# Preliminary investigation of mineral content of pollen collected from different Serbian maize hybrids – is there any potential nutritional value?

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#### **Abstract**

BACKGROUND: Bee pollen has already proved to be a good supplement rich in content of iron and zinc. Studies about application of flower pollen in food industry and medicine have been started. Bearing in mind prevalence of maize as crop culture its pollen will be easily available. The mineral composition of pollen of seven Serbian maize hybrids was analyzed in order to establish its nutritional value and benefits of its implementation in the human diet using ICP method.

RESULTS: Presence of twenty four different macro- (nine) and micronutrients (fifteen) was detected. The most common minerals were phosphorus and potassium, while arsenic, cobalt, lead, nickel and molybdenum were found in some samples.

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CONCLUSION: Comparing obtained results with recommended or tolerable dietary intake references for adults it was found that maize pollen can be used as very good source of zinc, iron, chromium and manganese for humans. According to selenium content pollen samples proved to be moderately good source of this important micronutrient. Contents of some elements (Fe, Zn, Mn, Cr, Se, Al and V) showed significant differences depending on hybrid type. In some samples increased concentrations of aluminum and vanadium were recorded which may pose a potential problem due to their toxicity.

**Keywords** maize; pollen; minerals; nutritional value

#### INTRODUCTION

Zea mays L., commonly known as maize or corn, is usually outcrossing with a low rate of self-pollination<sup>1</sup>. The viability period of individual pollen grains is short, and on a whole field basis, cross pollination could occur over a seven-day period<sup>2</sup>. Despite being a wind-pollinated plant<sup>3</sup>, it is not rare that bees, or some other insect pollinators visit maize and use it as a source of pollen, especially during the low blooming period of zoophilous plants<sup>4-7</sup>. In accordance with this, Kostić et al.<sup>8</sup> reported about presence of maize pollen in several samples of bee-collected pollen from Serbia. In addition, Feil and Schmidt<sup>9</sup> found that maize tassel is being visited by honeybees, however, since they have not been observed on female inflorescences, pollination by honeybees is excluded.

The chemical composition of pollen and its use for nutritional, medicinal and other purposes have been analyzed earlier, especially in the last decade, but to a limited extent. The first, preliminary, report of general chemical composition of maize pollen was given by Anderson and Kulp<sup>10</sup>. By examining pollen (ten minerals) from five different corn

genotypes, Pfahler and Linskens<sup>11</sup> found significant differences in content of Al, Ca, Fe and K. Recently some studies were conducted on the application of floral<sup>12</sup> or bee-collected pollen<sup>13-17</sup> in the nutrition of farm animals raised for meat production. Krystijan et al.<sup>18</sup> used bee-collected pollen as a food supplement in the biscuit making processes. Also, Atwe et al.<sup>19</sup> suggested that it is possible to use pollen grains as modular system for painless oral vaccination.

It is known that maize pollen is of poor quality as a source of amino acids and proteins for humans<sup>20</sup>, but there is no information on the nutritional value coming from minerals contained. This type of research is especially important if it is known that bee-collected pollen is the best source of minerals comparing to the other bee products<sup>21</sup> with a special emphasis on high iron and zinc content<sup>22</sup>. The present research has provided a detailed review of the mineral composition of pollen collected from seven maize hybrids from Serbia, referring to twenty four significant macro- and microelements, in order to establish its nutritional value and benefits of its implementation in the human diet.

# MATERIALS AND METHODS

## Study area and samples

Zea mays L. is anemophilous plant, belonging to the grass family (Poaceae). It is a monoecious plant producing separate male and female inflorescences. The pollen samples were collected from seven maize hybrids grown in Serbia, in June 2015, applying the method by Pfahler<sup>23</sup>. All samples originate from the study field (arable land) of Maize research Institute- Zemunsko Polje / Beograd (Belgrade) which represents leading institution in the development, production and introduction of new high-yielding, quality

maize hybrids and soybean cultivars, adapted to diverse agro-ecological conditions and growing practices, and suitable for various purposes and uses in Serbia and whole Balkan region. After collection, all samples were packed into the vacuum-bags filled with silica gel to prevent absorption of moisture in samples. Samples were stored and kept in refrigerator at temperature of 4°C until analysis.

Zemunsko Polje settlement (44°52'N - 20°19'E, Figure 1) is a part of Zemun municipality and is located in the easternmost region of Belgrade, near the highway to Novi Sad.

Pollen was collected from seven maize hybrids that were produced and developed in this Institute during past several decades - ZP Standard (yellow grain) (sample 1, S01), ZP 608K (sample 2, S02), ZP 611K (sample 3, S03), L 620/121-"sweet corn" (sample 4, S04), Lady Fingers (sample 5, S05), ZP Rumenka (sample 6, S06) and ZP 555 (sample 7, S07).

# **Analytical methods**

Determination of mineral elements was carried out using inductively coupled plasmaoptical emission spectrometry (ICP-OES) with previous microwave digestion of samples<sup>22</sup>.

# Statistical analysis

For statistical data analysis Statistica 8 program (StatSoft Co., Tulsa, USA) was used. Results are expressed as mean value of three measurements  $\pm$  standard deviation. The obtained results underwent to Duncan's multiple range test to determine if there are significant differences between samples and mineral content (p < 0.01).

#### RESULTS AND DISCUSSION

Comparing the content of selected macro- and microelements in the investigated hybrids, significant differences for certain minerals have been established. Macroelements [g kg<sup>-1</sup>] contained in a samples of maize pollen are given in Table 1.

ICP-OES analysis revealed that the two most common minerals in all pollen samples are phosphorus (1, 5, 6 and 7) and potassium (2, 3 and 4) (Table 1) Pfahler and Linskens<sup>11</sup>, also, found these two elements being the prevailing in the pollen of five different genotypes of maize. These results were expected if one takes into mind the importance of these two elements for the plant<sup>24</sup>.

High content of **phosphorus** especially is characteristic for seeds<sup>25</sup>. According to Food and Nutrition Board Standing Committee (NBSC) of US National Academies recommended dietary intake (RDI) referring to phosphorus<sup>26</sup> and potassium<sup>27</sup> for adults are 0.7 g day<sup>-1</sup> and 4.7 g day<sup>-1</sup>, respectively. The results have shown that consummation of about 100 g (101.9 g - 126.6 g) of pollen as a food supplement could meet daily requirements for phosphorus for adult person depending on type of hybrids. In case of potassium 100 g of pollen could meet 12 to 18% of daily intake.

# Table 1

The analyzed pollen samples have a relatively high **sulphur** content ranging from 2.39 to 3.04 g kg<sup>-1</sup>. Its high share is expected since this element plays an important role in biosynthesis of amino acids, proteins and some vitamins. Deficiency could lead to abnormal growth of some parts of plants or to the discoloration of tissues<sup>28</sup>. In human body it is the third abundant element and it is essential for animals and humans since only plants and bacteria can synthesize organic S-compounds<sup>29</sup>. Estimated dietary intake of sulphur for

human (in form of organic S from food) is  $2.8 \text{ g day}^{-1}$  27. In that case consummation of 100 g of maize pollen will fulfil 8.5 - 10.9% of daily intake.

With respect to the content of **magnesium**  $(0.73 - 1.03 \text{ g kg}^{-1})$  and **calcium**  $(0.45 - 0.65 \text{ g kg}^{-1})$ , a lower content of these two elements, and in particular of calcium, relative to the phosphorus, is a characteristic for the plant parts that are associated with the seed<sup>25</sup>. Bearing in mind the importance and role of these two elements in human body<sup>30</sup> RDI values for adults are relatively high  $-0.42 \text{ g day}^{-1}$  for magnesium and 1 g day<sup>-1</sup> for calcium<sup>26</sup>. Comparing the content of these two elements in the maize pollen with tolerable upper intake (TUI) level it can be realized that the consumption of 100 g of pollen per day satisfies about 20% (17.4-24.6%) of daily intake for magnesium and about 5% (4.5-6.5%) for calcium.

**Zinc** as a nutrient is relatively harmless in comparison with some other similar transition metals<sup>31</sup>. Main source of zinc in food for humans in developing countries are cereals and pulses while meat, dairy products and cereals are the most important source of zinc in diet of the inhabitants of the USA and other developed countries<sup>32</sup>. In crop plants, zinc plays an important role in cellular metabolic processes since it can participate in redox reactions because of presence of one unpaired electron<sup>33</sup>.

Similar properties possesses **iron** which is the reason why these two elements are competitive in nutrition process<sup>34</sup> and why the presence of ferrous ion in food (esspecialy in food suplements) can decrease absorption of **zinc**<sup>32</sup>. In humans, the main amount of iron is presented in form of hem group of hemoglobin and myoglobin, meanwhile, presence of zinc is associated with immune system, cell division and growth, prostate functionality in

men<sup>33</sup>. Comparing amounts of these elements in maize pollen (Zn: 0.041-0.062 g kg<sup>-1</sup>; Fe: 0.019-0.076 g kg<sup>-1</sup>) with RDI level for adult person (Zn – 0.011 g day<sup>-1</sup>; Fe – 0.008 g day<sup>-1</sup>) it can be observed that there is a significant difference for both elements depending on hybrid types. For instance, consumption of 100 g of "Lady fingers" hybrid maize pollen can meet almost 95% of daily needs for iron. On the other hand, ZP standard, ZP608K and ZP611K hybrids contain much smaller quantities of iron and could cater about 25-30% of daily intake of this mineral. In case of zinc, differences between hybrids are smaller (36.8-56.8%) but, still, it is pretty much high ratio comparing to recommended dietary allowance for zinc. Bearing in mind that bee-collected pollen has been proven to be the best source of zinc and iron of all bee products<sup>21</sup> and that consumption of Serbian bee-collected pollen could satisfy 30% of daily adult human requirement for iron and 15% for zinc<sup>22</sup> it can clearly notice that maize pollen is, also, an excellent source of these two nutrients for humans.

The presence of **sodium** in the investigated samples (0.0074-0.041 g kg<sup>-1</sup>) is of negligible nutritional importance considering the great daily need for this mineral in the diet (1.5 g day<sup>-1</sup>).

Young plant organs are rich in **manganese**, element that plays an important role in redox processes in plant (photosynthesis) and as important co-factor in 35 different enzymes<sup>35</sup>. Accordingly, relatively high amount of manganese in maize pollen has been found (0.014-0.021 g kg<sup>-1</sup>). In humans, Mn, also, plays an important role as different enzymes-co-factor<sup>26</sup>. Adequate dietary intake<sup>36</sup> is 0.0023 g day<sup>-1</sup>. Comparing this value to the content of manganese in the pollen, a high level of coverage of the human daily requirement (61.7-91.3%) can be observed, if consumed 100 g of maize pollen.

Results for the content of microelements ( $< 0.01 \text{ g kg}^{-1}$ ) presented in maize pollen samples are given in Table 2.

**Aluminum** is one of the most toxic metals for plants<sup>37-39</sup>. Generally, aluminum level in plants does not exceed 0.2 g kg<sup>-1</sup> on dry mass<sup>37</sup> but there are plants species known as Alaccumulator species such as tea plant<sup>40</sup> or oat<sup>38</sup> which can normally develop despite the high amount of this element in the substrate. Unlike tea plant and oat, maize represents Alsensitive plant species<sup>38</sup>. Considering the significant presence of aluminum in many soils in the form of aluminosilicates and hydroxides, its biggest influence is on the root of the plant and its proper development<sup>37,38</sup>.

#### Table 2

The main condition for the occurrence of soluble Al-forms which plants can absorb is acidic soil <sup>38,39</sup>. According to the obtained results, there are significant differences in Alcontent between different maize hybrids. Different types of aluminosilicate in combination with acidic soil character caused the occurrence of aluminum in maize samples. Tenfold greater amount of aluminum present in three (4, 5 and 6) of seven samples can be connected to a locally larger amount of aluminosilicate and more preferred pH-value on the part of the field where hybrids were grown. Considering that the transfer of aluminum is slow from the root to the aboveground plant parts<sup>41</sup> there are great chances that the contamination occurred under the anthropogenic influence. As a part of Belgrade, Zemunsko Polje is an urban area in the vicinity of the roads, railways and relatively close to the airport. As a confirmation of these allegations, Kostić et al.<sup>22</sup> reported about presence of aluminum in two bee pollen samples collected in Belgrade area. Toxicity of aluminum for

humans has been demonstrated through neurotoxicity and autoimmunity for adults and as vaccine adjuvants for children<sup>42</sup>. Since provisional tolerable weekly intake (PTWI) for adults is 0.07 g kg<sup>-1</sup> <sup>42</sup> it can be observed that in the case of consumption of two pollen samples (4 and 5) this limit will be overcome.

The presence of **boron** in drinking water and, generally, in food is undesirable because of its potentially harmful impact on human health<sup>43</sup>. According to NBSC<sup>36</sup> tolerant level of boron in food is 0.02 g per day. In this sense, entering of 100 g pollen as food supplement (0.0004 - 0.0006 g B) would not have a detrimental effect on health.

Copper, as an essential microelement for the functioning of many enzymes, especially in hematological and neurologic systems<sup>25,44</sup> in human body has recommended daily intake on the level of 0.0009 g day<sup>-1</sup> <sup>36</sup>. According to obtained results for maize pollen 19 to 30% of daily requirement may be satisfied through its consumption as food supplement. Although most part of the orally ingested copper wouldn't be absorbed excess dietary intake might cause accumulation in liver and intoxication of organism<sup>25</sup>.

In the case of **chromium**, with RDI of  $3.5 \cdot 10^{-5}$  g day<sup>-1</sup> <sup>36</sup> maize pollen has shown great ability to fulfill that requisite (25.7-68.6% of RDI). The presence of chromium in all samples is expected having in mind the presence in chromite mineral as well as its possible presence in garnets, epidote, and other minerals, which were also detected in surrounding soil. The importance of chromium for man is reflected in the influence of chromium (III) ions as essential micronutrients. On the other hand, Cr (VI) is carcinogen form but, it is important that it might be easily reduced in Cr (III), both in soil or in gastrointestinal tract. Absorbency of chromium in body is low, about 5%, similarly to copper<sup>45</sup>.

**Selenium** is important microelement with pronounced antioxidant capacity (synergistic with vitamin E). Organic forms of Se are more important than inorganic because of higher solubility<sup>25</sup>. RDI for this element<sup>46</sup> is 5.5·10<sup>-5</sup> g day<sup>-1</sup> which makes pollen samples as a potentially good source in diet (12.5-32% of RDI).

Amount of **vanadium** in samples 4 and 5 was about 15 times higher than in the other samples which may indicate anthropogenic contamination. Main source of vanadium in environment is combustion of fossil fuels or road fill materials and cement<sup>47,48</sup>. Even though still cannot be claimed there are indications that exposure to elevated concentrations of vanadium is harmful and potentially carcinogenic<sup>49</sup>. Because of that NBCS<sup>37</sup> gave relatively low tolerable daily intake level (0.0018 g day<sup>-1</sup>). Results have shown that consumption of 100 g of pollen may satisfy about 50% of this value in case of four samples or may significantly exceed that amount (about 900%) in case of samples with elevated concentration of vanadium. In case of some trace, toxic, elements, Campos et al.<sup>50</sup> suggest limit values in bee pollen – 0.0001 g kg<sup>-1</sup> for cadmium and 0.0005 g kg<sup>-1</sup> for **lead** and **arsenic**. In all samples these three elements were in allowed boundaries except in case of As in sample 2 with elevated concentration (0.00069 g kg<sup>-1</sup>).

Previously, Kostić et al.<sup>22</sup> reported about the presence of **strontium** in bee pollen for the first time in literature with mean value of 0.00138 g kg<sup>-1</sup>. In this case limits were a little bit lower (0.0004-0.00097 g kg<sup>-1</sup>) but, anyway, presence of this element in food supplements is undesirable because of its toxicity for humans. All other microelements were detected in traces or not detected at all in the investigated maize pollen samples.

The average mineral content in food can vary significantly depending on type. In Table 3 mineral content of different dairy products<sup>51</sup>, maize kernel and corn flakes<sup>52</sup> is shown together with values for macro and microelements in investigated pollen samples. If obtained results have been compared with these types of food it can be clearly observed higher content of K, P, Mg and S in pollen samples as well as for Zn, Fe, Mn, Cu, B and Al (Figure 2a and b).

#### Table 3

## **CONCLUSIONS**

According to obtained results there are significant differences between seven maize pollen of Serbian hybrids related to some macro and microelements: iron, zinc, manganese, aluminum, chromium, selenium and vanadium. Also, good nutritional potential of maize pollen was observed if it would be applied as food supplement in human diet. Floral pollen has shown better nutritional characteristics in sense of iron and zinc content comparing to bee pollen. Chromium and manganese presented in pollen can fulfill meaningful (Cr) or major part (Mn) of needs for this nutrient in diet. Presence of aluminum, vanadium and strontium in pollen can represent potential problem due to its toxicity to humans.

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Table capture

**Table 1**. The content of macroelements [g  $kg^{-1} \pm STDEV$ ] in pollen samples collected from seven maize hybrids grown in Zemun polje settlement (Belgrade, Serbia)

Sample's	K	P	S	Mg	Ca	Zn	Fe	Na	Mn
label									
S01	5.12 ±	6.41 ±	2.39 ±	0.73 ±	0.63 ±	0.056 ±	0.019 ±	0.007 ±	0.014 ±
	0.010a	0.010	0.010	0.002	0.003	0.001	0.0008a	0.0003	0.0005a
S02	6.84 ±	5.53 ±	2.45 ±	1.02 ±	0.59 ±	$0.041 \pm$	$0.019 \pm$	0.029 ±	$0.018 \pm$
	0.006	0.010	0.010	0.008a	0.001	0.001	0.0001a	0.0006	0.0003b
S03	6.07 ±	5.96 ±	2.54 ±	0.99 ±	$0.65 \pm$	$0.053 \pm$	$\boldsymbol{0.027} \pm$	$0.018 \pm$	$0.021 \pm$
	0.010	0.030	0.010	0.004b	0.001	0.0007	0.0009	0.0007	0.001
S04	7.76 ±	6.87 ±	3.04 ±	1.03 ±	$0.62 \pm$	$0.062 \pm$	$0.072~\pm$	$0.024 \pm$	$0.018 \pm$
	0.008	0.010	0.010	0.011a	0.003	0.001	0.016	0.0009	0.0009b
S05	6.43 ±	6.49 ±	2.70 ±	0.99 ±	0.51 ±	$0.045 \pm$	$0.076 \pm$	$0.041 \pm$	$0.016 \pm$
	0.015	0.020	0.015	0.010b	0.002	0.001a	0.001	0.001	0.0008c
S06	5.88 ±	6.61 ±	2.85 ±	0.92 ±	$0.45 \pm$	$0.045 \pm$	$0.052 \pm$	$0.013 \pm$	$0.015 \pm$
	0.011	0.009	0.010	0.003c	0.002	0.001a	0.0006	0.0003	0.0007a,c
S07	5.11 ±	6.79 ±	2.81 ±	0.92 ±	$0.40 \pm$	0.046 ±	$0.035 \pm$	0.014 ±	0.011 ±
	0.011a	0.011	0.012	0.002c	0.002	0.001a	0.001	0.0002	0.0005

Means with same letter in same column are not significantly different (p < 0.05)

**Table 2**. The content of microelements [mg kg<sup>-1</sup>  $\pm$  STDEV] in maize pollen samples

Sample's label	Al	В	Cu	Sr	Cr	Cd	Li	Sb
S01	6.85 ±	6.51 ±	1.89 ±	0.50 ±	0.113 ±	0.026 ±	0.0100 ±	0.080 ±
	0.07	0.05	0.01	0.01	0.001	0.0006a	0.0004	0.004a

S02	6.07 ±	5.04 ±	1.80 ±	0.95 ±	0.094 ±	0.009 ±	0.0190 ±	$0.080 \pm$
	0.02	0.04a	0.01	0.02a	0.001	0.0002	0.0004a	0.003a
S03	$8.07 \pm$	5.08 ±	2.33 ±	$0.97 \pm$	0.109 ±	$0.013 \pm$	$0.0180 \pm$	$0.098 \pm$
	0.19	0.11a	0.10a	0.01a	0.001	0.006	0.0008a	0.002
S04	74.29	5 90 1	2.24 +	0.74	0.222	0.020 +	0.0650	0.047
304	74.28 ±	5.80 ±	2.24 ±	0.74 ±	0.233 ±	0.028 ±	0.0650 ±	0.047 ±
	0.99	0.13b	0.09a	0.01	0.008a	0.0012a	0.0010	0.002
S05	89.65 ±	5.55 ±	2.73 ±	0.70 ±	0.239 ±	0.018 ±	0.0790 ±	0.014 ±
	0.83	0.10b	0.06	0.01	0.004a	0.0007	0.0015	0.001
S06	48.20 ±	4.82 ±	2.31 ±	0.42 ±	0.166 ±	$0.006 \pm$	$0.0420 \pm$	0.022 ±
	0.68	0.22a	0.02a	0.02b	0.003	0.0001	0.0015	0.001a
S07	$23.88 \pm$	5.85 ±	1.74 ±	$0.40 \pm$	$0.133 \pm$	n.d.*	$0.0230 \pm$	$0.024 \pm$
	0.99	0.28b	0.03	0.01b	0.002		0.0007	0.001a
	Se	Mo	V	Ni	Pb	Со	As	
S01	0.069 ±	0.0220 ±	$0.0100 \pm$	$0.0050 \pm$	0.0040 ±	n.d.	n.d.	
	0.002	0.0010	0.0005a	0.0002	0.0002			
S02	0.158 ±	0.026 ±	0.0110 ±	0.0070 ±	n.d.	n.d.	0.686 ±	
502	0.008a	0.0004	0.0002a	0.0002	n.u.	n.u.	0.003	
	0.000a	0.0004	0.00024	0.0002			0.003	
S03	0.176 ±	n.d.	0.0170 ±	n.d.	0.0490 ±	n.d.	0.032 ±	
	0.005b		0.0008		0.0013		0.001	
S04	0.155 ±	n.d.	0.1390 ±	n.d.	0.0070 ±	$0.070 \pm$	n.d.	
	0.007a		0.0068		0.0001	0.003		
S05	$0.073 \pm$	n.d.	$0.1650 \pm$	$0.0080 \pm$	n.d.	$0.017 \pm$	n.d.	

S06	$0.172 \pm$	$0.009 \pm$	$0.0940 \pm$	n.d.	n.d.	$0.0080 \pm$	n.d.
	0.004b	0.0001	0.0025			0.0001	
S07	0.176 ±	$0.007 \pm$	$\textbf{0.0410} \pm$	$\textbf{0.0110} \pm$	n.d.	n.d.	n.d.
	0.008b	0.0001	0.0006	0.0010			

\*n.d. - not detected

Means with same letter in same column are not significantly different (p < 0.05)

**Table 3**. The average content of macro  $[g \ kg^{-1}]$  and microelements  $[g \ kg^{-1}]$  in some of food types in comparation with maize pollen samples<sup>51,52</sup>

Food type	Milk	Cheeses	Butter	Yoghurt	Corn	Maize	Pollen	Higer

						flakes*	kernel*		(†)
									Lower
									(\psi)
									Equal
									(=)
	K	1.44-1.78	0.77-1.6	0.15	2.8	1.2	2.94	5.11-7.76	1
_	P	0.63-1.02	1-8.1	0.24	1.7	0.059	2.13	5.53-6.87	1
macroelements	Ca	1.07-1.33	0.73-12	0.15	2	0.13	0.08	0.40-0.65	↓ or =
eleme	Na	0.4-0.58	3-14.4	0.11	0.8	9.15	0.06	0.007-0.041	$\downarrow$
ents	Mg	0.09-0.16	0.09-0.45	0.02	0.19	/	/	0.73-1.03	1
	S	0.32	/	/	/	/	/	2.39-3.04	1
	Zn	0.74-1.45	5-53	1	7	/	/	41-62	1
	Fe	0.3-0.7	1-8	2	1	0.02	0.015	19-76	1
	Mn	0.013-0.04	<1	/	/			11-21	1
	Cu	0.02-0.3	<0.0033	0.0003	/			1.74-2.73	1
E.	Se	0.013-0.017	0.01-0.12	/	0.02			0.069-0.176	1
roele	Co	0.5-1.3						<0.07	$\downarrow$
microelements	Ni	0.004-0.06						<0.011	↑ or =
<b>9</b> 2	Mo	0.024-0.06						<0.026	1
	В	0.19-0.95						4.82-6.51	1
	Cr	0.01-0.04						0.094-0.239	1
	Al	0.46						6.07-89.65	<b>↑</b>

Fig. 1. The geographical map of the study area indicating experimental field



Figure 2. Comparation of mineral content in pollen and different types of food – a)macroelements b) microelements



