM Mars 5 Digestion Oven microwave mineralizer (CEM Corporation, Matthews, US). The content of cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), chromium (Cr), zinc (Zn) and manganese (Mn) was determined by AAS (AA-6800, Shimadzu Corporation, Kyoto, Japan). Because of minor differences in the obtained values, the results were presented as means for the years of the study. Zn : Cu and Mn : Zn weight ratios were calculated.

The results were processed statistically by analysis of variance (ANOVA) in the Statistica program. The significance of differences between means was evaluated by creating multiple confidence intervals in the Tukey's test at a significance level of α=0.05 (TIBCO Software 2017).

RESULTS AND DISCUSSION

Tables 1 and 2 present the results of laboratory analyses of heavy metal and micronutrient concentrations in the leaves of *D. tenuifolia* and *E. sativa*, depending on the type of plant cover. Both experimental factors, i.e. plant

Table 1

The effect of plant species and the type of plant cover on the heavy metal content of arugula leaves

Plant species	Type of plant cover	Cd	Pb	Ni
		(mg kg^{-1} DM)		
Diplotaxis tenuifolia (L.) DC.	PE film non-woven PP fabric without cover	0.294 0.262 0.181	0.836 0.546 1.932	1.923 2.813 3.442
Mean		0.245	1.105	2.726
Eruca sativa Mill	PE film non-woven PP fabric without cover	0.633 0.710 0.799	3.257 3.808 3.160	5.977 5.138 5.994
Mean		0.714	3.408	5.703
Mean for the type of plant cover	PE film non-woven PP fabric without cover	0.464 0.486 0.490	2.047 2.177 2.546	3.950 3.976 4.718
$LSD_{0.05}$ for: I – plant species II – type of plant cover I x II – interaction		 0.015 0.018 0.026	 0.226 0.277 0.391	 0.231 0.282 0.399

species and cover type, had a significant effect on the accumulation of all analyzed heavy metals (including micronutrients) in the edible parts of both plant species.

The Cd content of arugula leaves varied widely from 0.181 (uncovered *D. tenuifolia* plants) to 0.799 mg kg^{-1} DM (uncovered *E. sativa* plants). Average Cd concentrations were nearly three-fold higher in the leaves of *E. sativa* than in the leaves of *D. tenuifolia* (0.714 vs. 0.245 mg kg^{-1} DM). The Cd content of *E. sativa* leaves was significantly higher in plots where covers were not used compared with those covered with PE film. The leaves of *D. tenuifolia* covered with polyethylene foil, contained the most cadmium. In the experiments conducted by Moreno et al. (2001, 2002a) and Kalisz et al. (2012), Chinese cabbage grown under PP floating row covers had the highest Cd content.

Table 2

The effect of plant species and the type of plant cover on the micronutrient content of arugula leaves

Plant species	Type of plant cover	Cu	Cr	Zn	Mn
		(mg kg^{-1} DM)			
Diplotaxis tenuifolia (L.) DC.	PE film non-woven PP fabric without cover	4.453 4.315 3.936	5.468 7.168 8.147	86.58 107.1 67.08	47.04 43.13 51.58
Mean		4.234	6.927	86.90	47.25
Eruca sativa Mill	PE film non-woven PP fabric without cover	7.223 7.957 9.045	5.977 9.019 9.030	113.2 127.3 144.8	70.50 72.26 88.43
Mean		8.075	8.008	128.4	77.06
Mean for the type of plant cover	PE film non-woven PP fabric without cover	6.088 6.136 6.490	5.722 8.093 8.588	99.89 117.2 105.9	58.77 57.69 70.00
$LSD_{0.05}$ for: I – plant species II – type of plant cover I x II - interaction		 0.197 0.241 0.341	 0.351 0.429 0.607	 3.439 4.211 5.956	 3.185 3.901 5.517

The Pb content of the analyzed plant material ranged from 0.546 to 3.808 mg kg^{-1} DM, in the following order: *D. tenuifolia* plants covered with non-woven PP fabric < *D. tenuifolia* plants covered with perforated PE film < uncovered *D. tenuifolia* plants < uncovered *E. sativa* plants < *E. sativa* plants covered with perforated PE film < *E. sativa* plants covered with non-woven PP fabric. *Eruca sativa* tended to accumulate larger amounts of Pb. Average Pb concentrations were nearly three-fold higher in the leaves of *E. sativa* than in the leaves of *D. tenuifolia*. The greatest amount of lead was found in the edible parts of *D. tenuifolia* plants grown without cover, while in *E. sativa* – leaves covered with non-woven PP fabric. Moreno et al. (2001, 2002*a*) demonstrated that the leaves of Chinese cabbage grown under PP floating row covers had the lowest Pb content, and average Pb concentrations were 19% higher in uncovered plants and in plants covered with perforated PE film.

The Ni content of arugula leaves ranged from 1.923 (*D. tenuifolia* grown under PF film) to 5.994 mg kg^{-1} DM (uncovered *E. sativa* plants). Average Ni concentrations were more than two-fold higher in the leaves of *E. sativa* than in the leaves of *D. tenuifolia*. The Ni content of leaves was similar in plants of both species grown under both types of cover. Significantly higher Ni concentrations were noted in the leaves of uncovered plants. Díaz-Pérez (2013) found no correlations between shade levels and leaf concentrations of Ni in bell peppers.

The Cu content of leaves ranged from 3.936 in uncovered *D. tenuifolia* plants to 9.045 mg kg^{-1} DM in uncovered *E. sativa* plants. Average Cu concentrations were nearly two-fold higher in *E. sativa*. The Cu content of leaves in *E. sativa* and *D. tenuifolia* varied significantly depending on the cover type, as follows: perforated PE film < non-woven PP fabric < uncovered plants. In a study by Caruso et al. (2020), the Cu content of *D. tenuifolia* leaves decreased with decreasing light intensity. Hernandez et al. (2004) also observed that Cu concentrations were significantly higher in Chinese cabbage plants grown in the open air than in those grown under row covers of PP sheeting.

The Cr content of arugula leaves ranged from 5.468 (*D. tenuifolia* plants covered with PE film) to 9.030 (uncovered *E. sativa* plants) mg kg^{-1} DM, and it was more than 15% higher on average in *E. sativa* than in *D. tenuifolia*. Plant material harvested in plots covered with perforated PE film had the lowest Cr content, which was 41% and 50% lower compared with the leaves of plants covered with non-woven PP fabric and uncovered plants, respectively. In a study by Moreno et al. (2005), the Cr content of uncovered Chinese cabbage plants was 11% lower compared with plants covered with PE film, and 20% higher compared with plants covered with PP floating row covers.

Zinc content was highest in the edible parts of uncovered *E. sativa* plants (144.8 mg kg^{-1} DM) and lowest in uncovered *D. tenuifolia* plants (67.08 mg kg^{-1} DM). The leaves of *E. sativa* contained more Zn (128.4 mg kg^{-1} DM on average) than the leaves of *D. tenuifolia* (86.9 mg kg^{-1} DM on average). The Zn content of leaves in *E. sativa* and *D. tenuifolia* varied significantly depending on the cover type, as follows: perforated PE film < uncovered plants < non-woven PP fabric. Kalisz et al. (2012) investigated the effect of row covers of non-woven fleece on heavy metal accumulation in Chinese cabbage and found that the Zn content of leaves was similar in uncovered and covered plants. Caruso et al. (2020) reported that the Zn content of leaves in perennial wall rocket was higher when plants were grown under optimal light conditions.

In the present study, the Mn content of arugula leaves ranged from 43.13 (*D. tenuifolia* plants covered with non-woven PP fabric) to 88.43 mg kg^{-1} DM (uncovered *E. sativa* plants). The average Mn concentration was 63% higher in the leaves of *E. sativa*. Plant material harvested in uncovered plots had significantly higher Mn content than plants covered with PE film and non-woven PP fabric (by 19% and 21%, respectively). In studies investigating *D. tenuifolia* (Caruso et al. 2020) and *E. sativa* (Acikgoz 2011), the Mn content of leaves increased with increasing light intensity. Kalisz et al. (2012) observed no significant differences in the Mn content of Chinese cabbage grown in the open field or covered with non-woven fleece.

In the analyzed group of heavy metals, the maximum permissible levels in foodstuffs have been set only for Pb and Cr. In the present experiment, the concentrations of both heavy metals were below the maximum levels for leafy vegetables in all treatments (Commission Regulation (EU) No 488/2014, Commission Regulation (EU) 2015/1005).

The quality of edible plant parts is also affected by micronutrient ratios. According to TARIQ and MOTT (2006), the Zn:Cu ratio should remain in the range of 4.9 to 7.2. In the current study, the above ratio was too wide in all treatments, ranging from 15.66 (*E. sativa* plants covered with PE film) to 24.81 (*D. tenuifolia* plants covered with non-woven PP fabric). According to the cited authors, the optimal Mn:Zn ratio is 3.2 to 5.8. In the present experiment, the Mn:Zn ratio in the leaves of *D. tenuifolia* and *E. sativa* was too narrow, ranging from 0.40 to 0.77 (Table 3). Similar values were reported by PITURA and MICHAŁOJĆ (2015) for leafy vegetables: lettuce, curly kale and celery.

Table 3

The effect of plant species and the type of plant cover on weight ratios between micronutrients in arugula leaves

Plant species	Type of plant cover	Zn : Cu	Mn : Zn
Diplotaxis tenuifolia (L.) DC.	PE film non-woven PP fabric without cover	19.44 24.81 17.04	0.54 0.40 0.77
Mean		20.53	0.54
Eruca sativa Mill	PE film non-woven PP fabric without cover	15.66 16.00 16.00	0.62 0.57 0.61
Mean		15.91	0.60
Mean for the type of plant cover	PE film non-woven PP fabric without cover	16.41 19.10 16.32	0.59 0.49 0.66

Plants of the genera *Diplotaxis* and *Eruca* have a physiological tendency to accumulate excessive concentrations of nitrates(V) (SANTAMARIA 2006, WEIGHTMAN 2012). Therefore, the maximum permissible levels of nitrates in plants of the above genera are higher than in other leafy vegetable species (Commission Regulation (EU) No 1258/2011). According to the Commission Regulation, the maximum levels of nitrates(V) in the leaves of *D. tenuifolia* and *E. sativa* harvested from 1 April to 30 September and from 1 October to 31 March are 6000 and 7000 mg NO_3 kg FM, respectively.

In the current study, the concentrations of nitrates(V) in the edible parts of arugula plants ranged from 1114.2 to 1835.9 mg NO_3 kg^{-1} FM (Table 4), and they did not exceed the maximum permissible levels set for rucola (Commission Regulation (EU) No 1258/2011) in any of the treatments. The average nitrate(V) content of leaves was 26% higher in *D. tenuifolia* than in *E. sativa*. In the work of LENZI and TESI (2000), the leaves of *D. tenuifolia* had a two-fold higher content of nitrates(V) than the leaves of *E. sativa*. Nitrate(V) accumulation was significantly affected by the type of plant cover. In both analyzed plant species, nitrate(V) concentrations were highest in plants covered with non-woven PP fabric and lowest in uncovered

Table 4

The effect of plant species and the type of plant cover on the nitrate(V) content of arugula leaves

Plant species	Type of plant cover	Nitrates(V) (mg NO_3^- kg^{-1}) FM
Diplotaxis tenuifolia (L.) DC.	PE film non-woven PP fabric without cover	1368.1 1835.9 1182.5
Mean		1462.2
Eruca sativa Mill	PE film non-woven PP fabric without cover	1154.8 1209.4 1114.2
Mean		1159.5
Mean for the type of plant cover	PE film non-woven PP fabric without cover	1261,5 1522,7 1148.4
$LSD_{0.05}$ for: I – plant species II – type of plant cover I x II – interaction		 28.3 34.7 49.0

plants. This results from the fact that light conditions were least desirable under non-woven PP fabric covers. In all treatments, nitrate(V) concentrations were considerably below the maximum levels. Light transmission for PE film (0.03 mm) and non-woven PP fabric (17 g m^{-2}) is 79-90% and 72-83% on average, respectively. According to Colonna et al. (2016), leafy vegetables accumulate excessive levels of nitrates under low light conditions. Similar observations were made by Francke (2005) in *E. sativa* and Caruso et al. (2000) in *D. tenuifolia*. Santamaria et al. (2001) demonstrated that under conditions of low light availability, an increase in temperature stimulated nitrate accumulation in rocket leaves. Increased accumulation of nitrates(V) in the leaves of Chinese cabbage grown under floating row covers (in particular PP Agryl-P17) was also reported by Moreno et al. (2002*b*). The above results are consistent with the findings of Biesiada and Kędra (2012) who analyzed garden dill, and Govedarica-Lučić and Perković (2013) who tested various types of covering in different lettuce varieties.

CONCLUSIONS

1. The concentrations of all analyzed micronutrients and heavy metals in arugula leaves were affected by both experimental factors, i.e. plant species and cover type.

2. The leaves of *E. sativa* had a higher content of Cd, Pb, Ni, Cu, Cr, Zn and Mn than the leaves of *D. tenuifolia.*

3. The use of covers contributed to a decrease in the accumulation levels of micronutrients and heavy metals in the edible parts of arugula plants. The leaves of plants covered with PE film had the lowest content of Cd, Pb, Ni, Cu and Cr, whereas Mn content was lowest in the leaves of plants covered with non-woven PP fabric.

4. A widened Zn : Cu ratio was noted in all treatments, whereas Mn:Zn ratios were regarded as too narrow, relative to the normal ranges.

5. The concentrations of nitrates(V) were higher in the leaves of *D. tenuifolia*. Plants of both species covered with non-woven PP fabric accumulated the highest levels of nitrates(V).

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