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Content of Amino Acids in Maize and Yellow Lupine after Fluorine Application to Soil

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Abstract: Pollution of the natural environment with fluorine compounds makes the continuous monitoring of the content of this element necessary, not only in the air, water and soil, but also in food. A high level of fluorine contamination of soils can affect quality of the obtained yields, affecting the amino acid composition of protein. It often becomes a cause of reducing nutrition and feed value of agricultural yields. In the undertaken studies, the influence of fluorine application to the soil on the amino acid content in proteins accumulated in the above-ground parts (aerial) of *Zea mays* L. and *Lupinus luteus* L. was studied. The lowest and medium doses of fluorine had a significant positive effect on the content of exogenous and endogenous amino acids in the protein of the aerial mass of maize, for which the increase of sum of all amino acids was 7% and 8%, respectively, in relation to controls. The above-ground parts of yellow lupine were characterised by a much higher content of the tested amino acids than maize. The subsequent influence of soil contamination with fluorine on the amino acid content in yellow lupine did not have the same effect as in the main plant, and the influence of fluorine was somewhat limited. With respect to yellow lupine, it was demonstrated that the lowest fluorine dose (100 mg F kg^{-1} of soil), did not have a beneficial influence on the sum of all amino acids. The medium and highest doses of fluorine depressed in a small degree the content of the sum of these compounds in the dry mass of yellow lupine.

Keywords: fluorine; amino acids; *Zea mays* L.; *Lupinus luteus* L.

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1. Introduction

Fluorine belongs to the elements considered to be widespread in nature, because it is 13th in terms of the content of the elements in the crust of Earth [1]. The sources of fluorine in the environment are natural minerals, mostly fluorite, apatite, muscovite and biotite. It also permeates the natural environment due to rock weathering processes and leaching by atmospheric precipitations. Furthermore, the presence of fluorine in atmospheric air is associated with its natural emission, which occurs during volcanic eruptions and from the surface of oceans and seas. Fluorine compounds are examples of the many toxic substances that enter the natural environment as a result of human activity [2,3]. The main anthropogenic source of soil pollution with fluorine is the emission of this element from production of phosphate fertilisers, aluminium, steel, glass, and ceramics, as well as the emission of gases from municipal heat and power plants and from household furnaces and fireplaces [4–6]. In areas where such emission is more intensive, there is a risk of considerable accumulation of fluorine in soil and plants, which deteriorates the quality thereof. Hence, constant monitoring of the content of this element in agricultural crops is necessary. Fluorine is one of the trace elements necessary for proper functioning of living organisms. However, it is characterised by a very narrow safety margin between safe dose and dose above which symptoms of adverse effects occur. For humans and animals, water and food are the natural source of fluorine. The threat here is the constant consumption of plant products from areas exposed to increased emissions of this element. Particular attention should be paid to resistant plants and accumulating in tissues excessive concentrations of fluorine, threatening the safety of food and feed [7,8]. Plants are the first link in the ecological chain most exposed to fluorine. The effect of fluorine on plants should be considered from two points of view. On the one hand, the influence of fluorine in industrial gases and dust is mentioned. Plants absorb gaseous fluorine compounds contained in the air through the stomata through gas exchange. Plants are most adversely affected by fluorine compounds that fall directly onto the plants [9]. On the other hand, equally important is the influence of fluorine contained in soil and its influence on plants [10], which has not been fully explained in the literature. The uptake of fluorine from the substrate in areas with a natural content of this element is usually low, because the fluorine contained in the soil is most often in a form inaccessible to plants. However, in the case of soil contamination, uptake by plants in excessive amounts may occur [11]. The appearance of the first symptoms of a negative impact of fluorine pollution on plants depends on many environmental factors, such as the type and concentration of fluorine, distance from the emission source, duration of exposure and meteorological conditions [12]. A typical sign of the toxic effect of fluorine compounds on plant organisms is the appearance of light brown necroses progressing from the apex to the edge of the leaf blade. Chloroses appear inside the leaf, which change the leaf colour [13]. Before the appearance of the first symptoms of poisoning, growth is inhibited and yield is reduced, as well as increased susceptibility to viral diseases and the action of various pathogens [9].

The research carried us thus far on the impact of fluorine on plants has mainly concerned the immediate effect of this element on morphology, biomass yield, and the chemical composition of plants. As for crop chemical composition, the focus has been on the shaping of total content of nutrients, for example nitrogen has been analysed predominantly in terms of the total protein content rather than its quality [7,8,14,15].

While evaluating the nutritional value of agricultural crops, it is recommended to include both the total protein content and the amino acid composition of protein. The shares of exo- and endogenous amino acids in protein play an important role in the determination of the protein's nutritive quality. Deficiency of just one exogenous amino acid worsens the quality of proteins and consequently of yields [7].

The objective of this study is to explore and clarify the effect of fluorine application to soil on amino acid content in above-ground (aerial) mass of *Zea mays* L. and *Lupinus luteus* L.

2. Materials and Methods

2.1. Experiment Design

A pot trial was set up in a vegetation house belonging to University of Warmia and Mazury in Olsztyn (Poland). The soil used in the experiment originated from the arable horizon (0–25 cm). The soil had the textural composition of loamy sand (grain size in mm: <0.002–1.89%; 0.002–0.050–18.61%; >0.050–79.50%) and the pH equal 5.89 in H_2O and 4.43 in KCl. The other soil properties were as follows: hydrolytic acidity—30.7 mM(+) kg^{-1}; available nutrients content—phosphorus 43.2 mg P, potassium 124.5 mg K, magnesium 30.0 mg Mg kg^{-1} of soil; content of total—organic carbon 6.0 g kg^{-1}, nitrogen 0.62 g kg^{-1}, and fluorine 125 mg F kg^{-1} of soil.

The test plants were maize (*Zea mays* L.) of the Reduta variety (FAO 230) and yellow lupin (*Lupinus luteus* L.) of the Mister variety. The sensitivity of both crops to fluorine contamination of soil was determined based on earlier studies carried out prior to the establishment of the proper pot experiments. Maize and yellow lupine are plant species commonly cultivated in Poland due to their wide use in animal nutrition. High protein content and a beneficial amino acid composition make lupine seeds a component of animal mixtures. Yellow lupine contains about 40% total protein. Compared to other legume seeds, lupine protein is poorer in lysine, and richer in methionine and cysteine. Recently, we have observed an increase in the acreage of legume crops. Such an increase in interest is caused by the introduction of a subsidy system for the cultivation of legume plants. An additional advantage of sowing legumes is the role these plants play in crop rotation, enriching the soil with nitrogen.

The fluorine application to soil (commercial form of potassium fluoride) in the experiment was 0, 100, 200 and 300 mg F kg^{-1} of soil. The choice of fluorine doses was estimated according to total fluorine average content in the Polish soils [7]. Yellow lupine was the second crop after maize. Fluorine was applied to soil only under the main crop. In order to meet the nutritional demand of both crops, mineral NPK fertilisation, identical in all experimental treatments (urea (46%) 111 mg N, 46% triple superphosphate 48 mg P, 57% potassium salt 111 mg K kg^{-1} of soil) was carried out.

The dried and sieved (1 cm diameter) soil was used in the experiment. In total, each experiment was composed of 16 objects in 3 replicates. The fluorine and fertilisers were carefully mixed with the soil and put into properly labelled polyethylene pots. Immediately after the pots were filled with soil (9 kg per pot), including the appropriate doses of the pollutant and fertilisers, the test plants were sown (13 plants per pot). While growing the plants in pots, the soil moisture was maintained at 60% of the capillary water capacity. Weather data during the experiment were typical: the average air temperature was 14.5 °C, with the maximum recorded temperature being 20.7 °C and the minimum temperature—7.1 °C, average air humidity—62.9%, while the length of the daytime from 9 h 4 min to 16 h 18 min. The plants' above-ground parts were harvested at the stage of technological maturity (in case of maize—BBCH69, 64 days after sowing and yellow lupine—BBCH65).

2.2. Plants and Soil Analysis

The samples of plant and soil material samples were taken for laboratory analyses. The plant biomass obtained from each pot was whole cut and dried at a temperature of 60 °C. After drying, all material was milled. The soil was analysed before the start of the experiment. The granulometric composition in the soil was determined using the method of laser diffraction apparatus Mastersizer 2000 Hydro G dispersion unit (Malvern, UK) [16], the pH in 1 M KCl by the potentiometric method, hydrolytic acidity by Kappen method [17], total organic carbon (TOC) using a Shimadzu TOC-L CSH/CNS analyser (Shimadzu Corporation, Kyoto, Japan) with a solid sample module SSM-5000A (Shimadzu Corporation, Kyoto, Japan) [18], total nitrogen by the Kjeldahl method [19], the available forms of phosphorus and potassium by the Egner–Riehm method [20], magnesium by the Shachtschabel method [20] and total fluorine by X-ray fluorescence spectrometry method (XRF) using Philips WD-XRF PW 2004 (Philips Research Corporation, Eindhoven, The Netherlands).

Amino acids analysis was done by ion-exchange chromatography with post-column derivatisation with ninhydrin using an automatic amino acid analyser AAA400 by INGOS (Praha, Czech Republic) [21]. Hydrolysis of previously prepared samples was carried out in hydrochloric acid with a concentration of 6 M dm^{-3} at the temperature of 110 °C (383 K) for 3 h. The exogenous amino acids (arginine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine, as well as seven endogenous amino acids, that is, alanine, cystine, glycine, aspartic acid, glutamic acid, proline and serine) were analysed. The results of the determination of the content of amino acids in the aerial mass of both plants are presented relative to the dry matter and total protein. In our summary of the content of exogenous amino acids, the nutritional value of the protein in both plants was assessed. To this end, the Oser Index was applied [22]. According to this approach, the percent exogenous amino acid contents are compared with the concentrations of the same amino acids in the white of a whole hen egg. Finally, the nutritive value of the tested protein was expressed as a geometric mean for all the analysed exogenous amino acids. For calibration of amino acid analyser the amino acid standard solution was used Sigma (Sigma-Aldrich Co. LLC, Saint Louis, MO, USA).

2.3. Statistical Analysis

The results of the experiments were processed statistically in a Statistica 12.0 software package (Statsoft Inc., Tulsa, OK, USA), applying two-factorial ANOVA tests. The least significant differences (LSD) were calculated at the significance level of $p \leq 0.05$ by applying the Duncan's test [23]. The relationships (polynomial regression equations, Pearson's simple correlation) between fluorine and amino acid contents in the plants were calculated.

3. Results

The increasing fluorine doses affected the amino acids composition in the maize aerial mass (Table 1).

Table 1. Influence of fluorine application to soil on the amino acid content in aerial mass of maize.

Amino Acids	Soil Contamination with Fluorine in mg F kg^{-1} of Soil									
	0	100	200	300	r	0	100	200	300	r
	in g 100 g^{-1} Total Protein (16 g N)					in g kg^{-1} DM				
Exogenous										
Arginine	2.87	3.55	3.61	2.45	−0.28	1.20	1.64	1.67	1.12	−0.09
Phenylalanine	3.41	3.07	3.63	2.65	−0.52	1.43	1.42	1.68	1.21	−0.27
Histidine	1.65	1.64	1.90	1.29	−0.42	0.69	0.76	0.88	0.59	−0.19
Isoleucine	2.72	2.94	2.88	2.13	−0.64 *	1.14	1.36	1.33	0.97	−0.38
Leucine	5.99	6.29	6.46	4.69	−0.60 *	2.51	2.91	2.99	2.14	−0.34
Lysine	2.53	2.59	2.62	1.82	−0.71 **	1.06	1.20	1.21	0.83	−0.50
Methionine	0.91	0.99	0.61	0.39	−0.90 **	0.38	0.46	0.28	0.18	−0.83 **
Threonine	2.79	3.55	3.68	2.32	−0.26	1.17	1.64	1.70	1.06	−0.11
Tyrosine	1.96	1.51	1.47	1.32	−0.92 **	0.82	0.70	0.68	0.60	−0.97 **
Valine	3.96	4.26	4.32	3.07	−0.58 *	1.66	1.97	2.00	1.40	−0.37
Sum of exogenous amino acids	28.80	30.40	31.18	22.14	-	12.06	14.06	14.42	10.10	-
$LSD_{0.05}$			2.67					1.20		
Endogenous										
Alanine	4.94	5.23	5.17	3.75	−0.68 *	2.07	2.42	2.39	1.71	−0.43
Cysteine	0.02	0.04	0.07	0.02	0.16	0.01	0.02	0.03	0.01	0.13
Glycine	3.92	4.15	4.28	3.16	−0.55	1.64	1.92	1.98	1.44	−0.28
Asparagine acid	6.95	7.70	7.52	5.41	−0.60 *	2.91	3.56	3.48	2.47	−0.35
Glutamic acid	7.57	7.81	8.43	4.60	−0.63 *	3.17	3.61	3.90	2.10	−0.48
Proline	4.18	4.67	4.22	3.81	−0.57 *	1.75	2.16	1.95	1.74	−0.16
Serine	3.32	3.74	3.81	2.48	−0.52	1.39	1.73	1.76	1.13	−0.32
Sum of endogenous amino acids	30.90	33.34	33.49	23.23	-	12.94	15.42	15.49	10.60	-
$LSD_{0.05}$			2.87					1.30		
Sum of total amino acids	59.70	63.74	64.67	45.37	-	25.00	29.48	29.91	20.70	-
$LSD_{0.05}$			5.54					2.49		

LSD (least significant difference) for F application to soil. *—correlation coefficient (r) significant at $p \leq 0.05$; **—correlation coefficient (r) significant at $p \leq 0.01$.

The sum of exogenous amino acids converted to 100 g of total protein (16 g N) ranged from 22.14 in the object contaminated with 300 mg F kg^{-1} of soil to 31.18 g in the one where the dose of the pollutant was 200 mg F kg^{-1} of soil. These data show that the lowest and medium doses of fluorine had a positive effect on the exogenous amino acid content in the protein of the aerial mass of maize. By raising the soil contamination to the highest fluorine dose, i.e., 300 mg F kg^{-1} of soil, a 23% decrease in the analysed amino acid content relative to the control was caused. This relationship, that is a decrease in the exogenous amino acid content in the protein of the aerial mass in response to the highest fluorine dose, concerned all the determined amino acids that belong to this group.

An increase of exogenous amino acids in the aerial mass of maize grew due to medium doses of fluorine. Tyrosine was an exception, where a gradual decrease in its content was observed starting from the lowest fluorine dose. Statistical analysis of the results proved a significant negative correlation between the soil with fluorine pollution and the content of isoleucine (−0.64 *), leucine (−0.60 *), and valine (−0.58 *) as well as a highly significant negative correlation for tyrosine (−0.92 **), methionine (−0.90 **) and lysine (−0.71 **).

The sum of the determined exogenous amino acids calculated per 1 kg DM of maize varied between 10.10 in the plants treated with 300 mg F kg^{-1} of soil to 14.42 g kg^{-1} DM in the object polluted with the medium dose of fluorine, i.e., 200 mg F kg^{-1} of soil. The content of exogenous amino acids in the dry matter of maize in the presence of the growing fluorine pollution of soil fully corresponded to their content expressed per 100 g of total protein in the aerial mass of maize. In this case, the soil contamination with fluorine highly significantly correlated with the content of methionine (−0.83 **) and tyrosine (−0.97 **).

The sum of endogenous amino acids relative to 100 g of total protein in the aerial mass of maize varied from 23.23 in the object polluted with the highest dose of fluorine to 33.49 g in the variant treated with 200 mg F kg^{-1} of soil. The results show that the lowest and medium doses of fluorine had a positive effect on the content of these amino acids in maize protein. The application of the highest dose, 300 mg F kg^{-1} soil, contributed to a decrease in the concentration of most of the analysed endogenous amino acids in comparison to the object not contaminated with fluorine. An analysis of correlations revealed a significant negative correlation between the dose of fluorine and the content of alanine (−0.68 *), aspartic acid (−0.60 *), glutamic acid (−0.63 *) and proline (−0.57 *).

The highest content of endogenous amino acids converted per dry matter was noted where the soil was polluted with the medium and higher doses of fluorine. When the soil pollution was increased by applying the highest fluorine dose, the endogenous amino acid content decreased by 18% in comparison with the control. The endogenous amino acid content in dry matter of maize against the background of the increasing soil pollution with fluorine was approximately the same as the one converted per total protein in the aerial mass of maize.

The total content of the exo- and endogenous amino acids determined in the aerial mass of maize ranged from 20.70 g in the object polluted with the highest dose of fluorine to 29.91 g kg^{-1} DM in the series polluted with 200 mg F kg^{-1} of soil. Thus, it was revealed that the lowest and medium pollution of soil with fluorine had a positive effect on the analysed amino acid content, both exo- and endogenous, in the dry matter of maize. Simultaneously, it was shown that the application of the highest fluorine dose contributed to a decrease in the analysed amino acid content in the aerial mass of maize.

The Oser Index shows the nutritive value of protein. The Oser Index calculated on the basis of the content of exogenous amino acids varied from 36 (highest fluorine dose) to 52 (100 mg F kg^{-1} of soil) (Figure 1).

The amino acid content in the protein of yellow lupine aerial mass was also dependent, albeit to a lesser extent, on the soil contamination with fluorine (Table 2). Of note, however, is that yellow lupine was grown as a second crop (after maize) and therefore a residual effect of soil pollution with fluorine was analysed in this series of the trials.

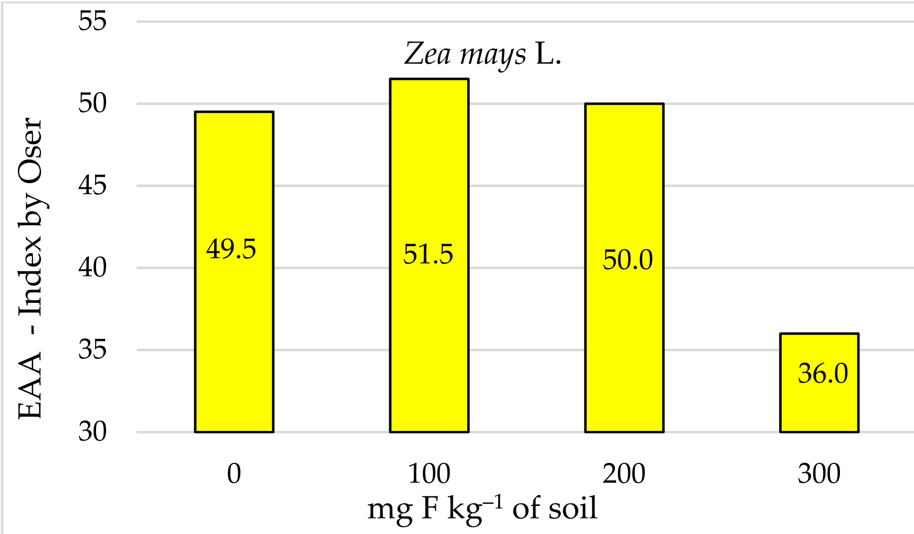

Figure 1. Nutritive value of protein accumulated in the aerial mass of maize depending on fluorine application to soil.

Table 2. Residual effect of fluorine application to soil on the amino acid content in aerial mass of yellow lupine.

Amino Acids	Soil Contamination with Fluorine in mg F kg^{-1} of Soil (Residual Effect)									
	0	100	200	300	r	0	100	200	300	r
	in g 100 g^{-1} Total Protein (16 g N)					in g kg^{-1} DM				
Exogenous										
Arginine	4.30	4.62	4.80	4.76	0.89 **	10.40	11.95	10.65	10.53	−0.16
Phenylalanine	3.39	3.04	3.30	3.29	−0.03	8.21	7.87	7.33	7.28	−0.96 **
Histidine	2.31	2.15	2.47	2.39	0.53	5.58	5.56	5.48	5.29	−0.93 **
Isoleucine	2.89	2.69	3.15	3.26	0.79 **	6.98	6.97	6.98	7.21	0.77 **
Leucine	5.75	5.38	6.32	6.86	0.85 **	13.90	13.93	14.03	15.18	0.83 **
Lysine	4.33	3.88	4.40	4.41	0.39	10.48	10.04	9.76	9.76	−0.93 **
Methionine	0.46	0.48	0.65	0.85	0.95 **	1.11	1.24	1.44	1.87	0.96 **
Threonine	3.60	3.42	4.02	4.10	0.83 **	8.71	8.86	8.93	9.08	0.99 **
Tyrosine	3.27	2.80	3.02	2.89	−0.58 *	7.90	7.25	6.69	6.39	−0.99 **
Valine	3.86	3.64	4.26	4.52	0.85 **	9.34	9.43	9.45	10.00	0.86 **
Sum of exogenous amino acids	34.15	32.12	36.39	37.33	-	82.61	83.10	80.74	82.59	-
$LSD_{0.05}$			3.30					n.s.		
Endogenous										
Alanine	3.73	3.51	4.44	4.58	0.86 **	9.02	9.08	9.86	10.13	0.95 **
Cysteine	<0.01	<0.01	<0.01	<0.01	0.00	0.01	0.01	0.01	0.01	0.00
Glycine	3.71	3.49	4.05	4.00	0.70 *	8.97	9.02	8.98	8.84	−0.71 **
Asparagine acid	17.93	16.87	14.96	13.55	−0.99 **	43.38	43.65	33.19	29.98	−0.93 **
Glutamic acid	8.05	7.52	8.83	8.52	0.61 *	19.48	19.45	19.6	18.85	−0.67 *
Proline	4.57	4.46	5.33	5.15	0.79 **	11.05	11.54	11.83	11.40	0.53
Serine	3.93	3.26	3.79	3.79	0.05	9.50	8.43	8.42	8.39	−0.79 **
Sum of endogenous amino acids	41.93	39.10	41.42	39.59	-	101.40	101.10	91.89	87.60	-
$LSD_{0.05}$			n.s.					9.01		
Sum of total amino aids	76.08	71.22	77.81	76.92	-	184.00	184.20	172.60	170.10	-
$LSD_{0.05}$			n.s.					n.s.		

LSD (least significant difference) for F application to soil; n.s.—no significant *—correlation coefficient (r) significant at $p \leq 0.05$; **—correlation coefficient (r) significant at $p \leq 0.01$.

The sum of exogenous amino acids per 100 g of total protein (16 g N) of yellow lupine ranged from 32.12 in the object polluted with 100 mg F kg^{-1} of soil to 37.33 g in the object treated with 300 mg F kg^{-1} of soil. Our comparison of the experimental data showed that the medium and highest soil contamination with fluorine affected positively the exogenous amino acid content in yellow lupine protein. The growing pollution of soil with fluorine contributed to a rise in the content of arginine, histidine, isoleucine, lysine, methionine, threonine and valine. With the two remaining exogenous amino acids, phenylalanine and tyrosine, a reverse correlation was noted, i.e., the content of these compounds decreased alongside the increasing doses of fluorine.

The exogenous amino acid content per 100 g of protein determined in the aerial mass of yellow lupine did not correspond to their concentrations in the dry matter. The sum of exogenous amino acids converted to 1 kg of DM varied from 80.74 in the series contaminated with 200 mg F kg^{-1} of soil to 83.10 g kg^{-1} DM in the one where the lowest fluorine dose, 100 mg F kg^{-1} of soil, was applied. As the soil pollution with fluorine increased, so did the content of arginine, isoleucine, leucine, methionine, threonine and valine. The concentrations of phenylalanine, histidine, lysine and tyrosine followed a contrary trend.

In regard to the endogenous amino acids, their sum per 100 g total protein was from 39.10 in the series polluted with 100 mg F kg^{-1} of soil to 41.93 g 100 g^{-1} of protein in the control, i.e., in the soil unpolluted with fluorine. The data obtained demonstrate that the growing soil contamination with fluorine did not have any considerable influence on the total content of these amino acids in the total protein of yellow lupine. As the soil pollution with fluorine increased, an increase was observed only in the concentrations of the following amino acids: alanine, glycine, glutamic acid and proline. The concentrations of aspartic acid followed a contrary trend, and its content decreased by 24% relative to the control in the series treated with the highest fluorine dose.

Likewise, the sum of endogenous amino acids per 1 kg of DM of yellow lupine was only weakly dependent on the fluorine soil contamination. As the soil pollution with this element increased, so did the content of alanine and proline. The concentrations of glycine, aspartic acid, glutamic acid and serine followed a contrary trend. The sum of the analysed endogenous amino acids calculated per 1 kg DM of yellow lupine ranged from 87.60 g per 1 kg DM to 101 per 1 kg DM in the control series.

The total content of the analysed amino acids, both exogenous and endogenous, in the dry matter of yellow lupine, was the highest and approximately the same in the series treated with the lowest dose of fluorine and in the control. The medium and highest doses of fluorine depressed the content of the sum of these compounds by 6 and 8%.

Based on the content of exogenous amino acids, the nutritive value of yellow lupine protein was calculated, and compared in relative values to hen's egg protein (Figure 2). The index values varied from 56 (lowest fluorine dose) to 63 (highest fluorine dose). These results show that the medium and highest level of fluorine pollution of soil tested in terms of residual effects considerably improved the nutritive value of protein accumulated in the aerial mass of yellow lupine.

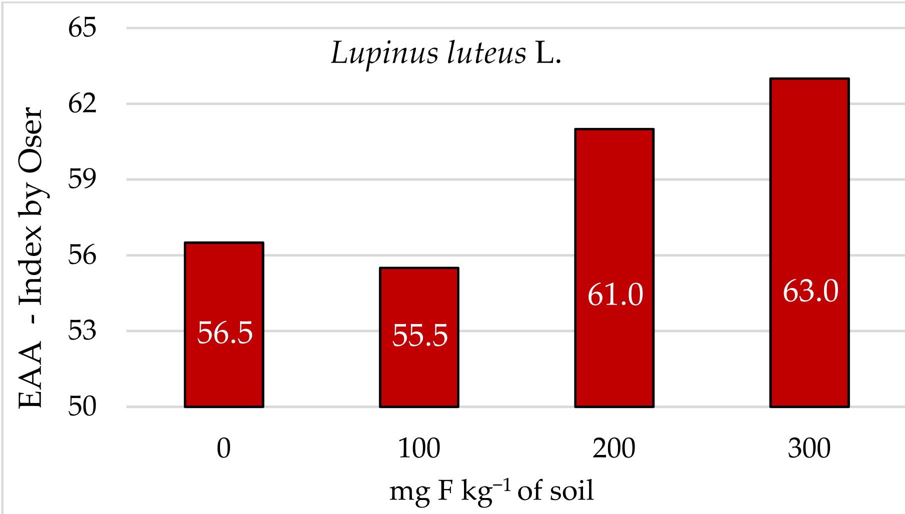

Figure 2. Nutritive value of protein accumulated in the aerial mass of yellow lupine depending on fluorine application to soil.

4. Discussion

The literature contains data indicating diverse effects of soil contamination with fluorine on the amino acids of crops. Our results are the same as in these findings, as some of our data are completely divergent.

Changes in the exogenous amino acid content in the aerial mass of maize induced by the growing soil pollution with fluorine tended to follow a parabolic curve. Tyrosine was an exception, where a gradual increase in its content was observed starting from the lowest fluorine dose. A gradually decreasing content of tyrosine was also noted by Li and Ni [24] in *Camellia sinensis* L., in contrast to the other amino acids, whose concentrations were observed to increase under the increasingly severe fluorine pollution of soil. At the same time, it was demonstrated that the content of the sum of the analysed amino acids continued to increase only up to the concentration of 4 mg L^{-1} of the xenobiotic. Higher levels of fluorine contamination of soil caused only a slight reduction in the total content of amino acids compared to the control. Our findings are similar to exogenous amino acid accumulation in the aerial mass of maize. Doses of 100 and 200 mg F kg^{-1} of soil of fluorine had a positive effect on the content of exogenous amino acids whereas the highest fluorine dose, i.e., 300 mg F kg^{-1} of soil, contributed to a decrease in the content of the analysed amino acids relative to the control. The reduced content of protein under the influence of fluorine may be attributed to the depressed synthesis of this protein, its greater degradation, and the utilisation of protein for the processes carried out in order to generate energy in response to the metabolic stress induced by fluorine.

The presence of fluorine also raises the content of proline, which is an important stress indicator in protein synthesis, which is confirmed by our research results. Li et al. [25] drew the conclusion from their research that the accumulation of L-proline in *Camellia sinensis* was strictly associated with the presence of some trace elements, including fluorine. The study conducted by Cai et al. [26] provides evidence supporting the above claim, as it demonstrated a positive correlation between the content of proline in *Camellia sinensis* leaves with the concentration of fluorine. The content of this amino acid increased by 17, 32, 47, 67 and 113% at the presence of 1, 5, 10, 20 and 50 mg L^{-1} F, respectively. These authors concluded that proline and betaine play an important role in the osmotic regulation in plants. The activated adaptive mechanisms aim to protect the plant from the stress caused by fluorine. The results reported by Pelc et al. [14] point to an increase in the content of free proline under the influence of 10 mM NaF in most of the tested plant seedlings. The highest increase in the proline content was observed in radish, sunflower and tomato plants. A gradual increase in the proline content was also demonstrated by Das et al. [27] in an experiment with tomato plants, as well as by Zouari et al. [28], who carried out a study on olive trees. In that research, the highest dose of fluorine, equal to 80 mM NaF, caused an increase in the proline content by 133% (leaves) and by 83% (roots) compared to the unpolluted objects. Saleh and Abdel-Kader [29] reported that the content of proline in *Helianthus annuus* was positively correlated with the content of fluorine. In an experiment on *Abelmoschus esculentus*, L. Ahmed et al. [30] observed a gradual increase in the content of proline in response to the growing doses of NaF within the range of 50 to 300 mg kg^{-1} and recorded the highest proline content in the objects with the highest fluorine dose. The same relationship was proven by Mezghani et al. [31] in their experiment on *Morus alba* L. and by Singh et al. [32], who completed a study on leaves of *Solanum melongena*. An experiment by Gadi et al. [33] demonstrated that the cellular stress induced by the presence of fluorine contributed to an increase in proline in *Vigna radiata* seedlings from 27 to 126% after the application of fluorine doses from 0.1 mM to 1 mM NaF. The results delivered by Datta et al. [34] from their experiment on *Cicer arietinum* L. showed an over twofold increase in the content of proline after the application of the highest 4 mM dose of fluorine relative to the control. Maitra et al. [35] proved the effect of high fluorine pollution on the level of proline in *Vigna radiata*. The results obtained in their study showed that the highest content of this amino acid appeared under the effect of the highest dose of fluorine, which equalled 1.75 mg L^{-1}. An interesting observation was reported by Eyini et al. [36] from their trial on *Azolla microphylla* kaulf. and *Azolla filiculoides* Lam. These researchers demonstrated a gradual rise in the content of L-proline in both plant species, although the increase was more evident in *Azolla filiculoides*. Moreover, both of these plant species accumulated by 33.3% more proline than *A. microphylla*. Based on this observation and their research findings, the cited authors suggested that *Azolla filiculoides* was more sensitive to exposure to fluorine, and the higher content of proline in *A. filiculoides* was closely correlated with the weak growth of this species. Greenway and Munns [37] supported this suggestion, implicating that plants accumulate large quantities of proline in a situation when their growth is distinctly inhibited. In response to the cellular stress induced by the presence of fluorine, plants strive to survive rather than grow. Hanson et al. [38] obtained similar results. Moreover, the presence of fluorine also raises the content of proline, which is an important stress indicator in protein synthesis, which is confirmed by our research results.

Our research revealed that the lowest and medium pollution of soil with fluorine had a positive effect on the total content of the exo- and endogenous amino acids, in the dry matter of maize. A similar trend was noted by Singh et al. [39], who investigated the effect of sodium fluoride on the content of amino acids in wheat (HUW-234). Thus, the use of NaF up to the highest dose of 200 mg kg^{-1} had a positive impact on the total concentration of amino acids in analysed plants. At the same time, an increase in the amino acid proline by 43% took place under the influence of 200 mg NaF kg^{-1} relative to the control.

The amino acid content in the protein of yellow lupine aerial mass was also dependent, albeit to a lesser extent, on the soil contamination with fluorine. The weaker response of the yellow lupine plants may be due to the fact that it was a successor plant and only the residual effect of fluorine contamination was investigated in this series of experiments. The total content of the analysed amino acids, both exogenous and endogenous, in the dry matter of yellow lupine, was the highest and approximately the same in the series treated with the lowest fluorine dose and in the control. It should be noted that yellow lupine (as a legume plant) has the ability to fix nitrogen from the atmospheric air, which presumably results in a higher content of amino acids in plants. Chakrabarti and Patra [40] determined an average increase of 27% in the amino acid contents they analysed in the three organs of *Oryza sativa* L. submitted to the research. In another experiment, by Pal et al. [41], the total content of free amino acids in leaves of different vegetables demonstrated a decreasing tendency relative to the control series.

Due to the fact that the cultivation of maize and yellow lupine has recently gained popularity in Poland in areas exposed to fluorine emissions, there is a risk of accumulation of this element in agricultural crops and thus a strong impact on their chemical composition.

That is why it seems so important to monitor crops in such areas for the sake of food safety [42].

The problem of fluorine in the natural human environment is of great importance; it threatens practically all living organisms. An important issue that awaits a full solution is the time of neutralisation of fluorine compounds and their circulation in nature. This would allow a full assessment of the degree of risk for plants, animals and people. The current concentration of fluorine in the environment is still far from the critical values [7]. However, fluorine in combination with other toxic agents can cause deterioration of the quality of agricultural crops in some areas, which can threaten food safety.

The proper amino acid composition of protein is more important, because the lupine, and especially the maize, fulfill a very essential role in the optimal coverage of energy and caloric protection for animals and people [43]. Maize is one of four plants (sugar cane, maize, wheat and rice), whose production is over half of the global plant production. Current production of maize is 12% of global production of cultivated plants [44]. The area of maize cultivation is growing three times faster than rice and wheat. Maize has the second place in the world in global production, after sugar cane [44]. Maize and lupine belong to terrestrial land plants that cover 82% of the world caloric requirement in food [45]. Forecasts indicate that the share of maize in global plant production will increase in the following years [46]. More than half of the growth of plant production will be in the United States, China and Brazil. It is predicted that global yield of maize by 2030 will increase by 10%, compared to the base period (2018–2020). The concentration of global maize production will be the largest in the United States (30%), China (22%), Brazil (9%), the European Union (5%) and Argentina (5%) [46]. Maize and lupine are components for many feed and food products. For this reason, it is very important that they are grown in conditions which protect them before moving environmental pollutants to feed and food.

5. Conclusions

The soil pollution with fluorine affected the amino acid content in the aerial mass of both plants, especially in maize. Lowest and medium doses of fluorine had a significant positive effect on the content of exogenous and endogenous amino acids in the protein of the aerial mass of maize, for which the increase of sum of all amino acids was 7% and 8%, respectively, in relation to controls. Moreover, statistical calculations showed a significant influence of soil contamination with fluorine on the total amino acid content in maize plants, while in relation to yellow lupine this influence was statistically insignificant. The obtained results indicate that soil contamination with medium and high doses of fluorine slightly increased the total content of some amino acids in the total protein of the aerial parts of yellow lupine. It should be noted that the above-ground mass of yellow lupine was characterised by a much higher content of the tested amino acids compared to maize. The subsequent influence of soil contamination with fluorine on the amino acid content in yellow lupine did not have the same effect as in the main plant, and the influence of fluorine was somewhat limited. With respect to yellow lupine, it was demonstrated that the lowest fluorine dose (100 mg F kg^{-1} of soil) did not have a beneficial influence on the sum of all amino acids. The medium and highest doses of fluorine depressed to a small degree the content of the sum of these compounds in the dry mass of yellow lupine.

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