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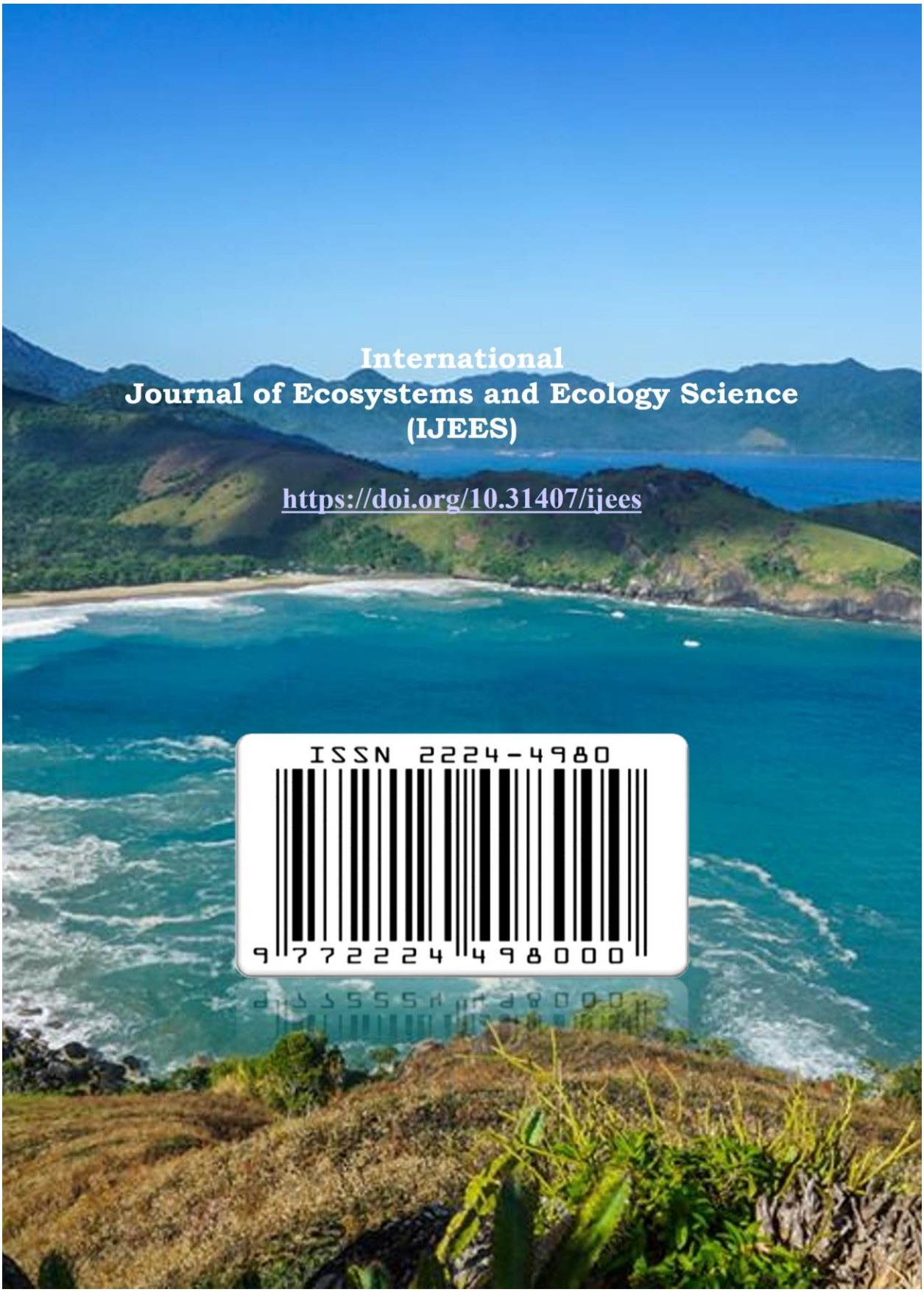
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Table of contents:

Sukru Dursun[1*], MARMARA SEA POLLUTION WITH ENVIRONMENTAL IMPACT, page 1-6;
DOI: https://doi.org/10.31407/ijees13.101

Nguyen Trong Hung[1*], Dinh Tran Ngoc Huy[2*], Tran Thi Tra Phuong[3*], Le Ngoc Nuong[4], Ninh Thi Nhung[5*], TECHNOLOGY APPLICATIONS IN PATIENT TREATMENT AND SURGERY SOLUTION FOR CANCER TREATMENT AT BAI CHAY HOSPITAL, QUANG NINH, page 7-12;
DOI: https://doi.org/10.31407/ijees13.102

Dinh Cong Hoang[1], Phan Huy Duong[2*], To Tha Hien[3], THE ROLE OF INSTITUTIONAL REFORM IN THE PRIVATE SECTOR DEVELOPMENT: EVIDENCE IN A TRANSITION COUNTRY, page 13-26;
DOI: https://doi.org/10.31407/ijees13.103

Nguyen Dinh Trung[1*], Nguyen Trong Diep[2*], Le Ngoc Nuong[3*], Phan Anh[4*], Le Thi Han[5], ANALYSIS OF INDUSTRIAL CLUSTER DEVELOPMENT, FRAMEWORK AND RELEVANT REGULATIONS, page 27-32;
DOI: https://doi.org/10.31407/ijees13.104

Nguyen Trong Diep[1*], Nguyen Dinh Trung[2*], Dinh Tran Ngoc Huy[3*], Ly Lan Yen[4*], Pham Thi Hong Nhung[5], IMPROVEMENT OF INDUSTRIAL CLUSTERS (IC) INFRASTRUCTURE IN HANOI, CONSUMER PROTECTION IN IC AND REGULATIONS IN INDUSTRIAL CLUSTER (IC) DEVELOPMENT, page 33-38;
DOI: https://doi.org/10.31407/ijees13.105

Petro Boiko[1], Nataliia Kovalenko[2*], Yevgen Yurkevych[3], Nataliia Valentiuk[3], Serhii Albul[3], THE HISTORY, CURRENT STATE AND PROSPECTS FOR THE IMPLEMENTATION OF ELEMENTS OF BIOLOGIZATION FOR THE EFFICIENT CULTIVATION OF CORN IN ORGANIC FARMING OF THE SOUTHERN STEPPE OF UKRAINE, page 39-58;
DOI: https://doi.org/10.31407/ijees13.106

Massimo Zucchetti, NUCLEAR PEACE IN UKRAINE: A ROADMAP, page 59-62;
DOI: https://doi.org/10.31407/ijees13.107

Pham Hung Nhan[1*], Dinh Tran Ngoc Huy[2*], THE ECONOMIC TRANSFORMATION IN CA MAU PROVINCE IN THE INDUSTRIAL TIME AND MODERNIZATION, 1997 - 2017 YEARS, page 63-72;
DOI: https://doi.org/10.31407/ijees13.108

Trinh Quoc Vinh[1*], Dinh Tran Ngoc Huy[2*], Yakutseny Sergey Pavlovich[1*], DIGITAL TWIN OF SOLID MINERAL DEPOSITS, DIGITAL TWIN OF SUBSOIL USE: IS IT NEEDED AND WHY, page 73-78;
DOI: https://doi.org/10.31407/ijees13.109

Nguyen Dinh Trung[1*], RECOMMENDATIONS FOR POLICIES ON INFRASTRUCTURE CONSTRUCTION FOR INDUSTRIAL ZONES IN HANOI CITY, page 79-86;
DOI: https://doi.org/10.31407/ijees13.110

Trinh Quoc Vinh[1*], Sergey Yakutseny[1*], Dinh Tran Ngoc Huy[2*], CLASSICAL TOXICOLOGY AND MAIN TASKS OF GEOTOXICOLOGY AND ANALYSIS OF ENVIRONMENTAL RISKS IN THE DEVELOPMENT OF HYDROCARBON RAW MATERIALS, page 87-94;
DOI: https://doi.org/10.31407/ijees13.111

Minh Ngoc DAO[1], Thi Hong Viet BUI[1*], MEASUREMENT OF VIETNAM CULTURAL RESOURCES ATTRACTIVENESS: THE CASE OF VIETNAM, page 95-108;
DOI: https://doi.org/10.31407/ijees13.112

Ngo Ngoc Diem[1*], DISCUSSION ON EXISTING PROBLEMS AND RECOMMENDATION BASE FOR PROVISION OF ENVIRONMENTAL CRIMINES IN CRIMINAL LAW, page 109-116;
DOI: https://doi.org/10.31407/ijees13.113

Bouricha Zineb[12], Chikhaoui Mira[1,3,*], Abdelhadi Si Ameur[1,2], PRELIMINARY STUDY ON THE REPRODUCTION PARAMETERS OF CATTLE IN SOME DAIRY FARMS IN THE WEST OF ALGERIA, page 117-122;
DOI: https://doi.org/10.31407/ijees13.114

Flora Qarri[1], Sonila Shehu[2*], Pranvera Lazo[2], PHYSICO-CHEMICAL CHARACTERISTICS OF SEA WATER OF BEACHES ALONG VLORA BAY, ALBANIA, page 123-128;
DOI: https://doi.org/10.31407/ijees13.115

Leila Soudani[*], Meriem Chafaa, Mohamed Islem Bouacha, Omar Safa, Moulkheir Selmani, Mhamed Maatoug, ASSESSMENT OF NOX CONTENTS BY MEANS OF A SENTINEL LICHEN *XANTHORIA PARITIENA L* IN THE TOWN OF TIARET, ALGERIA: POLLUTION CLASSES AND MAPPING, page 129-136;
DOI: https://doi.org/10.31407/ijees13.116

Aleko Miho[1*], IMPORTANCE OF SOCIO-ECOLOGICAL RESEARCH AND THE SCIENCE-POLICY INTERFACE IN ENVIRONMENTAL SUSTAINABILITY IN ALBANIA, page 137-142;
DOI: https://doi.org/10.31407/ijees13.117

Iryna Mosiychuk[1], Iryna Beznosko[1*], Julia Turovnik[1] Alla Lishchuk[1], Tatiana Gorgan[1], Yurii Ternovyi[2], FORMATION OF MICROBIAL COMPLEX OF THE SOIL IN AGROCENOSE OF SPRING BARLEY USING ECOLOGICALLY SAFE CULTIVATION TECHNOLOGIES, page 143-154;
DOI: https://doi.org/10.31407/ijees13.118

Amina Belkhemas[1*], Abdellatif Niar[1], Bouabdellah Benallou[2], Abdelkader Difallah[1], Sabrina Ait Abdelkader[2], Mohamed Badrane[3], PHYSICOCHEMICAL QUALITY DETERMINATION OF PASTEURIZED AND UHT MILK MARKETED IN TIARET REGION, ALGERIA, page 155-160;
DOI: https://doi.org/10.31407/ijees13.119

Liudmyla Lazarieva[1], Larysa Akymenko[1], Hanna Postoienko[1*], Volodymyr Postoienko[1], Lesia Nikitina[1], Dmytro Zasiekin[2], Serhii Razanov[3], Vitalii Nedosekov[2], Sergey Amons[4], Alla Razanova[3], Lyudmyla Symochko[5,6], SPECIFIC QUALITY INDICATORS OF MONOFLORAL LINDEN HONEY, page 161-168;
DOI: https://doi.org/10.31407/ijees13.120

Sabrina Ait Abdelkader[1*], Bouabdellah Benallou[1], Mohamed Amine Ayad[2], Amina Belkhemas[1], Yassine Hadj Boussada[2], sara Nesrine Benouadah[3], DIAGNOSIS OF EQUINE POST-BREEDING ENDOMETRITIS: ULTRASONOGRAPHY, MICROBIOLOGY, CYTOLOGY, AND CORRELATION TO FERTILITY IN TIARET REGION, WESTERN ALGERIA, page 169-178;
DOI: https://doi.org/10.31407/ijees13.121

Sakina Zerizer[1*], Faris AlHajri[2], Rayene Aras[1], Mohamed Tarek Benosmane[1], El-roumeissa Siari[1], Sara Khelfi[1], CATALASE ACTIVITY AND IMMUNOSTIMULATORY OF HOT WATER AND VITAMIN C, page 267-274;
DOI: https://doi.org/10.31407/ijees13.135

Serhii Razanov[1*], Tetiana Holubieva[2], Yuriy Tkalich[3], Lyudmyla Symochko[4,5], Yustyna Zhylishchych[6], Oleh Bakhmat[7], Uliana Nedilska[7], Halyna Lysak[6], Halina Ohorodnichuk[1], Igor Holovetskyi[2], Nataliia Kachmar[6], IMPACT OF MINERAL SUBSTANCES CONCENTRATION ON HEAVY METAL CONTENT IN POLYFLORAL HONEY, page 275-280;
DOI: https://doi.org/10.31407/ijees13.136

IMPACT OF MINERAL SUBSTANCES CONCENTRATION ON HEAVY METAL CONTENT IN POLYFLORAL HONEY

Serhii Razanov[1*], Tetiana Holubieva[2], Yuriy Tkalich[3], Lyudmyla Symochko[4,5], Yustyna Zhylishchych[6], Oleh Bakhmat[7], Uliana Nedilska[7], Halyna Lysak[6], Halina Ohorodnichuk[1], Igor Holovetskyi[2], Nataliia Kachmar[6]

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ABSTRACT

The effect of the mineral content of polyfloral honey produced by bees in the conditions of the northern Polissia of Ukraine (the zone affected by the Chernobyl nuclear power plant accident) on the level of accumulation of ^{137}Cs, Pb and Cd in it was studied. It was established that the specific activity of ^{137}Cs and the concentration of Pb and Cd in polyfloral honey depended on the content of mineral substances (ash) in this product. In the polyfloral honey produced by bees from the nectar of autumn pollinators (heather, various grasses), a higher specific activity of ^{137}Cs and the concentration of Pb and Cd were found in comparison with the same products obtained from spring nectar pollinators (apple, cherry, cherry, white acacia, spring rhinoceros). At the same time, it was found that the content of mineral substances in polyfloral honey produced by bees from autumn honey combs was 64.7% higher compared to this product produced from spring nectar pollen cones. The artificial reduction of ash content in polyfloral honey by 2.4 times due to its processing using sorption technologies contributed to the improvement of the quality of this product. In particular, a decrease in the specific activity of ^{137}Cs by 47.5% and the concentration of Pb by 59.5% and Cd by 41.2% was observed in the processed honey. At the same time, a decrease in sucrose by 1.18 pp, ash by 2.4 times and an increase in the content of amino acids by 58.8% was observed in honey.

Keywords: radiocesium, heavy metals, bees, pollution, toxicants, specific activity, sorbent, processing, chemical composition, amino acids.

INTRODUCTION

Bee honey is a treasure trove of highly nutritious and curative and preventive biologically active substances, its composition includes: carbohydrates, proteins, vitamins, minerals, etc. (Aljohar et al, 2018; Mărgăoan et al, 2021).

The main value of honey is determined by carbohydrates, which make up 80%, among which glucose and fructose predominate and only a small proportion of sucrose, the amount of which, depending on the botanical origin of the plants, is from 3 to 7% (Aljohar et al, 2018). Non-carbohydrate substances make up the second part of the dry substances of honey, most of which are represented by proteins. Their share varies between 0.3-0.4%. Proteins of plant origin enter honey during the collection of nectar with pollen (Erban et al, 2019; Lewkowski et al, 2019). The content of protein substances in honey also depends on the type of honey plants, the breed of bees, the period of its harvesting, etc. Paddy honey contains twice as many proteins as flower honey. Spring honey has two times less protein than summer and autumn honey. Experimental studies have established that the main part of the protein in honey is amino acids. In particular, 22 amino acids were found in honey, the main of which are alanine, arginine, valine, threonine, proline, phenylalanine, leucine, lysine, isoleucine and others (Machado De-Melo et al, 2018; Biluca et al, 2019; Balasubramanyam, 2020). Organic and inorganic acids were also detected in honey, the content of which is 0.3 and 0.03%, respectively. Organic acids include citric, malic, lactic, and gluconic acids. Acetic, pyruvic, and inorganic salt and phosphorous are represented in smaller quantities.

Honey also includes enzymes, the content of which largely depends on the type of plant (Silva et al, 2016; Orčić et al, 2017). High-quality honey contains amylase, catalase, invertase (Machado De-Melo et al, 2018). The diastase number of honey of different varieties is on average 15 units. Gote (White, 2016). Honey from heather and buckwheat has the highest level of diastase, which is from 20 to 50 units. Gote. Mineral substances in honey contain 0.27%. According to the State Standard 19792–87, 0.1-0.5% of mineral substances in flower honey is allowed, and 0.3-1.0% in fall honey. It is known that the content of mineral elements within one variety of honey varies depending on the mineral composition of the soil on which honey plants grow and the time of nectar collection (Bondarchuk et al, 2008; Haidera et al, 2022).

Honey also contains vegetable pigments that enter it together with nectar. Fat-soluble pigments are derivatives of carotene, xanthophyll, chlorophyll. Honey contains a large number of vitamins, namely: B_1, B_2, B_3, B_4, B_5, B_6, B_s, K, P, PP (Mishra et al, 2020). Due to its special chemical and biological composition, honey is a valuable food and pharmacological product (Oryan et al, 2016). It is now widely used in medicine in combination with medicinal preparations (Meo et al, 2017). Honey is characterized by high taste and nutritional qualities, its long-term consumption increases the immunobiological reactivity of the body, resistance to infectious diseases, improves the bactericidal and anti-inflammatory properties of the body. Honey has a high antimicrobial effect, which mostly applies to gram-positive cocci, bacteria, and bacilli (Visweswara et al, 2017). In connection with the high value of honey and the wide range of its use in food and medicine, the demand for it on the market is noticeably increasing. At the same time, the requirements for its quality and safety are increasing (Razanov et al, 2020; Razanov et al, 2022). It is well known that the quality and safety of honey depends on the ecological state of the environment in the area of nectar-pollen-bearing lands, the current state of which in some areas is characterized by contamination with various toxicants, some of which, when they enter the food chain from the soil and plants, reach acute toxic levels in human metabolism and animals can have toxic and carcinogenic effects in minimal concentration (Bondar et al, 2019; Dursun et al, 2020; Razanov et al, 2022). In particular, this phenomenon is observed in conditions saturated with industrial enterprises, a high level of motor vehicle load and chemical production (Pan et al, 2010; Razanov et al, 2022). It has been proven that honey plants are characterized by different intensity of accumulation of such toxicants as radionuclides and heavy metals (Razanov, 2009; Gucol, 2020; Razanov et al, 2021). The lowest concentration of radionuclides was found in honey-bearing plants of the aster family, and relatively higher in labium and rosaceae. It was established that the activity of ^{137}Cs in honey also depends on its botanical origin. High radioactivity was detected in pink and creeping clover, as well as thyme, even higher in heather. Honey from honey plants belonging to the legume family is also characterized by high activity of radionuclides. The least amount of it was found in honey, which is obtained from spring honey plants - dandelion, safflower, rapeseed. Honey produced by bees from white acacia is also characterized by a low level of radionuclide content. Even in the environmentally safe zone of enhanced radiation control, the highest content of ^{137}Cs and ^{134}Cs was noted in honey obtained from clover (4.1-7.8 Bq/kg). Honey from rapeseed (1.8-2.7 Bq/kg) and white acacia (2.0-2.75 Bq/kg) is characterized by a low level of radiocesium contamination (Razanov, 2009).

In modern conditions of man-made load, a certain accumulation of toxic heavy metals, in particular, Pb, Cd, Zn, Cu, etc., has been found in honey. The content of these toxicants in honey largely depends on the level of soil pollution in nectar-pollen-bearing lands, the botanical origin of plants, etc. It was found that honey produced from buckwheat nectar contains 5 times more Pb compared to similar products obtained from white acacia (Razanov, 2009). Taking into account the growing local level of toxicants in the environment, especially in the zone of technogenic influence

on nectar-bearing lands, there is a need to study the features of the accumulation of radionuclides and heavy metals in honey and measures to improve its quality.

MATERIALS AND METHODS

The study of the specific activity of ^{137}Cs and the concentration of Pb and Cd in polymorphic honey depending on the level of mineral substances in it was carried out in the conditions of the northern Polissia of Ukraine in the zone of local contamination of nectar-bearing soils with radionuclides and heavy metals as a result of the accident at the Chernobyl nuclear power plant. To study the influence of the mineral fraction of polyfloral honey on the specific activity of ^{137}Cs and the concentration of Pb and Cd in this product, polyfloral honey harvested in three periods of the active bee season (spring, summer, autumn) was studied. In the spring period, honey was produced by bees from the nectar of apple, cherry, cherry, white acacia and spring rhinoceros; in the summer - from the nectar of the heart-leaved linden, broad-leaved linden, Ivan-tea and various herbs, and in the autumn - mostly from the nectar of heather and various herbs. Research on improving the quality of polyfloral honey produced in the conditions of man-made pollution of nectar-pollen-bearing lands by reducing the content of mineral substances (ash) in it was carried out according to a scheme that included: pumping polyfloral honey contaminated with ^{137}Cs, Pb and Cd, dissolving it in distilled water in a ratio of 1:1; passing the resulting mixture through the sorbent; processing of this mixture by bees; pumping out the matured honey produced by the bees from the processed mixture. A mixture of distilled water and honey was fed to bee colonies (1 liter) per day. After processing the mixture into honey by bees, it was pumped out and the chemical composition, specific activity of ^{137}Cs and concentration of Pb and Cd were studied. The specific activity of ^{137}Cs in polyfloral honey was determined spectrometrically; concentration of Pb and Cd - by atomic adsorption; amino acid composition – by the method of ion-exchange liquid column chromatography. Research materials were processed using generally accepted statistical methods with the definition of Student's criterion. At the same time, the average arithmetic values (M), the mean square deviation (m) and the reliability of the difference between the average values (criterion P) were calculated. Conventional designations are used to show the probability in the tables: $P<0.05$; $P<0.01$; $P<0.001$ in the paper, respectively, marked with asterisks (*, **, ***).

RESULTS AND DISCUSSION

The research results showed that the polyfloral honey produced by bees from the nectar of spring, summer and autumn nectarifera was characterized by a different concentration of mineral substances, which ranged from 0.17% to 0.28%. The lowest concentration of mineral substances - 0.17% was observed in honey from spring nectary cones, while the concentration of mineral substances was higher by 23.5% and 64.7%, respectively, from summer and autumn nectary cones. At the same time, a direct dependence of the content of mineral substances in honey and its specific activity of ^{137}Cs and the concentration of Pb and Cd was established. In particular, with a mineral content of 0.17% in honey, the specific activity of ^{137}Cs was 92.7 Bq/kg. An increase in mineral substances in copper by 0.04% pp and 0.11 pp increased the specific activity of ^{137}Cs by 19.7% and 30.5%, Pb – by 63.6% and 2.5 times, and Cd – by 33.3 and 2.3 times, respectively (Table 1).

Table 1. Specific activity of ^{137}Cs and concentration of Pb and Cd in polyfloral honey, (n=3, M±m).

The content of minerals in honey, %	Specific activity of ^{137}Cs, Bq/kg	Pb concentration, mg/kg	Cd concentration, mg/kg
0.17 ± 0.04	92.7 ± 0.32	0.011 ± 0.008	0.006 ± 0.0005
0.21 ± 0.02	111 ± 0.21	0.018 ± 0.004	0.008 ± 0.0007
0.28 ± 0.07	121 ± 0.14	0.028 ± 0.003	0.014 ± 0.002

Taking into account the influence of the concentration of heavy metals in honey on the content of ^{137}Cs, Pb and Cd in it, we proposed a method of purifying honey from these toxicants. The essence of which was to reduce the mineral content of honey by passing a mixture of honey dissolved in distilled water (1:1) through a sorbent and bees processing this mixture into marketable honey.

Table 2. Specific activity of ^{137}Cs and concentration of Pb and Cd in processed copper, (n=3, M±m).

Product	Ash content	^{137}Cs, Bq/kg	Pb	Cd
Polyfloral honey	0.26±0.01	191.3±1.98	0.069±0.009	0.017±0.005
The mixture (honey, distilled water) before passing through the sorbent	0.12±0.03	112.6±0.31***	0.033±0.0007***	0.009±0.0003***
The mixture (honey, distilled water) after passing through the sorbent	0.07±0.002	52.5±0.33***	0.016±0.0009***	0.006±0.0005***
Honey produced from a mixture (honey, distilled water) passed through a sorbent	0.11±0.03	100.5±1.52***	0.028±0.001***	0.01±0.001**

As a result of using this method, the activity of radionuclides in copper naturally decreased. Thus, in the mixture of honey dissolved in water, the specific activity of ^{137}Cs decreased by 1.7 (P<0.001) times. After filtering the mixture (honey dissolved in water), the specific activity of ^{137}Cs decreased by 2.1 (P<0.001) times compared to this mixture before passing it through the sorbent. After processing by bees of the mixture passed through the sorbent, the activity of ^{137}Cs in honey increased by 1.9 times due to the removal of water by bees and thickening of this product.
Similar changes were characteristic of the concentration of Pb and Cd in copper during its processing. Thus, dissolving polyfloral honey in distilled water (1:1) reduced the concentration of Pb in this mixture by 2.1 (P<0.001), and Cd – by 1.9 (P<0.001) times. After passing this mixture through the sorbent, the content of Pb and Cd in it decreased by 2.06 (P<0.001) and 1.5 (P<0.001) times, respectively. Processing of the honey mixture passed through the sorbent by bees increased the concentration of Pb by 1.75 times and Cd by 1.66 times in the produced honey. In general, as a result of a 2.4-fold decrease in the amount of minerals in honey due to its processing (Fig. 1), the specific activity of ^{137}Cs decreased by 47.5% (P<0.001), and the concentration of Pb and Cd – by 59.5 (P<0.001) and 41.2% (P<0.001), respectively, compared to honey that was not subject to purification by filtration through a sorbent and repeated processing by bees.

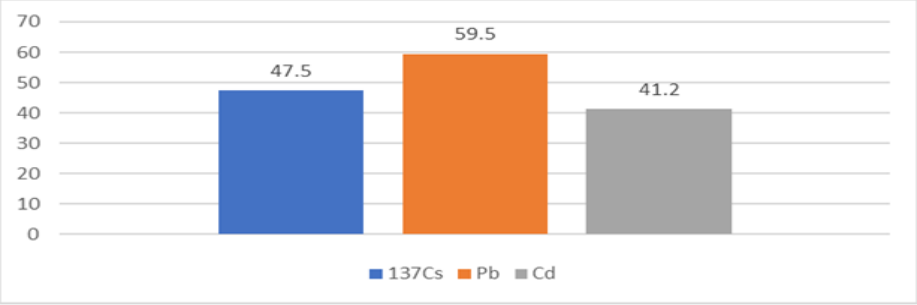

Figure 1. The intensity of reduction of toxicants in honey as a result of its processing

In connection with the processing of honey by bees, which included dissolving it in distilled water, passing the resulting mixture through a sorbent and thickening this mixture, there was a need to study the effect of these technological operations on the chemical composition of the honey obtained
The research results shown in Table 3 showed that the proposed technology of processing polyfloral honey by bees to some extent affected its chemical composition and increased its quality and safety.

Table 3. Content of individual substances in polyfloral honey after processing (n=3, M±m)

Substances	Substance content in honey, %	
	befor processing (control)	after processing (experiment)
Invert sugar	92.93±0.72	92.67±1.19
Saccharose	3.08±0.1	1.90±0.09**
Nitrogenous substances	0.40±0.01	0.57±0.01**
Amino acids	0.22±0,014	0,35±0,027
Ash	0.26±0.01	0.11±0.03***

In particular, the content of nitrogenous substances in the completely dry substance as a result of honey processing increased by 0.17 pp. (P<0.01), while the ash content, on the contrary, decreased by 0.15 pp. (P<0.001).
Changes in the carbohydrate composition of honey produced from a honey solution passed through a sorbent and reprocessed by bees were also revealed, namely, the content of invert sugar in it decreased by 0.26 pp, sucrose - by 1.18 pp. (P<0.01). An increase in the content of nitrogenous substances in reprocessed honey caused changes in its amino acid composition. It should be noted that a noticeable increase of 58.8% in the total amount of amino acids was observed in the processed honey. The highest increase in amino acids in processed honey was observed for lysine by 2.5 times, aspartic acid by 2.4 times, glutamic acid by 2.5 times, valine by 2.7 times, methionine by 2.6 times, leucine by 2.8 times, and phelalanin by 3.6 times.

CONCLUSIONS

- As a result of the conducted research, a direct dependence of the content of mineral substances in polyfloral honey and the accumulation of ^{137}Cs, Pb and Cd in it was established.
- In particular, it was established that the increase in mineral substances in polyfloral honey from 0.17% to 0.28%, the amount of ^{137}Cs, Pb and Cd increased by 30.5%, 2.5 and 2.3 times, respectively.
- An artificial 2.4-fold decrease in mineral substances in polyfloral honey as a result of its processing reduced the specific activity of ^{137}Cs by 47.5% and the concentration of Pb by 59.5%, Cd by 41.2%.
- At the same time, an increase in nitrogenous substances in processed honey by 0.17 pp was observed. and the total number of amino acids by 58.8% and a decrease in sucrose by 1.18 pp.

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