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# The Use of Natural Bee Products as Bioindicators of Environmental Pollution-The Detection of Heavy Metals

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**ABSTRACT**

Can be pollen used as a biological indicator of environmental pollution. So this work aims to determine some of the heavy metals such as Pb, Cr, Cd, Zn, and Cu in bee pollen from different areas in the Pelagonija region. The concentration of heavy metals in pollen depends on the geographical area, plants, and soil. And also, the composition of pollen varies with the source location, source plant, etc. The concentrations of transition metals copper (Cu), zinc (Zn), cadmium (Cd), and chromium (Cr) Lead (Pb) were measured by Gas Chromatography. The samples were collected from four different places, as well as a general picture of the phenomenology of the targeted sites, and accordingly obtained the ecological picture of the possible contamination. Also, the data of the composition of the soil were collected from the same locations, and are taken into consideration. Pollen collected by bees (Apis mellifera Macedonica sub-species) is used as a bioindicator for further eco monitoring. The concentration of heavy metals contents in pollen is varies from sample to sample, due to the difference in the geographical area, various plants species, and soil. The composition of pollen varies with the source location, source plant, and handling. We have collected samples during the same season from the bee apiaries, located in industrial-urban and rural areas. The availability of heavy metals in plants depends on soil reaction, mineral colloids, soil humidity, microbiological activity, and organic matter content. Hence pollen can be used as a biological indicator of environmental pollution.

**Keywords:** Bee powder, Bioindicator, Heavy metals, Contamination, Pollution.

## INTRODUCTION

Bees and their products have been suggested as biological indicators of environmental pollution in many studies. The pollen powder collected by honey bees from flowers is known as honey bee pollen[1]. Pollen is stored in the chambers of the hives by honeybees. Bee pollen is a ball or pellet of pollen collected in the outdoors by worker honeybees and utilized as the hive's major food supply. All of the additional nutrients required for honeybee development and growth are found in pollen. It is a wide-ranging plant product. Bees use it as a raw material to make bee bread. Pollen colours ranged from bright yellow to dark brown. Pollen grains can be as large as 2.5–250 m in diameter and vary in shape, colour, size, and weight depending on the plant species. Proteins, amino acids, carbohydrates, lipids, fatty acids, phenolic compounds, enzymes, and coenzymes, as well as vitamins and bio elements, were discovered in pollen powder[2]. Study on Pollen is important as it provides a "real" picture of the atmosphere; however, it is difficult to provide clear ideas about pollen and pollution interactions because multiple components are found mixed in various types of pollutants in different regions of the world, and finding a single experimental device for pollen-pollutant detection is currently difficult[3]. Pollen powder from various floristic components has a composition of fifty percent total carbohydrates, 2-16 percent polysaccharides and dietary fibers, 6-28 percent proteins, 4-8 percent lipids, and 6 percent free amino acids[4]. Because of the high concentrations of flavonoids and other phenolic components in bee pollen powder, it possesses anti-inflammatory, antiviral, antioxidant, antifungal, antibacterial, and immune-stimulating characteristics[1,5-6]. Selected heavy metals that have been studied, or potentially toxic elements, lead, cadmium, copper, zinc, chromium is noticed in foods, and generally, their concentrations are higher than the approved limit[7-8]. The Food and Agriculture Organization (FAO) of Macedonia, as well as the World Health Organization (WHO), have both registered and have made the legal norms (Official Gazette of RM-No.102/2013) in favours of it[9]. A high concentration of heavy metals can reason health problems. Pollen powder is one of the bee products that can be used as a bioindicator[10] because its final product contains a collection of several factors, soil, water, plants, air, etc.[10-13].

## EXPERIMENTAL

For experimentation purposes, we have ensured the following steps.

### Sampling

#### a-Sampling of pollen-

The pollen powder samples were collected from the bee apiaries from four different locations of the Pelagonija region, and conserved in the vacuum-packed bags at the temperature ($<-4^{0}$C). All samples are collected in the same season [from May 2020 to October 2020]. Half gram (0.5 g) samples are taken in macerated in a porcelain plate for the test, and then added the $HNO_3$ 69%, MERCK Trace Pure (7 mL+2 mL $H_2O_2$), and Alkaloid. Left the sample mixture in the oven (CEM Mars 5 Digestion Oven) for 24 hours. The solutions are filtrated by (Munktell Filter-Qualitative Filter Papers) and put into plastic laboratory pumpkins of 25 mL for the experiment[14].

#### b-Sampling of soil-

From the same locality, soil samples were also collected for the detection of Potentially Toxic Elements (PTE), as well as an overview of the flora from the same regions.

### The Region Selected for sampling

The region selected for the collection of experimental samples is ecologically polluted due to the different established industries. The region situated geographically at the latitudes of 40°50″ and 42°20″ N and longitudes of 20°27′ and 23°05″ E, and further, we subdivided it into two groups: two lowland areas near to river areas around the village of Novaci (41°2′34.49″ N, 21°27′35.81″ E, 600 m a.s.l.), the village of Makovo (41°7′4.91″ N, 21°36′31.6″ E, 620 m a.s.l.) and two mountain areas or highland areas named the village of Rapeš (41°6′9.73″ N, 21°38′50.18″ E, 802 m a.s.l.) and the village of Orle (41°8′53.82″ N, 21°36′48.28″ E,780 m a.s.l.).

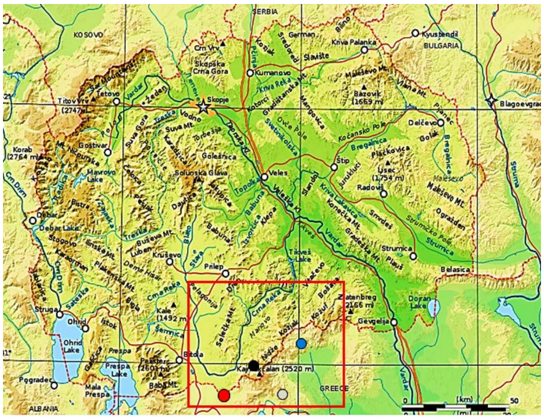


**Fig. 1. The geographical map of the Republic of Macedonia (taken from Google Maps). The landmarks are such as the red spot is for the village Novaci and the white spot is for village Makovo (both are lowland areas) and village Orle is a black spot whereas village Rapes blue (both are highland areas)**

**Instrumentation**

The Potentially Toxic Elements (PTE) were examined by the application of atomic emission spectrometry with inductively coupled plasma, ICP-AES (VARIAN spectra AA240) applied an ultrasonic nebulizer CETAC (ICP/U-5000AT+) for better results and sensitivity. The Rosh-Drying oven DZLG-9023A, Soxhlet apparatus (Sigma-Aldrich Z556173), Kjel Digester K-449 and Buchi Scrubber K-415, Büchi Kjeldahl and Sistem Kjel Flex K-360, HPLC -MKC EN 14463:2010, and electronic balance were used the sample analysis.

**Phytosociology**

In the phytosociological literature, the maximum and permissible limit scale is used that is proposed by Braun Blanquet. This scale contains not only numerical grades but also descriptive, but it does not have a mathematical data processing application.

**Experiments**

The samples analysis was carried out by an accredited laboratory and test methods. The concentrations of the potentially toxic elements such as lead, cadmium, copper, zinc, chromium, were examined in the pollen samples, soil, and the agricultural products of the region with help of the instruments discussed above.

For the Physical and chemical composition of bee pollen samples, 1 g of bee pollen is taken at $105^0$C, and firstly examined the ash content by using Rosh-Drying oven DZLG-9023A. For the test of the lipid concentration, two-gram (2 g) pollen samples are taken at (>78°C) for 5 h and determined with the help of Soxhlet apparatus (Sigma-Aldrich Z556173) automated method applied and using diethyl ether (80 mL) as a solvent. The concentration of nitrogen components was determent with the classic method by Kjeldal method, taking 1 g of sample for the test. A KjelDigester K-449 and Buchi Scrubber K-415 are used for homogenization, and digestion a Büchi Kjeldahl and Sistem KjelFlex K-360 is used. For the determination of fructose and glucose in the sample powder HPLC spectrometer is used. The HPLC spectrometer used for the purpose is ISO Standards and MKC EN 14463:2010 by Agilent technologies G7129A/1260.

**Data analysis**

Data were analysed using packages software SPSS 19.0. Means and Standard deviation, one-way ANOVA test was used, the statistical significance was $p<0.05$. The same data analysis package was used for bee products[15-16].

## RESULTS AND DISCUSSIONS

It was assumed that bee powder is an ecologically pure product that can be a bioindicator[14]. of environmental pollution. The physical-chemical composition of bee pollen, the mean composition data of lipid, percentage of heavy metals in food grains and soil, of the target location, may confirm the above assumption. The current study compels us to accept the said assumption that the bee pollen powder can be used as a high degree of the eco-monitoring system with the targeted locations[17].

The results of phytocoenological structure obtained from the different target locations are presented in Table 1.

The results show that the finding of Lipids is in order of Orle 7.31 mg/kg>Rapeš, 5.38 mg/kg>Makovo 4.44 mg/kg> and Novaci 3.49 mg/kg. Orle has the highest among all 7.31mg/kg.

The Potentially Toxic Elements such as lead, cadmium, copper, zinc, chromium are analyzed quantitatively of the soil samples from the four different targeted locations of study[8].

However, in principle, these elements are part of human food, but they should be under the authorized limit. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO), as well as the government of Macedonia (Official Gazette of RM-No.102/2013), have fixed the optimum limit of human food and its product.

According to the regulation of WHO (World Health Organization), the optimum limit of Cu should be 20.0 mg/kg, but the above-obtained results showed a very low concentration of Cu only 2.01 mg/kg. Even much lower than the results obtained by Harmanescu (2007), according to his findings from different areas are as Agricultural area <4.47, The Industrial area <4.58, and in the desert area <5.34 mg/kg.

**Table 1: Phytocoenological findings of the target locations**

| Sequence number of the recording | 1 | 2 | 3 | 4 | 1 | | | | 2 | | | | 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locality | v. Novaci | v. Makovo | v. Orle | v. Rapeš | v. Novaci | v. Makovo | v. Orle | v. Rapeš | v. Novaci | v. Makovo | v. Orle | v. Rapeš | v. Novaci | v. Makovo | v. Orle | v. Rapeš |
| Надморска висина (m) | 600 | 620 | 780 | 802 | | | | | | | | | | | | |
| Exposure | E | E | E | E | | | | | | | | | | | | |
| Slope (0) | 25 | 25 | 35 | 35 | | | | | | | | | | | | |
| Coverage (%) | 60 | 60 | 40 | 50 | | | | | | | | | | | | |
| Surface image | 100 | 100 | 100 | 100 | | | | | | | | | | | | |
| Geological basis | clayey | clayey | clayey | clayey | Degree of presence | | | | Mean cover value | | | | Frequency (%) | | | |
| *Sequence number of the recording | 10 | 10 | 10 | 10 | | | | | | | | | | | | |
| *Medicago sativa- alfalfa* | 3.3 | 3.4 | 2.2 | 1.1 | V | IV | II | I | 37,5 | 37,5 | 17,5 | 5,0 | 100 | 80 | 40 | 20 |
| *Trifolium pratense- red clover* | 3.3 | 3.2 | 1.1 | 2.1 | V | IV | I | II | 37,5 | 37,5 | 5,0 | 17,5 | 100 | 80 | 20 | 40 |
| *Cirsium arvense – creeping thistle* | 3.2 | 1.3 | 1.1 | 1.1 | V | IV | II | I | 37,5 | 17,5 | 5,0 | 5,0 | 100 | 80 | 40 | 20 |
| *Clematis vitalba L. – traveller's joy* | 3.3 | 2.3 | 2.1 | 1.2 | IV | III | II | II | 37,5 | 17,5 | 17,5 | 5,0 | 80 | 60 | 40 | 40 |
| *Campanula medium-canterbury bells* | 3.2 | 1.2 | + | 1.1 | IV | III | I | II | 37,5 | 17,5 | 0,1 | 5,0 | 80 | 60 | 20 | 40 |
| *Salvia nemorosa – balkan clary* | 2.2 | 1.2 | 1.3 | 3.1 | IV | III | II | V | 17,5 | 5,0 | 5,0 | 37,5 | 80 | 60 | 40 | 100 |
| *Heliantus annus – sunflower* | 3.4 | 1.1 | + | + | IV | I | I | I | 37,5 | 5,0 | 0,1 | 0,1 | 80 | 20 | 20 | 20 |
| *Medicago sativa – alfalfa* | 3.3 | 2.3 | 1.1 | + | V | IV | II | I | 37,5 | 17,5 | 5,0 | 0,1 | 100 | 80 | 40 | 20 |
| *Gossypium - cotton* | 1.2 | 1.2 | + | + | III | II | I | I | 5,0 | 5,0 | 0,1 | 0,1 | 60 | 40 | 20 | 20 |
| *Rubusidaeus L. - red raspberry* | + | 1.1 | 2.3 | 2.2 | II | II | IV | V | 0,1 | 5,0 | 17,5 | 17,5 | 40 | 40 | 80 | 100 |
| *Ulmis campetris L .- elm* | 1.2 | 1.1 | 3.3 | 3.4 | III | III | IV | IV | 5,0 | 5,0 | 37,5 | 37,5 | 60 | 60 | 80 | 80 |
| *Corylus colurna L. - hazel* | 1.1 | 1.2 | 3.4 | 2.3 | II | II | IV | IV | 5,0 | 5,0 | 3,5 | 17,5 | 40 | 40 | 80 | 80 |
| *Trifolium repenss L. – white clover* | 3.2 | 3.4 | 2.2 | + | IV | IV | III | II | 37,5 | 37,5 | 37,5 | 0,1 | 80 | 80 | 60 | 40 |
| *Tymus serpyllum – thyme* | 1.2 | + | 1.2 | + | III | II | IV | V | 5,0 | 0,1 | 5,0 | 0,1 | 60 | 40 | 80 | 100 |
| *Salvia verticullata L. – ring sage* | 1.3 | 1.3 | + | + | III | III | II | II | 5,0 | 5,0 | 0,1 | 0,1 | 60 | 60 | 40 | 40 |
| *Borago officinalis L. – borage* | 1.2 | 1.1 | 1.1 | + | III | II | III | II | 5,0 | 5,0 | 5,0 | 0,1 | 60 | 40 | 60 | 40 |
| *Rubus fruticosus L .- rubus* | 2.1 | 1.1 | 3.1 | 3.3 | II | II | IV | IV | 17,5 | 5,0 | 37,5 | 37,5 | 40 | 40 | 80 | 80 |
| *Rubus idaeus L. – raspberry* | + | 1.1 | 3.3 | 3.3 | I | II | V | IV | 0,1 | 5,0 | 37,5 | 37,5 | 20 | 40 | 100 | 80 |
| *Lotus corniculatus - bird's-foot trefoil* | 2.3 | 1.2 | 1.1 | + | II | II | II | II | 17,5 | 5,0 | 5,0 | 0,1 | 40 | 40 | 40 | 40 |
| *Cucurbita pepo - pumpkin* | 2.4 | 1.2 | r | r | IV | II | I | I | 17,5 | 5,0 | 0 | 0 | 80 | 60 | 20 | 20 |
| *Stachys annua L. –wight basil* | 1.1 | 1.3 | 1.1 | 1.1 | II | III | II | II | 5,0 | 5,0 | 5,0 | 5,0 | 40 | 60 | 40 | 40 |
| *Lytrum salicaria L. – purple loosestrife* | 1.1 | 1.2 | + | + | II | III | I | II | 5,0 | 5,0 | 0,1 | 0,1 | 40 | 60 | 20 | 40 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mentha longifolia – horse mint* | 2,4 | + | 1,1 | 2,3 | III | I | II | III | 17,5 | 0,1 | 5,0 | 17,5 | 60 | 20 | 40 | 60 |
| *Rosa canina L. - dog-rose* | 3.3 | 3.3 | 3.4 | 3.4 | III | III | IV | V | 37,5 | 37,5 | 37,5 | 37,5 | 60 | 60 | 80 | 100 |
| *Robinia pseudoacacia L. - black locust* | 2.3 | 2.2 | 1.1 | + | IV | IV | II | I | 17,5 | 17,5 | 5,0 | 0,1 | 80 | 80 | 40 | 20 |
| *Lavandula officinalis spp. – lavender* | 2.1 | 1.1 | 3.1 | 2.1 | II | III | IV | III | 17,5 | 5,0 | 37,5 | 17,5 | 40 | 60 | 80 | 60 |
| *Humulus lupulus - hops* | 1.1 | 1.2 | 2.4 | 2.4 | II | II | IV | IV | 5,0 | 5,0 | 17,5 | 17,5 | 40 | 40 | 80 | 80 |
| *Castanea sativa - sweet chestnut* | 2.1 | 2.1 | 1.1 | 1.1 | IV | IV | I | I | 17,5 | 17,5 | 5,0 | 5,0 | 80 | 80 | 20 | 20 |
| *Iris germanica L. - bearded iris* | 1.1 | 1.1 | 1.1 | 1.1 | I | II | III | III | 5,0 | 5,0 | 5,0 | 5,0 | 20 | 40 | 60 | 60 |
| *Campanula roduntifolia L. – harebell* | + | 1.1 | 1.1 | r | I | II | II | I | 0,1 | 5,0 | 5,0 | 0 | 20 | 40 | 40 | 20 |
| *Melisa officinalis L.- lemon balm* | 1.2 | 1.1 | + | + | III | III | I | I | 5,0 | 5,0 | 0,1 | 0,1 | 60 | 60 | 20 | 20 |
| *Mentha spicata - diosmos* | 1.3 | 1.2 | 1.3 | 1.3 | II | II | III | III | 5,0 | 5,0 | 5,0 | 5,0 | 40 | 40 | 60 | 60 |
| *Prunus domestica L. – plum* | + | + | 2.3 | 1.2 | I | I | IV | II | 0,1 | 0,1 | 17,5 | 5,0 | 20 | 20 | 80 | 40 |
| *Salix alba – white willow* | 1.1 | 1.1 | 1.1 | 1.1 | I | III | I | I | 5,0 | 5,0 | 5,0 | 5,0 | 20 | 60 | 20 | 20 |
| Prunus cerasifera- cherry plum | 1.1 | r | 1.1 | + | II | II | III | I | 5,0 | 0 | 5,0 | 5,0 | 40 | 20 | 60 | 20 |
| *Polygonium persicaria – redshank* | 1.3 | 1.1 | r | + | III | II | I | I | 5,0 | 5,0 | 0 | 0,1 | 60 | 40 | 20 | 20 |
| *Saturea montana l.- winter savory* | 1.2 | 1.1 | 2.2 | 2.1 | III | II | IV | IV | 5,0 | 5,0 | 17,5 | 17,5 | 60 | 40 | 80 | 80 |
| *Solidago virgaurea L. - european goldenrod* | 1.2 | 1.3 | 1.2 | + | III | III | II | I | 5,0 | 5,0 | 5,0 | 0,1 | 60 | 60 | 40 | 20 |
| *Salvia officinalis L. -common sage* | 1.3 | 1.1 | 1.1 | r | IV | V | III | II | 5,0 | 5,0 | 5,0 | 0 | 80 | 100 | 60 | 40 |
| *Calluna vulgaris - hull* | 2.1 | 2.1 | 1.2 | 1.1 | III | II | IV | IV | 17,5 | 17,5 | 5,0 | 5,0 | 60 | 40 | 80 | 80 |
| *Satureja montana -winter savory* | 1.1 | + | 1.1 | 1.2 | II | II | III | III | 5,0 | 0,1 | 5,0 | 5,0 | 40 | 40 | 60 | 60 |
| *Castanea sativa - sweet chestnut* | r | r | 1.1 | 1.2 | I | I | III | II | 0 | 0 | 5,0 | 5,0 | 20 | 20 | 60 | 40 |
| *Rubus fruticosus L. - blackberry* | 1.2 | 1.1 | 2.2 | 3.4 | I | II | IV | V | 5,0 | 5,0 | 17,5 | 37,5 | 20 | 40 | 80 | 100 |
| *Rubus idaeus L. - red raspberry* | 1.1 | 1.2 | 3.2 | 3.3 | I | II | IV | V | 5,0 | 5,0 | 37,5 | 37,5 | 20 | 40 | 80 | 100 |
| *Tymus serpulum – wild thyme* | 1.1 | 1.2 | 3.4 | 3.4 | II | II | IV | V | 5,0 | 5,0 | 37,5 | 37,5 | 40 | 40 | 80 | 100 |
| *Juglans nigra L. - black walnut.* | + | r | 3.3 | 3.4 | I | I | IV | IV | 0,1 | 0 | 37,5 | 37,5 | 20 | 20 | 80 | 80 |
| *Hipericum perforatum - perforate St John's-wort* | + | r | 1.1 | 3.3 | I | I | II | IV | 0,1 | 0 | 5,0 | 37,5 | 20 | 20 | 40 | 80 |
| *Bruns spruce - bruns* | 1.1 | + | + | 1.1 | I | I | II | III | 5,0 | 0,1 | 5,0 | 5,0 | 20 | 20 | 40 | 60 |
| *Pinus silvestris – wight pinus* | 1.1 | + | 1.1 | 2.3 | I | I | II | IV | 5,0 | 0,1 | 5,0 | 17,5 | 20 | 20 | 40 | 80 |
| *Alnus glutinosa – black alder* | + | + | + | 1.3 | I | I | I | III | 0,1 | 0,1 | 0,1 | 5,0 | 20 | 20 | 20 | 60 |

**Table 2: Physical-chemical composition of the collected bee pollen from the target locations**

| Component | Novaci | Makovo | Orle | Rapeš |
|---|---|---|---|---|
| Lipid | 3.49±0.67 | 4.44±1.25 | 7.31±0.52 | 5.38±1.14 |
| Protein | 18.10±1.61 | 21.65±0.22 | 24.47±1.42 | 26.45±3.02 |
| Fructose | 11.73±0.28 | 12.70±0.61 | 11.60±0.92 | 14.53±0.61 |
| Glucose | 8.42±0.06 | 8.49±0.21 | 12.56±1.12 | 14.17±1.65 |
| Moisture | 22.46±0.09 | 23.36±0.12 | 25.34±0.16 | 26.31±0.31 |
| Ash | 2.18±0.04 | 2.31±0.01 | 2.35±0.05 | 2.48±0.06 |

Note. Data are given as means ± SD (medians); * p< 0.05, ** p < 0.01, *** p < 0.001; c -Novaci, d - Makovo, c- Orle, d -Rapeš

**Table 3: The concentration of heavy metals Cu, Pb, Cd, Cr, and Zn (mg /kg) in the bee powder samples from the target locations**

| | Four different Areas are targeted | | | |
|---|---|---|---|---|
| Element | v.Novaci | v.Makovo | v.Orle | v.Rapeš |
| Cu | 2.01±0,42 | 1.60±0,24 | 1.62±0,37 | 1.85±1,31 |
| | (2.04) | (1.5) | (1.5) | (1.62) |
| Pb | 0.78±0.31 | 1.10±0.80 | 1.21±0.69 | 1.07±0.55 |
| | (0.72) | (1.03) | (0.9) | (0.82) |
| Cd | 0.5±1.12 | 0.12±0.40 | 0.02±0,10 | 0.03±0.25 |
| | (0,60) | (0.7) | (0.78) | (0.64) |
| Cr | 1.14±1.12 | 0.80±0.40 | 0.60±0.10 | 0.55±0.25 |
| | (0.78) | (0.7) | (0.6) | (0.64) |
| Zn | 38.50±9,24 | 34.05±7.70 | 29.68±4.58 | 27.84±3,61 |
| | (37.58) | (32.17) | (30.14) | (28.33) |

Note. Data are given as means ± SD (medians); * p< 0.05, ** p < 0.01, *** p < 0.001; c - Novaci, d - Makovo, c- Orle, d - Rapeš.

**Table 4: The results of the Concentration Potentially Toxic of elements Cu, Pb, Cd, Cr and Zn (mg/kg) of the soil samples from the four different targeted locations**

| Element | Area | | | |
|---|---|---|---|---|
| | Novaci | Makovo | Orle | Rapeš |
| Cu*** | 15.41 ± 2.48 c*d*** | 26.64 ± 1.52 acd*** | 12.08 ± 1.85 d** | 8.95 ± 0.74 |
| | -16.4 | -26.85 | -12.1 | -9.05 |
| Pb*** | 17.15 ± 1.17 cd*** | 14.01 ± 0.84 cd*** | 11.62 ± 1.02 d*** | 8.26 ± 0.71 |
| | -17.55 | -14.15 | -11.45 | -8.2 |
| Cd*** | 0.25 ± 0.05 | 0.60 ± 0.09 cd*** | 0.36 ± 0.09 a* | 0.42 ± 0.05 a*** |
| | -0.27 | -0.58 | -0.36 | -0.41 |
| Cr*** | 38.28 ± 0.83 bd***c* | 34.47 ± 2.14 d*** | 36.02 ± 1.78 d*** | 27.67 ± 1.46 |
| | -38.45 | -33.8 | -36.8 | -27.8 |
| Zn*** | 29.57 ± 2.01 | 53.26 ± 2.02 ac*** | 43.52 ± 1.62 a*** | 72.03 ± 1.55 abc** |
| | -29.15 | -53.55 | -43.4 | -72.2 |

Note. Data are given as means ± SD (medians); * - p< 0.05, ** - p < 0.01, *** - p < 0.001; c - Novaci, d - Makovo, c - Orle, d - Rapeš

The study found the concentration of Cd in bee powder is above the optimum limit in the low land areas, the data shows as in v. Novaci the average concentration of Cd is 0.5mg/kg, and in v. Makovo Cd is 0.12mg/kg.

The Cd concentration in the soil from the different locations, mentioned by the same said author was as in the order of 0.5mg/kg in Novaci> 0.1mg/kg in Makovo> 0.03mg/kg in Rapeš> 0.02mg/kg in Orle. Similar results were presented by Sattler D.A.G, and S. Sandikci Altunatmaz as well.

The amount of Cr was found in Novaci 1.14mg/kg> in Orle 0.80mg/kg> in Makovo 0.60mg/kg> in Rapeš 0.55mg/kg respectively. But as compared to the value found by the Kostić and *et al.,*[4] is much lower, is between 0.170 mg/kg to 0.465 mg/kg.

The mean amount of Zn in bee powder was found in order of from minimum to maximum as 27.84 mg/kg in Rapeš,<29.68 mg/kg in Orle< 34.05 mg/kg in Makovo<38.50 mg/kg in Novaci. According to WHO (World Health Organization), the optimum limit of Zn is specified as 60.0 mg/kg. Similar results were reported by Hamza and co-authors of Jordan as well.

The concentration of Pb in bee powder were found very low in all four target locations, the results are found as 1.21mg/kg in Orle>0.78mg/kg in Novaci>0.07mg/kg in Rapeš>0.10mg/kg in Makovo. It has been confirmed from the current study that the concentration of heavy metals in Pollen's powder is depended on the type of plant, soil, and the local geographical structure. Pollen powder is one of the bee products that can be used as a bioindicator, which includes a compilation of several stages, soil, water, plants, and air. A higher or lower concentration of the above investigated heavy metals can cause human health problems[9,18-20,11-13,21-29].

## CONCLUSION

The focus of the present work is to identify heavy metals like Pb, Cr, Cd, Zn, and Cu in bee pollen from various parts of the Pelagonija region. Bee pollen powder is a substance collected by bees from flowers. Bees and their products have been suggested as biological indicators of environmental pollution in many studies. We can be concluded from the collected data that heavy metal levels are associated with the type of plant, soil, and geographical conditions. The concentrations of Cd and Cr elements were found to be higher than expected. Steps should be taken to protect the ecological environment, the submitted results are evidence of the alarming situation of pollution in the existing region.

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### Conflict of Interest

The author declares that there is no conflict of interest.

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