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Bioindication for Detecting Environmental Risks in Agrocenoses Contaminated with Heavy Metals

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ABSTRACT

The relevance and scientific and methodological approaches to the use of the bioindication method for assessing environmental risks in agrocenoses contaminated with toxic substances (in particular, heavy metals) were substantiated in the article. The study is based on the use of bee honey and heavy metal transfer coefficients in the sequential chain "soil – honey plant – bee honey". This ensures control over the content of toxicants in the soil and inflorescences of honey plants. Toxicant transfer coefficients can be used in the soil-plant-beekeeping products chain as indicators for developing environmental risk management tools. Bioindication is important for contaminated agricultural landscapes that are used as raw materials and forage lands for beekeeping and other environmental objects. The use of the bioindication method makes it possible to assess the environmental risks of toxic substance pollution of agroecosystems and natural biocenoses and to outline the boundaries of toxicant pollution of a certain area for the safe placement of apiaries and obtaining high-quality bee honey.

Keywords: bee honey, bee products, heavy metals, bioindication, environmental risks, agrocenosis, transition coefficient.

INTRODUCTION

The environment suffers from extreme man-made pressure in the areas where powerful industrial enterprises, large cities, transportation links, agrochemical warehouses, etc. are located. At the same time, agricultural technologies for growing crops involve the use of large quantities of mineral fertilizers and pesticides to fertilize and protect crops from pests and diseases [Kulidzhanov et al. 2022]. These anthropogenic factors lead to significant contamination of agrocenoses and natural biocenoses with toxicants such as heavy metals and pesticides [Shumygai 2022, Ruschioni et al. 2013, Zhou, Taylor and Davies 2018]. It is not always possible to conduct analytical laboratory testing to identify all pollutants that cause negative changes in the environment. Therefore, the use of bioindication methods (honeybee monitoring) to assess the environmental risks of the negative impact of pollutants on biota has a great practical importance. It is known that the contamination of agricultural forage bee lands with heavy metals, pesticides, radionuclides and other harmful substances leads to environmental risks of toxicant accumulation in the environment [Lishchuk et al. 2022a, Lishchuk et al. 2022b, Lishchuk et al. 2023, Moklyachuk et al. 2017]. This is one of the main factors in the accumulation of hazardous toxic substances in beekeeping products, which negatively affects the quality of honey, wax, pollen, and propolis [Finger et al. 2014, Orsi et al. 2018, Cebotari, Gliga and Buzu 2016, Solomakha et al. 2022].

Bees and bee products are a unique object that can be used to obtain a wide range of characteristics for environmental monitoring, including honeybee monitoring [Parikh, Rawtani & Khatri 2021, Ruschioni et al. 2013, Girotti et al. 2020]. Honeybee monitoring is considered as biological monitoring using bioindication methods. It involves assessing the functioning of bee colonies and the accumulation of pollutants (including heavy metals) in bee organisms and bee products. Heavy metals can enter bee products from the air, soil, pollen or nectar.

Honey bees are both a biological indicator that can be used to assess environmental pollution with toxic substances and a passive bioaccumulator. This is due to the fact that particles of pollutants accumulate in the bee's body and in bee products. The main criteria for contamination of the apiary territory with toxic elements are the number of dead flight bees and pollutants in hives and beekeeping products [Girotti et al. 2020, Costa et al. 2019]. Bees are an ideal subject for monitoring large areas, as they constantly come into contact with the environment within a radius of 2–3 km from the apiary to collect nectar and pollen; at the same time, they accumulate and carry to their nests various pollutants contained in plants, soil, atmospheric air and translocated in honey plants. Therefore, apiaries can be used as a kind of monitoring network. In the areas where additional information is needed to assess the environmental situation, it is advisable to use mobile apiaries.

Due to their physiological characteristics, bees are able to accumulate some toxic elements in their own tissues or concentrate them in bee products. Bee tissues, honey, wax, pollen, propolis, which accumulate heavy metals, pesticides, radio nuclides and other harmful substances, can be used as indicators of environmental pollution [Quigley et al. 2019, Badiou-Bénéteau et al. 2013]. In the process of honey production, bees collect nectar, thicken it and thereby increase the concentration of heavy metals in honey products [Bargańska, Ślebioda and Namieśnik 2016, Sager 2020]. At the same time, many researchers have proven that bee products are more contaminated with toxicants than the plants from which they are harvested [Spirić et al. 2019, Quigley et al. 2019, Orsi et al. 2018, Finger et al. 2014].

In accordance with the EU requirements, mandatory standards of state regulations on the quality of beekeeping products, their environmental cleanliness and safety are being tightened worldwide. In the EU, the basic legislative requirements for the quality and safety of bee honey are set out in Directive 2014/63/EU of the European Parliament and of the Council [Directive 2014/63/EU]. Directive 2014/63/EU sets out the main provisions on the requirements to be met by the quality of honey entering the EU internal market. The requirements set out general rules for the labeling and composition of honey, which guarantee the quality of the food product for consumers.

In Ukraine, honey quality indicators are regulated by DSTU 4497:2005 "Natural Honey. Technical specifications" [DSTU 4497:2005], which specifies physical, chemical, biochemical, minimum indicators of diastase activity, water mass fraction, total and active acidity, etc. The safety requirements of DSTU 4497:2005 for heavy metals determine the limitations of the content of lead, cadmium, arsenic elements in honey (no more than 1.00 mg/kg, 0.05 mg/kg and 0.50 mg/kg, respectively). However, these regulatory documents do not provide for honey safety indicators, namely maximum permissible concentrations (MPCs) of other heavy metals, active ingredients of modern pesticides, nitrates and nitrites, radionuclide contamination, etc. Moreover, there is no standardization of pollutants in other bee products, such as propolis, wax, pollen (bee pollen), cerago (bee bread), etc. It is worth noting that the problem of regulatory control over the quality of honey and bee products in Ukraine remains unresolved due to the lack of legally approved regulation of hazardous toxicants [Adamchuk et al. 2020]. That is why, it is important to constantly monitor the content of toxic substances, including heavy metals, in bee products.

Today, there is no sufficiently well-grounded, environmentally safe method for assessing environmental risks in the toxic-contaminated agrocenoses that use agricultural forage bee lands. Therefore, the aim of the study was to investigate a bioindication method for detecting and assessing environmental risks of heavy metal-contaminated agrocenoses.

MATERIALS AND METHODS

The study was conducted at the Institute of Agroecology and Nature Management of the National Agrarian Academy of Sciences of Ukraine (IAP NAAS) in accordance with the scientific and technical program “Developing scientific foundations for managing environmental risks in agricultural production for growing crops”. Transfer coefficients of heavy metals Pb, Cd, Cu, Zn, Ni, Co, Cr are calculated (K_t) in the links of the sequential chain “soil – honey plant – bee honey”. The average values of three years of data on the concentration of toxicants in samples of soil, honey plants and bee honey from three districts of Cherkasy region (Katerynopil, Zvenyhorod, Talniv) in the Forest-steppe were used for the calculations. The content of heavy metals (Pb, Cd, Cu, Zn, Ni, Co, Cr) in the sequential chain “soil – honey plant – bee honey” was investigated using the bioindication method.

The study used honey samples from nine beekeeping apiaries in the above-mentioned districts of Cherkasy region with different degrees of anthropogenic pressure on the environment. These regions are characterized by the same type of soil (regraded chernozem), a set of honey plants, weather and climatic conditions, and differ in the presence of different sources of anthropogenic pressure on the environment. To study the territory, samples of soil, plants, and honey were collected within the possible flight radius of bees from the apiary under study.

Soil and plant sampling

Soil samples were collected in agrocenoses under crops of the most common honey crops (buckwheat and sunflower), as well as from biocenoses (herbs) using the envelope method from a depth of 0–20 cm in accordance with existing requirements and guidelines [Dospekhov 1973, Sozinov and Priester 1994]. The inflorescence samples of honey plants of sunflower, buckwheat, and herbs were collected according to the generally accepted methods. The number of replications of sampling was three times.

Sampling of bee honey

For analysis, honey samples were taken with special samplers, transferred to prepared glass containers in triplicate according to the generally accepted methods [Feshchenko 2006]. The weight of an average honey sample depends on the batch volume and research method and is about 0.2 kg. All collected honey samples must have: the number of the original sample, the number of the field where the honey plants grew, the name and origin of the honey, the date of collection. Honey samples must be analyzed immediately after collection or as soon as possible, avoiding changes in its density. Depending on the origin of the honey, its density or crystallization state, it is advisable to use appropriate samplers for sampling, namely: samples of liquid honey should be taken with a tubular aluminum sampler; dense honey – with a dipstick for plastic oil from different layers; crystallized honey – with a conical dipstick; a part of 20 cm^2 should be cut out of one frame of dense honey [Feshchenko 2006].

Determination of the content of heavy metals (Pb, Cd, Cu, Zn, Ni, Co, Cr) in soil samples, buckwheat, sunflower and herb inflorescences and in the corresponding samples of bee honey varieties was carried out by atomic absorption on an atomic absorption spectrophotometer according to the methods approved by the Ministry of Health of Ukraine in accordance with DSTU 7670:2014 “Raw materials and food products. Preparation of samples. Mineralization to determine the content of toxic elements” [DSTU 7670:2014]. The method for determining heavy metals in honey samples is based on dry mineralization and acid extraction of samples for further determination of copper, lead, cadmium, zinc, chromium, nickel, and cobalt. Dry mineralization involves the complete decomposition of organic matter by burning raw materials in an electric furnace under controlled temperature conditions, followed by determination of the concentration of elements in the mineralized solution by atomic absorption spectrophotometry in accordance with DSTU 7670:2014. Statistical processing of the results of analytical studies was carried out using standard statistical programs, i.e. Microsoft Excel and S-plus.

RESULTS AND DISCUSSION

Biological testing methods are known to help diagnose negative changes in the environment at low concentrations of pollutants. A bioindicator can be used to determine the localization of pollutants in the environment, monitor the rate of change in the environment, investigate the degree of harmfulness of substances to wildlife, and predict further development of the ecosystem. The main task of bioindication is to use the available methods and criteria that could adequately reflect the level of anthropogenic impact, taking into account the complex nature of pollution, as well as diagnose early changes in the most sensitive components of biotic communities. Bioindication using bee honey (honeybee monitoring) is carried out at such levels of biosphere organization as agrocenoses and natural biocenoses and allows solving environmental monitoring tasks in cases where the combination of anthropogenic pressure factors on biocenoses is difficult or inconvenient to measure directly.

Therefore, to assess environmental risks in agrocenoses contaminated with heavy metals, the principle of the bioindication method was used, which consists in studying the accumulation of pollutants in bee honey. Calculations of transfer coefficients (K_t) of the heavy metals in the sequential chain "soil – honey plant – honey bee" were carried out in several stages:

- stage 1 – the chain "soil – honey plant – honey plant";
- stage 2 – the chain "honey plant – bee honey";
- stage 3 – the chain "soil – bee honey ".

Calculations of transfer coefficients ($K_{t\,soil-plant}$) of heavy metals in the "soil–honey plant" chain were based on the ratio of the average toxicant content in plant samples (A) to their content in soil (B) according to the formula:

$$K_{t\,soil-plant} = A_1/B_1 \qquad (1)$$

where: $K_{t\,soil-plant}$ – transfer coefficients of heavy metals from soil to plants; A_1 – heavy metal content in plants, mg/kg; B_1 – heavy metal content in soil, mg/kg.

The calculations of the coefficients of transfer *Kt* of soil-plant heavy metals from soil to inflorescences of honey plants of grasses, sunflower, and buckwheat are presented in Table 1.

The accumulation of different amounts of heavy metals in plants is due to their physiological cumulative ability to accumulate toxicants in roots, vegetative or reproductive organs in different ways, and depends on the level of intake, properties and concentration of elements in the soil. As it can be seen from Table 1, the indicators $K_{t\,soil-plant}$ indicate an active transition of heavy metals in the chain "soil – honey plant" and high cumulative properties of plants, especially in terms of cadmium accumulation (K_t= 0.7÷0.44), copper (K_t = 0.64÷1.84) and zinc (K_t = 0.82÷1.47), while the lowest conversion rates are typical for cobalt (K_t = 0.03÷0.05) and chromium (K_t = 0.01). Fluctuations in the coefficients of transfer from soils to plants within a fairly wide range cause significant differences in the accumulation of heavy metals in the honey-bearing parts of these plants and in the transformation in the tissues of bees and bee products.

Transfer coefficients ($K_{t\,plant-honey}$) of heavy metals from plants into bee honey

Calculation of toxicant transfer coefficients in the plant-bee honey chain ($K_{t\,plant-honey}$) was carried out by the ratio of the concentration of heavy metals in honey plants to their content in honey according to the formula:

$$K_{t\,plant-honey} = A_2/B_2 \qquad (2)$$

where: $K_{t\,plant-honey}$ – transfer coefficients ($K_{t\,plant-honey}$) of heavy metals from plants into bee honey; A_2 – content of heavy metals in bee honey, mg/kg; B_2 – heavy metal content in plants, mg/kg.

The results of calculations of the coefficients of transfer of heavy metals from honey plants to bee honey ($K_{t\,plant-honey}$) are presented in Table 2. According to the calculated plant-honey coefficients $K_{t\,plant-honey}$, it is clear that heavy metals such as lead ($K_{t\,Pb\,plant-honey}$ = 0.33÷0.37) and chromium ($K_{t\,Cr\,plant-honey}$ = 0.31÷0.34) are most intensively transferred from honey plants to honey. For

Table 1. Transfer coefficients of heavy metals from soil to plants ($K_{t\,soil-plant}$)

The chain	$K_{t\,soil-plant}$						
	Pb	Cd	Cu	Zn	Ni	Co	Cr
The soil is an inflorescence of herbs	0.16 ±0.02	0.43 ±0.07	0.64 ±0.03	1.47 ±0.28	0.19 ±0.02	0.05 ±0.009	0.01 ±0.001
Soil - sunflower inflorescences	0.03 ±0.01	0.44 ± 0.05	1.84 ±0.39	1.16 ±0.09	0.18 ±0.02	0.03 ±0.001	0.01 ±0.003
Soil - buckwheat inflorescences	0.05 ±0.005	0.27 ±0.02	0.79 ±0.03	0.82 ±0.03	0.26 ±0.04	0.04 ±0.005	0.01 ±0.002

Table 2. Transfer coefficients ($K_{t\ plant-honey}$) of heavy metals from plants into bee honey ($K_{t\ plant-honey}$)

The chain	$K_{t\ plant-honey}$						
	Pb	Cd	Cu	Zn	Ni	Co	Cr
The soil is an inflorescence of herbs	0.33 ±0.01	0.11 ±0.01	0.04 ±0.008	0.08 ±0.004	0.16 ±0.02	0.14 ±0.02	0.34 ±0.03
Soil - sunflower inflorescences	0.36 ±0.02	0.08 ±0.01	0.04 ±0.001	0.07 ±0.006	0.14 ±0.01	0.13 ±0.01	0.33 ±0.04
Soil - buckwheat inflorescences	0.37 ±0.01	0.13 ±0.02	0.04 ±0.006	0.07 ±0.006	0.14 ±0.02	0.14 ±0.02	0.31 ±0.02

the convenience of using the transfer coefficients $K_{t\ plant-honey}$ for a preliminary forecast of heavy metal contamination of elements of the plant-honey chain, the averaged values $\check{K}_{t\ plant-honey}$ can be used, which were calculated using the indicators of Table 2 using existing statistical methods and are presented in Table 3.

Calculations of transfer coefficients ($K_{t\ soil-honey}$) of heavy metals from soil into bee honey

The transfer coefficients of heavy metals from soil into bee honey ($K_{t\ soil-honey}$) were calculated from the ratio of heavy metal content in bee honey samples to their concentration in the soil using the formula:

$$K_{t\ soil-honey} = A_3/B_3, \tag{3}$$

where: $K_{t\ soil-honey}$ – coefficient of transfer of heavy metals from soil to bee honey; A_3 – content of heavy metals in bee honey, mg/kg; B_3 – heavy metal content in the soil, mg/kg.

The results of calculations of the coefficients of transfer of heavy metals from honey plants to bee honey ($K_{t\ soil-honey}$) are summarized in Table 4. The high reliability of the K_t values for copper, zinc, and lead indicates that they can be determined by the proposed method. However, a small difference in the values of the mean error deviation for cadmium, cobalt, and chromium indicates that in this case the application of this method is quite limited. In this case, to determine the content of heavy metals, it is necessary to use sequential calculations of the transition coefficients $K_{t\ soil-honey}$ and $K_{t\ Pb\ plant-honey}$, which are shown in Tables 1 and 2.

Using the heavy metal transfer coefficients (K_t), it is possible to predict the limits of toxicant contamination in a particular area. If the concentration of heavy metals in honey samples from the study area does not exceed the maximum permissible concentrations, it can be assumed that the content of toxicants in this area will be within the limits that do not exceed the regulatory indicators of toxicant content. The transition coefficients (K_t), obtained by the bioindication method, indirectly characterize the degree of heavy metal contamination of the Cherkasy region and can be extrapolated to the entire territory of Ukraine.

According to [Adamchuk 2020], the environmental purity of beekeeping products depends on a number of factors: the level of soil and air pollution (content of heavy metals, radio nuclides, pesticides, and other toxic substances); species composition of honey plants; soil type; distance of the bee colony (apiary) from the source of pollution; season of product selection; compliance with the

Table 3. Indicators of the averaged values of heavy metal tranfer coefficients ($\check{K}_{t\ plant-honey}$) from plant to honey

$\check{K}_t$	Pb	Cd	Cu	Zn	Ni	Co	Cr
$\check{K}_{t\ plant-honey}$	0.35 ±0.02	0.11 ±0.01	0.04 ±0.005	0.07 ±0.005	0.15 ±0.02	0.12 ±0.04	0.33 ±0.03

Table 4. Coefficients of transfer of heavy metals from soil to bee honey ($K_{t\ soil-honey}$)

The chain	$K_{t\ soil-honey}$						
	Pb	Cd	Cu	Zn	Ni	Co	Cr
The soil is an inflorescence of herbs	0.050 ±0.006	0.050 ±0.010	0.030 ± 0.006	–	0.030 ±0.002	0.004 ± 0.002	0.005 ± 0.003
Soil - sunflower inflorescences	0.010 ±0.002	0.030 ± 0.010	–	–	0.020 ± 0.010	0.002 ± 0.001	0.003 ± 0.006
Soil - buckwheat inflorescences	0.020 ±0.003	0.030 ± 0.006	0.030 ± 0.006	0.060 ±0.004	0.040 ± 0.003	0.030 ± 0.001	0.004 ± 0.001

technological regulations for keeping bee colonies throughout the year; compliance with the sanitary and veterinary requirements for keeping apiaries; use of environmentally friendly preparations for the treatment and prevention of bee diseases; compliance with sanitary and hygienic standards at all stages of processing, packaging and storage of bee products. Therefore, it is important to assess environmental risks in agroecosystems, both soil and honey plants contaminated with heavy metals. Such studies allow predicting the limits of toxicant contamination of a certain area for the safe location of apiaries and the production of environmentally friendly beekeeping products.

A comparative analysis of the content of heavy metals in the soil and buckwheat inflorescences determined by the bioindication method and one of the generally accepted basic methods – atomic absorption – was carried out. The comparison analysis showed insignificant differences between the obtained concentrations for most heavy metals (Table 5). Thus, the insignificant error of the analyzed comparison methods proves that bioindication of heavy metals using bee honey is a convenient and objective method of environmental monitoring, in particular, agrocenoses and natural biocenoses.

Therefore, when analyzing the results of the study using the bioindication method, it should be noted that this method is promising and reliable. In addition to heavy metals, honeybee monitoring can be used to track the migration of other elements of anthropogenic pollution in ecosystems, including pesticides, radionuclides, etc. Given that the radius of flight activity of bees reaches about 3 km around the hive, and there are 30 to 60 thousand of such bees in one nest, it can be argued that the apiary is a ready-made monitoring network with concentrated, uniform and averaged collection of samples of nectar, pollen and other "bee material" contaminated with anthropogenic pollutants from the landfill. Therefore, it is possible to quickly deploy a monitoring network with interval sampling of bee products, in which pollutants are concentrated in an average form.

Comparison of the above two methods demonstrates the superiority of bioindication over basic physical and chemical methods due to the integral nature of bioindicators, which summarize all biologically important data on the environment without exception and fully reflect its ecological state; reveal the presence of a complex of pollutants in the environment. Under the conditions of constant anthropogenic load, bioindicators can respond to very weak impacts due to the accumulation of pollutants and record the rate of changes occurring in the environment, indicate the pathways and places of accumulation of various pollutants in ecological systems and possible ways of entering these substances into the human body.

Existing physicochemical methods allow determining the chemical composition and quantity of chemical compounds or their residual amounts in the environment. However, the complex impact of such chemical compounds on the biosystem and on humans can only be determined using bioindicators, since the biosystem, unlike laboratory methods, responds to even very low loads by accumulating these substances. In addition, known laboratory research methods are characterized by high labor intensity, the need for special equipment, considerable cost and long lead times. Compared to physicochemical methods, the method of bioindication using honey is considered an express method that allows for cheaper and faster surveys of large areas for heavy metals.

Table 5. Comparative analysis of the content of heavy metals in the soil and buckwheat inflorescences using different research methods

Chemical element	Heavy metal content in soil, mg/kg		Heavy metal content in buckwheat inflorescences, mg/kg	
	Atomic absorption method	Bioindication method	Atomic absorption method	Bioindication method
Pb	5.84 ± 0.02	5.50 ± 1.00	0.29 ± 0.02	0.31 ± 0.05
Cd	0.14 ± 0.01	0.13 ± 0.01	0.040 ± 0.001	0.04 ± 0.006
Cu	9.72 ± 0.03	10.86 ± 1.05	7.80 ± 0.09	8.20 ± 0.80
Zn	39.50 ± 2.30	39.53 ± 6.33	32.79 ± 3.60	33.85 ± 5.40
Ni	20.47 ± 3.21	17.6 ± 1.20	5.60 ± 0.60	4.67 ± 0.30
Co	5.86 ± 0.52	3.75 ± 0.03	0.28 ± 0.03	0.34 ± 0.02
Cr	17.44 ± 0.23	17.50 ± 2.50	0.21 ± 0.02	0.21 ± 0.03

The disadvantage of the bioindication method is the limited period of honeybee monitoring due to the flight activity of bees (April – September), as its greatest efficiency coincides with the flowering period of honey plants.

Thus, honey bees and bee products, as bioindicators of the ecosystem state, are unique research objects that can be used to obtain a set of environmental characteristics of the environment. However, in order to identify the areas requiring of honeybee monitoring of heavy metal pollution, it is necessary to pay special attention to the location of environmentally hazardous facilities (agrochemical warehouses, factories, roads with constant traffic, quarries, etc.), i.e. sources that constantly create environmental risks of anthropogenic pressure on the environment.

CONCLUSIONS

The conducted studies have shown the possibility of using the bioindication method to assess the environmental risks of heavy metal pollution of agrocenoses. The coefficients of toxicant transfer in the soil-plant-beekeeping products chain can be used as indicators for the development of environmental risk management tools in contaminated agricultural landscapes used as a raw material base and forage lands for beekeeping, and other environmental objects. The use of the bioindication method makes it possible to assess the environmental risks of toxic substance pollution of agroecosystems and natural biocenoses and to outline the boundaries of toxicant pollution of a certain area for the safe placement of apiaries as well as the production of high-quality bee honey

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