

Jolanta MOLAS<sup>1\*</sup>, Roman PRAŻAK<sup>2</sup>, Anna KRZEPIŁKO<sup>3</sup>,  
Sławomir MICHAŁEK<sup>1</sup> and Agata ŚWIĘCŁO<sup>2</sup>

## NITRATE CONTENT IN LEAVES OF VEGETABLE AND HERBAL PLANTS FROM HOME GARDENS OF AGROTOURIST FARMS

### ZAWARTOŚĆ AZOTANÓW W LIŚCIACH ROŚLIN WARZYWNYCH I ZIELARSKICH Z OGRODÓW GOSPODARSTW AGROTURYSTYCZNYCH

**Abstract:** The objective of the research study was to compare of the nitrate content in leafy vegetables (lettuce, spinach) and herbal plants (basil, peppermint, oregano and thyme) from the home gardens of agrotourist farms located in the Central Roztocze region. The obtained results have shown that the content of nitrates in the studied plants depended on the plant species, physiological phase of plant development and the method of plant cultivation. The content of nitrates in the leaves of studied plant species was the following: lettuce > spinach > basil > peppermint > oregano >> thyme. Plants cultivated by conventional method accumulated the largest amounts of nitrates; when integrated method was used, less nitrates were accumulated and when organic method was used, the content of accumulated nitrates was the smallest. At the early stage of vegetative development the plants contained twice as much nitrates as the plants at the ripe vegetative stage. At the juvenile vegetative stage the content of nitrates exceeded the legally permissible content in lettuce, spinach and basil from conventional and integrated gardens. At the ripe vegetative stage the content of nitrates in all the studied plant species did not exceed the legally permissible content of these compounds in leafy vegetables with a short vegetative term.

**Keywords:** agrotourism, antinutritious components, leafy vegetables, nitrate(V), nitrate phytoaccumulation, nitrogen fertilization, spice plants, plant cultivation methods

Most agrotourist farms located in the Central Roztocze have home gardens in which vegetables and ornamental plants are grown. Herbal plants are grown less often but their

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<sup>1</sup> Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Akademicka 14, 20-950 Lublin, Poland, phone: +48 81 445 69 61.

<sup>2</sup> Faculty of Agrobioengineering, University of Life Sciences in Lublin, Akademicka 14, 20-950 Lublin, Poland, phone: +48 81 445 69 61.

<sup>3</sup> Faculty of Food Sciences and Biotechnology, University of Life Sciences in Lublin, Akademicka 14, 20-950 Lublin, Poland, phone: +48 81 445 69 61

\* Corresponding author: jolanta.molas@up.lublin.pl

cultivation has become more and more popular recently. In spite of the fact that these farms do not have certificates of ecological farms, the plants are usually grown by ecological methods, based on organic fertilization. The existing studies have shown that the plants cultivated by organic method have a much larger nutritional value and are healthier than plants cultivated by conventional method [1–7].

One of the main indicators of health of consumption plants is their content of nitrates [8, 9]. In spite of the fact that nitrates are antinutritive, they are not toxic by themselves and do not pose a direct threat to man's health. They are relatively quickly absorbed in the digestive tract and are eliminated in urine in unchanged form. However, some amount of nitrates are reduced to toxic nitrites by microflora of the digestive tract, which, in turn become nitrogen oxide and ammonia [10, 11]. While nitrites cause many dangerous diseases [8, 9, 12, 13], the products of their reduction (nitrogen oxide and nitric acid) prevent cardiological disorders, control blood pressure and increase insulin secretion, have anti-obesity and antibacterial effects [14–19].

In Poland and in other EU countries a permissible content of nitrates in vegetables and food products has been clearly defined by Ordinance of the Minister of Health [20] and European Commission Regulation (EC) [21]. However, the permissible content of these compounds in herbal plants used not only for cooking but also for medicinal purposes, has not been determined so far. It needs to be emphasized that in accordance with Ordinance of the Minister of Health [22] the vegetables and other plants cultivated on farms can be grown and introduced to the market without a quality control. Consequently, it is possible that the vegetables and herbal plants grown on such farms can have excessive amounts of nitrates, which significantly influences the biological value of food and pharmaceuticals as well as the quality of agrotourist services offered to customers.

In this study the content of nitrates in the leaves of vegetables and herbal plants depending on their species, physiological stage of development and cultivation method in home gardens of agrotourist farms were compared.

## Materials and methods

The experiments were conducted in the Central Roztocze (County of Zamojski, Province of Lubelskie) in home gardens of agrotourist farms. The Central Roztocze is a major tourist attraction in the County of Zamojski. The plants were grown in 6 agrotourist farms which have home gardens where vegetables and ornamental plants and recently also herbal plants have been cultivated. For several years plants have been cultivated in these gardens by the following methods:

- organic method, based exclusively on organic fertilization (these gardens are referred to as organic gardens #1 and #2 below);
- integrated method, based on organic fertilization supplemented with mineral fertilization to satisfy the physiological needs of plants (referred to as integrated gardens #3 and #4 below);
- conventional method, based on mineral fertilization (referred to as conventional gardens #5 and #6 below).

In all the studied gardens there was brown soil, formed on loess. In the year preceding plant cultivation in organic and integrated gardens (#1, 2, 3 and 4) manure was used as a fertilizer whereas in conventional gardens (#5 and 6) mineral phosphorus and potassium fertilizers were used. In the year of cultivation (spring) compost and a solution of Humvit BIO preparation were used and in integrated and conventional gardens – mineral nitrogen and phosphorous fertilizers.

Research material were leafy vegetables and herbal plants with short vegetation term. The vegetables included lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) and herbal plants – basil (*Ocimum basilicum* L.), peppermint (*Mentha piperita* L.), oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.). The same cultivars of the particular species were grown in all the gardens. Plants were collected for research at two stages of their development, i.e. at the juvenile (early) vegetative stage (the second half of May) and at the ripe vegetative stage (at the beginning of July). The mass of single sample of leaves of lettuce, spinach, basil, peppermint and oregano and non-ligneous leafy shoots of thyme was 50 g. 5 single samples from each species were collected from every garden; they were cleaned, washed and flaked and then 5 g samples were made from them. Nitrate content in plants is determined many methods [23]. In our study the content of nitrates in plant fresh mass samples was determined by ionometric method. The analysis was carried out using nitrate ion-selective electrode (ISE) made by Orion. Measurements were taken in 0.4 M  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  solution, in which nitrate were extracted from plant material. Results were read directly from logarithmic scale of ion analyzer in  $\text{mg NaNO}_3 \cdot \text{dm}^{-3}$  of extracted solution. After taking into account the size of sample weight (5g mean mass) and the quantity of extraction solution ( $100 \text{ cm}^3$ ), the obtained result was multiplied by factor/number/20. Obtained results were presented in tables in  $\text{mg N-NO}_3 \cdot \text{kg}^{-1}$  of the biological material.

**Statistical analysis:** Variance analysis (ANOVA) of obtained data was carried out, followed by a test checking the significant difference with probability of 5 %,  $LSD_{5\%}$ .

## Results and discussion

Results of chemical analysis have shown that the content of nitrates in vegetables and herbal plants grown in home gardens of selected agrotourist farms in the Central Roztocze depended on the species, physiological stage of plant development and plant cultivation method (Table 1 and 2). The studies conducted so far have shown that the ability of plants to accumulate nitrates is their genotype [12, 13, 24–30]. This relation was also confirmed by the results of the experiments. As Table 1 and Table 2 show, leafy vegetables (lettuce and spinach) have more nitrates than all the other species of herbal plants. As regards susceptibility to accumulation of nitrates, the studied plants can be arranged in the following order: lettuce > spinach > basil > peppermint > oregano >> thyme. The content of nitrates depended also on plant organ. As a rule, larger content of nitrate occurs in assimilation and vascular tissues than in other tissues; also, more of these compounds are found in leaves, petiole and the stem than in the root, fruit and seeds [12, 24]. As a result, leafy vegetables usually accumulate the largest amounts of nitrate, root vegetables assimilate smaller amounts of nitrates and fruit and

Table 1

Nitrate content [ $\text{mg N-NO}_3 \cdot \text{kg}^{-1}$  f.m.] in vegetables and herbal plants at the early stage of their vegetative development

Plant species	Method of plant cultivation in garden						$LSD_{5\%}$
	organic		intergrate		convectional		
	1	2	3	4	5	6	
Lettuce	2080	1914	4603	4458	4772	4529	272
Spinach	1968	2055	3875	4117	4576	4850	279
Basil	1872	1738	3187	3099	3770	3972	193
Peppermint	1824	1692	2720	2864	3015	3127	167
Oreganum	1542	1597	2189	2075	2673	2459	219
Thyme	453	369	627	593	685	702	119
$LSD_{5\%}$	208	224	168	360	276	293	—

f.m. – fresh mass.

Table 2

Nitrate content [ $\text{mg N-NO}_3 \cdot \text{kg}^{-1}$  f.m.] in vegetables and herbal plants at the ripe stage of their vegetative development

Plant species	Method of plant cultivation in garden						$LSD_{5\%}$
	organic		intergrate		convectional		
	1	2	3	4	5	6	
Lettuce	1005	962	1590	1353	2117	1985	146
Spinach	1207	1065	1411	1476	2027	1879	153
Basil	1017	896	1107	1219	1757	1657	129
Peppermint	784	903	980	1005	1521	1397	131
Oreganum	753	679	792	934	1356	1309	147
Thyme	207	165	265	312	517	469	58
$LSD_{5\%}$	176	114	129	112	206	115	—

seed vegetables accumulate the least. The content of nitrates in the leaves of all the studies plants was many times larger than in non-ligneous leafy shoots of thyme (Table 1 and 2). The ability of consumption plants to accumulate nitrates and nitrites has been studied thoroughly because the share of these plants in man's diet is the largest (50–75%). It is estimated that about 85% of nitrates in man's diet come from vegetables [12, 13, 29]. Less is known about the content of nitrates in herbal plants and the issue has not been studied so thoroughly as in vegetables. Recorded content of these compounds at the ripe vegetative stage of herbal plants corresponds more or less to the content studied by other authors [26–28].

As study results presented in Table 1 and 2 show, the content of nitrates both in leafy vegetables and in herbal plants depended on the physiological stage of plant development. The content of nitrates in all the plants at early stage of their vegetative

development (Table 1) was about two times larger than at the ripe stage of vegetative development (Table 2).

It is a natural phenomenon and is connected with the relation between assimilative reduction of  $\text{NO}_3^-$  ions and the activity of photosynthesis and the supply of organic carbon skeletons for organic biosynthesis [31–34]. If the supply of carbon skeletons is small, the activity of reductase at the first stage of assimilative reduction of nitrates catalyzed by nitrate reductase (NR) may be hindered by the products of this reaction by way of feedback, which leads to excessive accumulation of nitrates.

Due to the fact that vegetables and potatoes are a source of as much as about 85% of nitrates in man's diet, both in Poland and in the EU legally permissible content of these compound in these plants has been defined [13, 20, 21]. Pursuant to these regulations the legally permissible limit of contamination with nitrate(V) in leafy vegetables with a short vegetation term is: in lettuce 2500–4000  $\text{mg NO}_3^- \cdot \text{kg}^{-1}$  of average mass, in fresh spinach 2500–3000  $\text{mg NO}_3^- \cdot \text{kg}^{-1}$  of average mass [20]. The legally permissible content of nitrates in herbal plants used for cooking and medicinal purposes has not been determined so far. Taking into account the fact that the vegetation term of the studied herbal plants is short and it is their leaves that are used, the assessment of the content of nitrates in their usable organs was conducted by comparing the measured content to the legally permissible content of N- $\text{NO}_3$  in lettuce and spinach (Table 3 and 4).

Table 3

The percentage of legally permissible content of nitrates in plants at the early stage of their development

Plant species	Method of plant cultivation in garden					
	organic		intergrate		convectional	
	1	2	3	4	5	6
Lettuce	52.0 <sup>a</sup>	47.8	115.1	111.4	119.3	113.2
Spinach	65.6 <sup>b</sup>	68.5	129.2	137.2	152.5	161.7
Basil	46.8 <sup>a</sup> (62.4) <sup>b</sup>	43.4 (57.9)	79.7 (106.2)	77.5 (103.3)	94.2 (125.7)	99.3 (132.4)
Peppermint	45.6 <sup>a</sup> (60.8) <sup>b</sup>	42.3 (56.4)	68.0 (90.7)	71.6 (95.5)	75.4 (100.5)	78.2 (104.2)
Oreganum	38.5 <sup>a</sup> (51.4) <sup>b</sup>	39.9 (53.2)	54.7 (72.9)	51.9 (69.2)	66.8 (89.1)	61.5 (82.0)
Thyme	11.3 <sup>a</sup> (15.1) <sup>b</sup>	9.2 (12.3)	15.7 (20.9)	14.8 (19.8)	17.1 (22.8)	17.5 (23.4)

100 % – legally permissible maximum content of nitrates: <sup>a</sup> in lettuce = 4000  $\text{mg N-NO}_3 \cdot \text{kg}^{-1}$  f.m. and <sup>b</sup> in spinach = 3000  $\text{mg N-NO}_3 \cdot \text{kg}^{-1}$  f.m. [20].

As Table 3 shows, the content of nitrates in very young plants of all the species does not exceed their permissible content in leafy vegetables whose vegetation term is short. Lettuce and spinach contained about 50–70% of permissible content of nitrates in their

biomass. Herbal plants contained from 9.2 to 60.8% of permissible content of N-NO<sub>3</sub> in lettuce and spinach. In leafy vegetables from integrated and conventional gardens the content of nitrates exceeded the permissible content, more so in spinach than in lettuce. The content of nitrates in the studied herbal plants from all the 6 gardens was smaller than the permissible content of these compounds in lettuce. In basil grown in integrated and conventional gardens and in peppermint grown in conventional gardens the content of N-NO<sub>3</sub> was larger (basil) or similar (peppermint) to legally permissible content of these compounds in spinach.

At the vegetative ripe stage no content of N-NO<sub>3</sub> exceeding the legally permissible content was observed in any of the species (Table 4). At the vegetative ripe stage the content of nitrates in leafy vegetables from organic, integrated and conventional gardens ranged from 38.5 to 84.7% of their legally permissible content in these plants (Table 4). In oregano, peppermint and basil from these gardens it ranged from 27.2 to 60.8% of the legally permissible content in leafy vegetables with a short vegetation term. In thyme the content of nitrates was the smallest and ranged from 6.6 to 20.7%. Regardless of plant cultivation method, all the studied plants at their mature stage met the criteria of health as regards the content of nitrates in their leaf biomass.

Table 4

The percentage of legally permissible content of nitrates in plants at the ripe stage of their development

Plant species	Method of plant cultivation in garden					
	organic		intergrate		convectional	
	1	2	3	4	5	6
Lettuce	40.2	38.5	63.6	54.1	84.7	79.4
Spinach	48.3	42.6	56.4	59.0	81.1	75.2
Basil	40.7	35.8	44.3	48.8	70.3	66.3
Peppermint	31.4	36.1	39.2	40.2	60.8	55.9
Oreganum	30.1	27.2	31.7	37.4	54.2	52.4
Thyme	8.3	6.6	10.6	12.5	20.7	18.8

100 % – legally permissible content of nitrates in lettuce and spinach at the ripe vegetative stage = 2500 mg N-NO<sub>3</sub> · kg<sup>-1</sup> f.m. [20].

Accumulation of nitrates in the studied vegetables and herbal plants depended to a large extent on their cultivation method (Tables 1–4). The content of these compounds in plants grown by organic method was the smallest; it was larger in plants grown by integrated method and the largest in those grown by conventional method. This relation was observed at both stages of plant vegetative development. These results correspond to the results of studies conducted by other authors, according to whom plants from ecological production, based on organic fertilization have a much smaller content of nitrates than those grown by conventional method [2, 3, 6, 7, 27, 28, 35, 36]. The results of conducted studies have also shown that vegetables and leafy herbs grown by integrated method accumulate relatively large, even excessive amounts of nitrates at the

early stage. It is a result of mineral fertilization applied in both cultivation methods before their vegetation, i.e. in spring. Mineral nitrogen fertilizers introduce  $\text{NO}_3^-$  ions into the environment of plant growth, preferred and directly absorbed by plants in spite of the fact that the process requires metabolic energy [37]. Organic fertilizers, on the other hand, have a slowed-down effect and the release of absorbable forms of nitrogen, including  $\text{NO}_3^-$  ions, is connected with microbiological decomposition of the fertilizer in the soil.

## Conclusions

1. The studied species of vegetables and herbal plants grown by organic, integrated and conventional methods in home gardens of agrotourist farms differed in respect of susceptibility to accumulation of nitrates. In terms of their susceptibility to accumulation of N- $\text{NO}_3$  these species can be arranged as follows: lettuce > spinach > basil > peppermint > oregano >> thyme.

2. The smallest amounts of nitrates were accumulated in the leaves of vegetables and herbal plants cultivated by organic method, larger amounts by plants cultivated by integrated method and the largest amounts by plants cultivated by conventional method.

3. The accumulation of nitrates in the leaves of all the six plant species was almost two times smaller at the ripe stage than at the early stage of their vegetative development. The content of nitrates was larger than the legally permissible content in Poland and the EU in leafy vegetables whose vegetation term is short only in the leaves of young lettuce, spinach and basil grown in the integrated and conventional gardens.

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## ZAWARTOŚĆ AZOTANÓW W LIŚCIACH ROŚLIN WARZYWNYCH I ZIELARSKICH Z OGRODÓW GOSPODARSTW AGROTURYSTYCZNYCH

<sup>1</sup> Wydział Ogrodnictwa i Architektury Krajobrazu,

<sup>2</sup> Wydział Agrobioinżynierii,

<sup>3</sup> Wydział Nauk o Żywności i Biotechnologii,

Uniwersytet Przyrodniczy w Lublinie, Lublin

**Abstrakt:** Celem przeprowadzonych badań było porównanie zawartości azotanów w liściach roślin warzywnych (sałata, szpinak) i zielarskich (bazylija, mięta pieprzowa, lebiodka pospolita zwana również oregano, tymianek) uprawianych w ogrodach przydomowych gospodarstw agroturystycznych, zlokalizowanych na Roztoczu Środkowym. Uzyskane wyniki wykazały, że poziom fitoakumulacji azotanów zależał od gatunku i fizjologicznej fazy rozwoju rośliny oraz metody uprawy. Zawartość azotanów w liściach badanych gatunkach roślin kształtowała się następująco: sałata > szpinak > bazylija > mięta pieprzowa > lebiodka pospolita, zwana oregano >> tymianek. Najwięcej azotanów gromadziły rośliny uprawiane metodą konwencjonalną, mniej metodą integrowaną, a najmniej metodą organiczną. Rośliny we wczesnej fazie wegetatywnej zawierały około dwukrotnie więcej azotanów niż rośliny w dojrzałej fazie wegetatywnej. W juwenilnej fazie wegetatywnej zawartości azotanów przekraczały prawie dopuszczalne ich zawartości w sałacie, szpinaku i bazylii z ogrodów konwencjonalnych i integrowanych. W dojrzałej fazie wegetatywnej zawartości azotanów we wszystkich badanych gatunkach roślin nie przekroczyły prawie dopuszczalnych zawartości tych związków w warzywach liściowych o krótkim okresie wegetacji.

**Słowa kluczowe:** agroturystyka, azotany(V), fitoakumulacja azotanów, metody uprawy roślin, nawożenie azotowe, rośliny przyprawowe, składniki antyodżywcze, warzywa liściowe