

THE STUDY OF RAPESEEDS ASH COMPOSITION IN THE CONDITIONS OF THE AGROECOLOGICAL EXPERIMENT

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ABSTRACT

A comparative analysis of the seeds ash composition of the breed Rif (*Brassica napus* L.) rapeseeds grown in the Lipetskaya region was held. The plants were grown in the conditions of the agroecological experiment using mineral (NPK and zeolite) and organic (hen droppings) fertilizers. 6 variants of the experiment were studied – the plants are grown without fertilizers application (the control); the mineral fertilizer (N₆₀:P₆₀:K₆₀) separately and together with zeolite (5 t.ha⁻¹); the zeolite in pure form (5 t.ha⁻¹); hen droppings (5 t.ha⁻¹) separately and together with zeolite (5 t.ha⁻¹). We studied the accumulation of 9 basic elements (in mass %) contained in *Brassica napus* seeds ash using the method of energy-dispersive X-ray spectroscopy. The accumulation order of the elements was determined: P ≈ K > Mg ≥ Ca > Mo > S > Zn > Mn > Fe. The proportion of P fluctuated from 10.852 to 11.855 mass %; the proportion of K – from 9.933 till 12.343 mass %. The rapeseeds contained Mg, Ca, and Mo in similar concentrations within the range of 4.0 -5.8 mass %. The combined application of zeolite with organic fertilizer ensured the accumulation of the minerals in the seeds. Correlations between the elements were established. High correlation between elements K and Mo was found (r = 0.96); P and Mg (0.86); P and Fe (r = 0.94); C and Mo (r = 0.86). The positive effect of the combined organic-mineral fertilizers with poultry farms wastes usage on the mineral elements accumulation in rapeseeds was stated. It is noted that the accumulation of P, Ca, Mo, and S in rape seeds leads to a decrease in Zn.

Keywords: *Brassica napus*; seeds; analytical scanning electron microscopy; Energy Dispersive X-ray Analysis (EDS); ash elements

INTRODUCTION

For the last twenty years, the world's rapeseed production has had a stable growth. This crop is an important source of renewable energy, and new conditions are created for the use of oilseed rape (Ralphe et al., 2006; Günnur and Nilgün, 2013). The main manufacturers are China, India, Canada, and the EU (Carre and Pouzet, 2014). This culture has been adapted to the western conditions of the USA. Edaphoclimatic conditions of Russia allow growing the rapeseed plants in all the regions (Karpachev, 2009). The seeds fund development demands the constant improvement of this culture cultivation technology in the concrete edaphoclimatic conditions taking into account the breed peculiarities, the reaction on the different technological elements including the reaction on both mineral and organic fertilizers (Pin Koh and Ghazoul, 2008; Rondanini et al., 2012). The researches results prove that the greatest impact on rapeseeds productivity is caused by the norm of a nitrogen fertilizer application, and the smallest – by the previous culture and the fertilizer type, as well as the interaction between these processing factors (Rathke et al., 2005). Organic animal waste is recognized as a valuable source of plant nutritional chemicals in farming systems and plays a certain role in the improvement

of the soil using organic substances (Schoenau and Davis, 2006). Therefore, the further development of rapeseed cultivation technologies with organic and mineral fertilizers usage is currently important. Also, there is growing interest in the use of rapeseed for food development. The focus is on the composition of the protein and lipids contained in rapeseed. (Gunhild et al., 2000; Arif et al., 2012). Moreover, there is very little research on the effect of zeolite and poultry manure when growing rapeseed on seed mineral composition.

Therefore, our research aimed to study the composition of ash elements in rapeseed under agroecological experiments.

Scientific hypothesis

Information on the mineral composition of the seeds of *Brassica napus* of the breed Rif grown in the Lipetskaya region is not available. We checked whether there are differences in the content of macro - and microelements in rapeseed depending on the use of mineral and organic fertilizers.

MATERIAL AND METHODOLOGY

Biological material

The experiments were conducted in the experimental field of Bunin Yelets State University in 2018 – 2019. The spring-planted rapeseed precursor plant is winter-planted wheat. The experimental area soil is black earth with the following agrochemical characteristic of the tilth-top layer: pH 4.88, humus content – 5.76%, total content of N – 0.288%, P – 197.2 mg.kg⁻¹, K – 124.7 mg.kg⁻¹, Ca – 25.7 mg.kg⁻¹ and Mg – 2.4 mg.kg⁻¹. The research object was the spring-planted breed Rif rapeseed, combining high potential for productivity and adaptability with high quality of oil and seeds and resistance to major diseases. The rapeseed was seeded on the following experiment scheme: 1 – control; 2 – N₆₀:P₆₀:K₆₀; 3 - zeolite, 5 t.ha⁻¹; 4 – hen droppings, 5 t.ha⁻¹; 5 – N₆₀:P₆₀:K₆₀ + zeolite 5 t.ha⁻¹; 6 – hen droppings 5 t.ha⁻¹ + zeolite 5 t.ha⁻¹.

Samples

The seeds used for analysis were cleaned of all foreign substances, such as dust, dirt, stones, immature and damaged seeds.

Preparing seeds for analysis

Preliminary dried at T = 40 – 50 °C seeds weighing with the mass of 10 g was mineralized in the muffle furnace Naberterm (Germany) at T = 400 °C. The received ash was dispersed by ultrasound at 18 kHz frequency for 15 minutes. The disperse even layer was applied on the object table covered with carbonic scotch.

EDS - analysis

The chemical composition of the basic ash components (P, S, K, Mn, Fe, Mg, Ca, Zn, Mo) was determined by the method of energy dispersive spectrometry (ESD) on the analytical scanning electron microscope (SEM) EVO LA 15 (Zeiss, Germany). While performing the elemental analysis the working distance (WD) is 10 mm. X-ray microanalysis data are presented in the form of standard protocols which contain the microstructure picture of the sample under study, the table of the spectra, and histograms (Figure 1). The fractional accuracy of the chemical analysis is spread in the following way: at the element concentration from to 5% the accuracy is less than 10%; from 5 till 10% the accuracy is less than 5%; at the element concentration more than 10% the accuracy is less than 2%. Analyzed 6 samples of rape seeds in 3 replicates. 3 ash areas of each sample were studied.

Statistical Analysis

The data obtained during the experiments were processed using mathematical methods of variation statistics using the Microsoft Excel software and Statistika 6; we used the Student-Fisher method. To assess the effect of growing conditions on the mineral composition of seeds the threshold of reliability of the obtained data is designated as ($p < 0.05$).

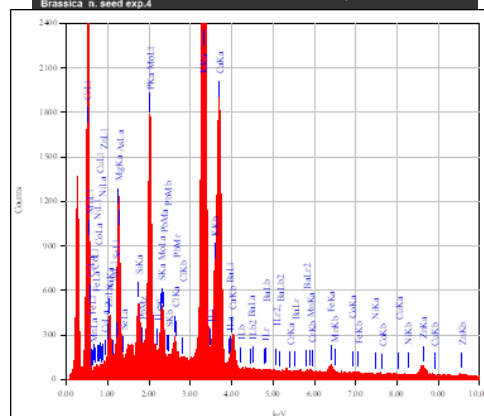
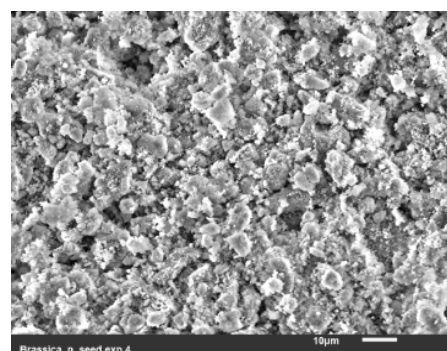


Figure 1 The microstructure picture of the sample under study (at the top) and the general view of the X-ray spectrum lines that show the elements presence in the analyzing area (at the bottom).

RESULTS AND DISCUSSION

We analyzed the content of 9 elements in the rapeseed ash - K, P, Mg, Ca, Mo, Mn, Zn, Fe, and S (shown in Table 1). The predominant elements were P and K.

The decreasing series of elements in the rapeseed ash is as follows: $P \approx K > Mg \geq Ca > Mo > S > Zn > Mn > Fe$. Our results are comparable to the data obtained by the authors (Cahsir et al., 2005). Studies (Gins et al., 2018) showed that the main elements in the seeds of amaranth are also K, Ca, but the order of their accumulation is different.

The proportion of P is from 10.852 mass % (control) till 11.855 mass % (zeolite 5 t.ha⁻¹). High content of P in the rapeseed ash was also noted in the experimental variants using hen droppings 5 t.ha⁻¹; N₆₀:P₆₀:K₆₀ + zeolite 5 t.ha⁻¹ and hen droppings 5 t.ha⁻¹ + zeolite 5 t.ha⁻¹. (Figure 2). The content of P in seeds is an important and only source available to maintain the initial growth of seedlings of future plants (Manske et al., 2001; Masoni et al., 2007; Fageria, Baligar and Jones, 2010). The accumulation of P in the seeds is determined by bioavailability, the ability of the roots to absorb P from the soil, and plant growth rates (Lynch 2007; Hammond and White 2008; White, 2013).

According to White and Veneklaas (2012), increasing P content in seeds can improve plant formation and increase yield rape. K in the seed ash contains from 9.933 mass % (zeolite 5 t.ha⁻¹) till 12.34 mass % (N₆₀:P₆₀:K₆₀) (Figure 2).

It was determined that the accumulation of K in the control was 25% lower than in the variant with the mineral fertilizer (N₆₀:P₆₀:K₆₀) and 20% lower than in the variant with the combined mineral fertilizer (N₆₀:P₆₀:K₆₀ + zeolite 5 t.ha⁻¹) application. K is a macroelement that is responsible for the regulation of the majority of metabolic reactions that

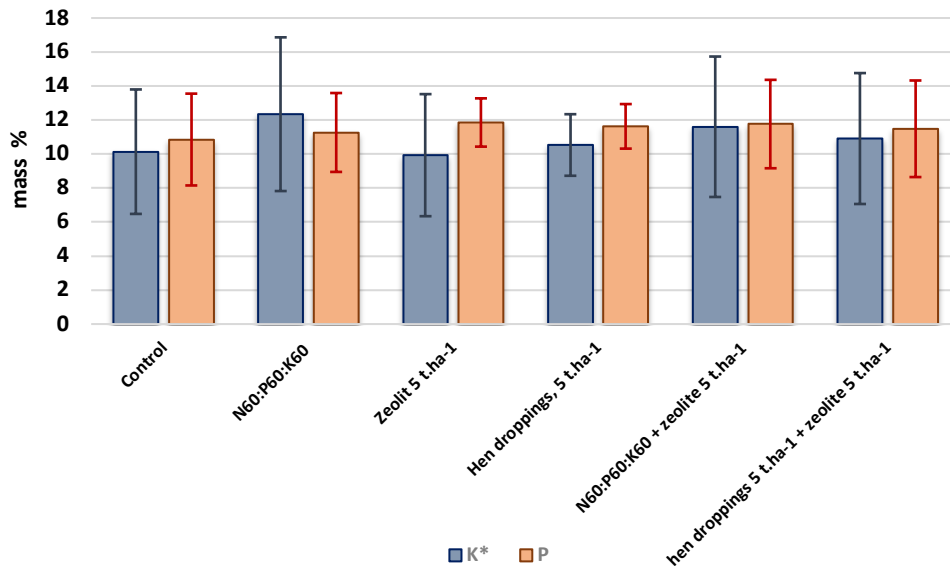


Figure 2 The comparative concentration of K and P in the ash of the rape.

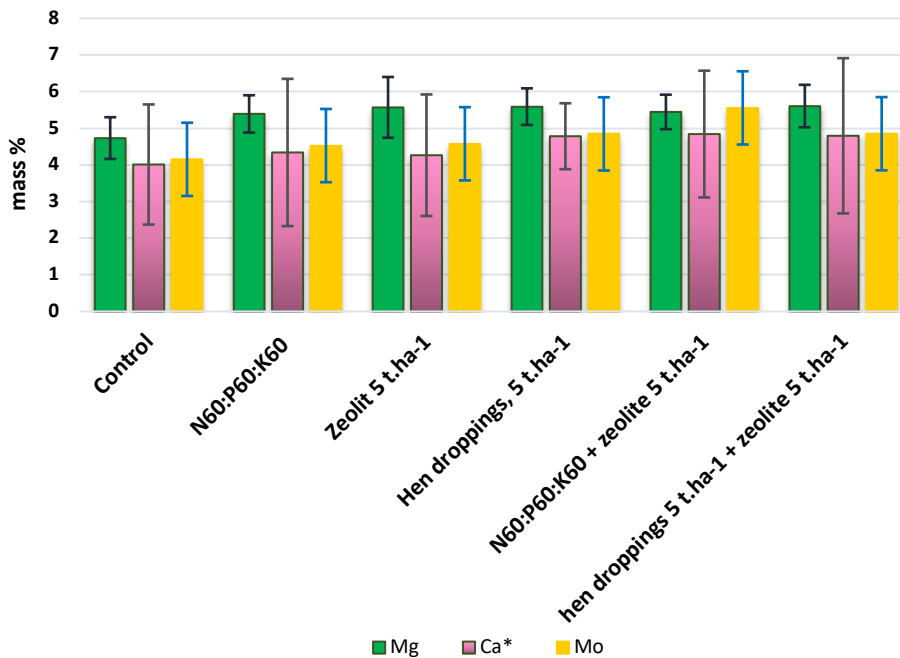


Figure 3 The comparative concentration of Mg, Ca and Mo in the ash of the rape.

low in living organisms. K controls osmotic pressure transmembrane potential, charges equilibrium, cathode-anion balance, pH – everything that the homeostasis of cells and tissues consists of.

In the ionic form, K can be found in all the organs, tissues, and cell structures in concentrations that exceed the concentration of other ions (Meathnis et al., 1997).

In Figure 3 the comparative data of Mg, Ca, and Mo concentrations are presented. Mg activates a large number of enzymes that take part in the processes of CO₂ and N assimilation. Mg is necessary for the keeping up of the cathode-anion balance and pH regulation. In the cell wall and the seeds membrane, Mg²⁺ is coordinately connected with carboxylic groups of pectin substances and takes part in the creation of the inner physiological environment of plants. Mg, Ca and N are localized in the seed membrane. ATP, phosphoinositol (phytin) in combination with Mg are

accumulated in the seeds in the form which is comfortable for storage (Nechaev, Trauberg and Kochetkova, 2007). The concentration of Mg in the rape seed fluctuates from 4.733 mass % (control) to 5.575 (zeolite 5 t.ha⁻¹). In the variants of the experiment with the mineral and organic N application the concentration of Mg in the rapeseeds increases by 20 – 25% in comparison with the control.

Ca is indispensable within the plant for the stabilization of cell walls and maintenance of membrane integrity. A high proportion of calcium is located in the cell walls. It is involved in the regulation of the cation/anion-balance (IPI Bulletin). The concentration of Ca in the rapeseed fluctuates from 4.012 mass % (control) to 4.843 mass % (N₆₀:P₆₀:K₆₀+ zeolite 5 t.ha⁻¹).

Mo fulfills many useful functions for the organism: it is a cofactor and an activator of oxidases (xanthine oxidase and serine oxidase), which takes part in the synthesis of the

Table 1 The elemental composition of *Brassica napus* seeds, breed Rif, mass % in the ash.

Elements	Experience options					
	Control	N ₆₀ :P ₆₀ :K ₆₀	zeolite, 5 t.ha ⁻¹	hen droppings, 5 t.ha ⁻¹	N ₆₀ :P ₆₀ :K ₆₀ + zeolite 5 t.ha ⁻¹	N ₆₀ :P ₆₀ :K ₆₀ + hen droppings, 5 t.ha ⁻¹
K	10.14	12.34	9.96	10.52	11.61	10.91
min – max	6.75 – 13.17	7.52 – 7.92	6.64 – 10.27	7.33 – 4.21	7.47 – 16.09	4.77 – 13.91
V %	36.1	34.5	35.2	34.5	35.5	42.1
P	10.85	11.27	11.85	11.69	11.73	11.85
min – max	8.58 – 4.21	8.32 – 3.42	9.03 – 4.74	9.18 – 14.61	9.16 – 14.22	5.98 – 12.89
V %	23.9	21.6	23.8	22.3	22.1	30.52
Mg	4.73	5.39	5.58	5.19	5.15	5.21
min – max	4.77 – 6.06	4.18 – 5.41	4.98 – 6.13	4.67 – 5.84	4.55 – 5.68	3.89 – 5.24
V %	10.9	10.8	10.1	9.6	9.2	12.4
Ca	5.82	4.34	4.07	4.78	4.89	4.77
min – max	3.15 – 6.11	3.87 – 7.81	2.55 – 5.72	2.94 – 6.84	3.24 – 6.39	2.55 – 7.54
V %	37.9	34.6	38.2	37.5	35.8	43.8
Mo	5.65	4.53	4.18	4.85	5.56	4.75
min – max	1.74 – 8.09	2.02 – 9.67	1.4 – 7.16	1.56 – 8.69	1.52 – 7.85	1.68 – 8.75
V %	75.8	74.8	75.2	74.2	74.1	75.3
S	1.28	1.18	1.11	1.25	1.15	1.16
min – max	0.97 – 1.51	0.98 – 1.63	0.48 – 1.51	0.67 – 1.97	0.9 – 1.43	0.97 – 1.48
V %	25.8	23.6	26.7	23.9	23.9	17.4
Zn	0.31	0.16	0.27	0.21	0.16	0.22
min – max	0.08 – 0.26	0.09 – 0.65	0.13 – 0.42	0.08 – 0.36	0.07 – 0.26	0.11 – 0.61
V %	62.4	73.3	61.2	63.3	61.1	72.2
Mn	0.06	0.06	0.12	0.31	0.19	0.15
min – max	0.04 – 0.11	0.03 – 0.09	0.06 – 0.24	0.04 – 0.24	0.07 – 0.37	0.08 – 0.24
V %	52.9	50.2	69.7	67.4	67.5	52.4
Fe	0.03	0.03	0.19	0.11	0.22	0.16
min – max	0.01 – 0.07	0.01 – 0.06	0.09 – 0.32	0.03 – 0.12	0.12 – 0.31	0.09 – 0.27
V %	40.6	52.1	55.5	57.2	58.4	60.1

Note: Means within a column with at least one identical superscript are not significantly different by Student's *t*-test (*p* < 0.05).

Table 2 Correlation matrix for the 9 elements in the ash of the seed *Brassica napus*

Elements	P	Mg	Ca	Mo	S	Zn	Mn	Fe
K	-0.03	0.19	0.32	0.96	0.57	0.76	-0.11	0.13
P		0.85	0.61	0.69	-0.49	-0.28	0.58	0.94
Mg			0.72	0.57	-0.17	0.001	0.56	0.76
Ca				0.86	0.17	-0.23	0.78	0.72
Mo					0.06	-0.23	0.60	0.78
S						0.52	0.16	-0.50
Zn							0.16	-0.52
Mn								0.67

amino acid (Avtsyn et al., 1991). The concentration of Mo in the rapeseed fluctuates from 4.178 mass % (control) to 5.558 mass % (N₆₀:P₆₀:K₆₀+zeolite 5 t.ha⁻¹).

Sulfur plays an indispensable role in rape plant metabolism as a component of proteins and glucosinolates. S is essential for protein formation, important for high protein content in rapeseeds. Not only the amount of protein but also the quality of protein is influenced by the S-status of plants. Because of the central role of sulfur and nitrogen in the production of proteins, there is a close relationship between the supplies of S and N in plants (Blake-Kalff et al., 2000). The concentration of S in the rapeseeds is rather high and varies from 0.993 to 1.275 mass %. Depending on the experimental variant the content of S in the seeds

fluctuates insignificantly (Table 1). The proportion of Zn does not exceed 0.305% mass and also fluctuates insignificantly depending on the variant of the experiment. Mn is a co-factor and activator of many enzymes and possesses antioxidant activity. In the rapeseeds the concentration of Mn is 0.058 mass % (control) – 0.303 mass % (hen droppings 5 t.ha⁻¹). In the experimental variants with the application of the fertilizer, the concentration of Mn is 2 – 6 times higher than the control (Table 1). Organic Fe is an essential compound for the human organism this element is a part of catalytic centres of many oxidation-reduction enzymes (Shmalko and Roslyakov, 2011).

In the rapeseeds, the concentration of Fe varies from 0.034 mass % (the control) to 0.159 – 0.215 (the variant with the

complex composition of fertilizers), Table 1. Thus, we have found that the content of manganese and iron in rape seeds increases significantly when grown in the variants of the experiment with the introduction of zeolite and hen droppings. **Fordoński et al. (2016)** research also showed that the micronutrient content (Mn, Zn, Cu, and Fe) of winter oilseed rape increased significantly in response to an increase in nitrogen fertilization doses.

The low variation coefficient 10 – 25% is determined for P, Mg, and S; K and Ca are characterized with the middle variation coefficient 35 – 36%. The low and average variation coefficient is typical for biologically significant elements of seed rape and indicates the accumulation stability of the elements. Zn, Mn, and Fe are marked with the high variation coefficient

Calculated correlation coefficients between elements (Table 2). It is established that there is a high correlation between elements, for example, K and Mo ($r = 0.96$); P and Mg (0.86) and P and Fe ($r = 0.94$); C and Mo ($r = 0.86$). The mean correlation is between Ca and Mn, Mo, and Fe ($r = 0.78$); between K and Zn, Mg and Fe ($r = 0.76$); between P and Mo ($r = 0.69$); Mn and Fe ($r = 0.67$); P and Ca ($r = 0.61$); Mo and Mn ($r = 0.60$); S and Zn ($r = 0.56 - 0.57$). Weak correlation found between between S and Zn ($r = 0.52$); between K and Ca ($r = 0.32$); K and Mg ($r = 0.19$); Ca and S ($r = 0.17$) and S and Mn ($r = 0.16$).

It is noted that the accumulation of P, Ca, Mo, and S in seeds leads to a decrease in Zn. These results are consistent with data from **Szczepaniak et al. (2017)**.

CONCLUSION

Research conducted in agro-ecological experience the influence of zeolite and chicken droppings on the formation of the mineral composition of rapeseed has been established. Using the method of the energy dispersive X-ray spectrometry the new data about the variety of the rapeseeds mineral composition were received, the proportion of the elements in the ash was determined, the variation coefficients were calculated. In variants of the experiment with hen droppings, 5 t.ha⁻¹, and N₆₀:P₆₀:K₆₀ +hen droppings, 5 t.ha⁻¹ the content of P, Ca, Mo, S, Mn, and Fe are 2 – 4 times higher than in the control. The influence of the experimental variants on the accumulation of potassium in rape seeds has not been established. A high correlation has been established between the elements K and Mo, P and Mg, P and Fe, C and Mo. It was noted that the accumulation of P, Ca, Mo, and S in rape seeds leads to a decrease in the Zn content.

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Funds:

The study was carried out with the financial support of RFFR and the Lipetsk region administration within the framework of the scientific project No. 19-44-480003.

Acknowledgments:

Author Motyleva S. (ID 26489) thanked the SAIA agency for the financial support provided and the opportunity to carry out research at the Institute for Biodiversity and Biosafety of the Faculty of Agrobiological and Food Resources of the Slovak Agricultural University of Nitra, where are obtained the main results presented.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

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