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# Effect of feeding honey bees *Apis mellifera* with nanocerium dioxide on the mineral composition of honey, wax and the bees' bodies

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Restrictions or complete prohibition of antibiotic use in livestock production, and particularly in beekeeping, require the search for alternative means to control infectious diseases in honey bee colonies. Therefore, nanotechnology products are gaining importance as they are capable of enhancing bees' resistance to diseases by disrupting bacterial cell walls or membranes at low concentrations, while potentially remaining safe for humans and the environment. Nanocerium dioxide (nano-$CeO_2$) is one such nanoproduct with these properties, including acting as antioxidant and antibacterial agent. Although nano-$CeO_2$ has been identified as a potential product against infectious diseases in honey bees, there is no information on its effects on honey composition and hive products, nor its broader impact on honey bee health. Thus, the aim of the study was to evaluate the quality of honey and the mineral composition of bee bodies, honey, and wax following the feeding of bee colonies with nano-CeO2 via sugar syrup. For the experiment, control and experimental groups of medium-strength bee colonies were formed at the NSC Institute of Beekeeping named after P. I. Prokopovich (Kyiv, Ukraine). Colonies in the experimental group were fed sugar syrup supplemented with nano-$CeO_2$ at a concentration of 1 mM, while the control group received syrup without the nano-additive. The experiment assessed honey quality indicators, such as diastase activity, as well as the content of Ce, Mg, Zn, and Se in bees' bodies, honey, and wax using inductively coupled plasma optical emission spectroscopy. Feeding bee colonies with sugar syrup containing nano-$CeO_2$ did not affect the levels of water, proline, Mg, or Zn in honey but increased its diastase activity by 32.8%. Feeding bees nano-$CeO_2$ increased Ce content by 2.84-fold and decreased Se content by 24.1% in bee bodies, forming the following order of mineral component distribution: Mg > Zn > Ce > Se. The use of nano-$CeO_2$ in bee colonies did not affect the accumulation of Mg, Zn, or Se in honey, but increased the Ce content by 19.14-fold. Under the influence of nano-CeO2, Ce content increased 1.51-fold and Se content 1.91-fold in wax. Ce concentration in honey showed a strong positive correlation with its content in bee bodies, whereas Se content in bee bodies showed a strong inverse correlation with its level in wax. Spring feeding of bee colonies with sugar syrup supplemented with nano-$CeO_2$ showed no effect on the main honey quality indicators, while demonstrating a high capacity for Ce accumulation in bees' bodies, honey, and wax. Overall, these findings provide a foundation for the development of functional and safe treatment products against bee diseases, as well as beekeeping products enriched with nano-$CeO_2$ as an antimicrobial agent.

*Keywords*: beekeeping products; microelements; macroelements; nanoproducts; cerium; magnesium; zinc; selenium.

## Introduction

Honey bees (*Apis mellifera*) serve as essential pollinators of entomophilous plants that account for more than 35% of global food production (Khalifa et al., 2021; Schuhmann et al., 2022). However, honey bees are increasingly exposed to multiple stressors, including agrochemical exposure, infectious diseases, and abiotic factors such as extreme climatic conditions, that contribute to severe colony losses reported in Ukraine and globally (Leska et al., 2021; Omelchun et al., 2025a). Addressing this issue is further complicated by restrictions, and in some cases complete bans, on the use of antimicrobial agents in beekeeping, particularly antibiotics used to treat highly damaging diseases, like European and American foulbrood (Bayer et al., 2018; Voitsitskiy et al., 2019). Therefore, novel tools, such as nanotechnologies, are being explored as potential alternatives against infectious diseases (Faisal et al., 2021; Elqady et al., 2025). Nanoproducts are composed of ultrafine particles of roughly 1–100 nm in size and characterized by a high surface-area-to-volume ratio and unique physical, chemical, and biological properties that differ from those of bulk materials (Khan et al., 2022). Nanoproducts are widely used across the chemical, pharmaceutical, food, and agricultural industries due to their potentially low toxicity to animals, including beneficial insects and humans, and their unique functional properties (Arslan & Akbaba, 2020; El-Sayied Ali et al., 2024; Mamatha et al., 2024; Wojtczak et al., 2024). Their applications include nanofertilizers, nanopesticides, and drug and vaccine delivery systems (Kaur et al., 2014; Peters et al., 2016). Furthermore, rare-earth elements have proven to be highly effective immunomodulators, antioxidants, and antimicrobial agents based on *in vivo* and *in vitro* assays. In particular, nanocerium dioxide (nano-$CeO_2$) has demonstrated the ability to enhance antioxidant defense enzymes in tissues and to potentiate the activity of antibacterial agents (Charbgoo et al., 2017). Additionally, nano-$CeO_2$–based nanomaterials have been shown to possess intrinsic virucidal, bactericidal, and fungicidal properties (Abou-Shaara et al., 2021). Interestingly, treatment of human colorectal carcinoma and lung cancer cell cultures with $CeO_2$ nanoparticles promoted apoptosis, resulting in inhibition of cancerous cells (Datta et al., 2020; Gunasekaran et al., 2025). The positive effects of nano-$CeO_2$ on severe and incurable diseases are also supported by studies in which feeding mice nano-$CeO_2$ for 14 weeks showed its potential for the treatment and prevention of Alzheimer's disease (Sofranko et al., 2022). Consequently, investigations into the effectiveness of nanoparticles and nanoproducts against infectious, invasive, and non-infectious diseases remain highly relevant for both human and animal health, including honey bees. Equally important is assessing the potential of rare-earth metal nanoparticles, such as Ce, to accumulate in beekeeping products and exhibit antioxidant properties that may benefit both bees and consumers of bee products (Priscilla et al., 2025; Zhang et al., 2025).

Although unique beneficial properties of nanoproducts have been reported in different biological models, their innocuous and beneficial capacities in insect models remain controversial. For example, El-Samad et al. (2024) reported 1,763 documents in a review on the accumulation of nanoparticles of various elements in insects. Most of the reported studies focused on the effects of nanoparticles on *Aedes aegypti* (yellow fever mosquito), *Culex quinquefasciatus* (southern house mosquito), *Bombyx mori* (silkworm), and *Anopheles stephensi* (Asian malaria mosquito), and described negative effects, like neurotoxicity and loss of vision. In addition, a study conducted in *Drosophila melanogaster* (fruit fly) showed that metal nanoparticles entering the cell induced the formation of reactive oxygen species (ROS), including hydroxyl ions, peroxide ions, superoxide anions, singlet oxygen, and hypochlorous acids, resulting in cellular damage (Mishra & Panda, 2021). However, Demir et al. (2022) reported that nano-$CeO_2$ at concentrations of 0.01–10.0 mM did not exhibit toxic, genotoxic, or teratogenic effects on *D. melanogaster*. Furthermore, studies on the effects of nano-$CeO_2$ on *Acheta domesticus* (crickets), *Epilachna varivestis* (Mexican bean beetles), and *Podisus maculiventris* (spined soldier bugs) also confirmed the ability of nano-$CeO_2$ to accumulate in their bodies without causing apparent toxicity (Majumdar et al., 2016). In addition, feeding *D. melanogaster* with nano-$CeO_2$ at a concentration of 1 mM restored superoxide dismutase activity, indicating potential beneficial effects in insect models of neurodegenerative diseases (Sundararajan et al., 2021).

Currently, the literature provides limited information on the effects of nano-$CeO_2$ on honey bees. A study on feeding *Apis mellifera carnica* bees with nano-$CeO_2$ at doses of 2–500 mg/L over a 9-day period showed no significant effect on insect survival even at 500 mg/L. However, biochemical changes in their tissues were detected at doses as low as 2 mg/L; honey bees fed with nano-$CeO_2$ during the summer showed a significant increase in acetylcholinesterase and glutathione S-transferase activity compared to winter bees, although the health implications of these changes remain to be investigated (Kos et al., 2017).

Thus, findings on the effects of nano-$CeO_2$ in insect organisms and other animal models remain inconclusive. Consequently, further research is warranted to clarify its impacts on honey bee health, the potential for residues in bees, honey, and other hive products, and its prospective use as an antimicrobial agent. Therefore, the objectives of this study were to evaluate honey quality by measuring key indicators, including the mass fraction of water, diastase activity, and proline content, in honey from colonies exposed to nano-$CeO_2$ fed in sugar syrup. In addition, the study aimed to determine the mineral composition of bee bodies, honey, and wax from these treated colonies. Finally, the study sought to calculate human intake of cerium from honey collected from nano-$CeO_2$–treated colonies in order to estimate potential exposure levels, although there are currently no established recommendations or safety thresholds for cerium consumption.

## Materials and methods

The experiment was conducted from April 26, 2023 to May 24, 2023 at the P. I. Prokopovych Institute of Beekeeping of the National Academy of Agrarian Sciences of Ukraine. The Ukrainian steppe breed of honey bees (*Apis mellifera*) was used in the study. Two groups of medium-strength bee colonies were formed using the method of analogous groups: a control group and an experimental group, with 6 colonies in each (Table 1). All colonies had 9–11 frames covered with adult bees.

The nano-$CeO_2$ preparation in the form of a sol was kindly provided by Oleksandr B. Shcherbakov, Candidate of Chemical Sciences and Senior Researcher at the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. For colony feeding, appropriate dilutions of the sol were prepared ex tempore.

Sugar syrup used for feeding the bee colonies during the equalization and main experimental periods had a concentration of 50%. Sugar syrup consumption by the bee colonies was monitored using a gravimetric method.

During the equalization period of the experiment, the selected honey bee colonies were evaluated for colony strength, productivity, health status, and consumption of sugar syrup. Based on the recorded data, only colonies with the most similar characteristics were retained in each group in order to minimize experimental variability; these colonies were therefore considered equalized and treated as analogs. During the main experimental period, the effectiveness of feeding nano-$CeO_2$ to the experimental group of bee colonies was assessed in comparison with the control group, as well as with the data obtained during the equalization period, which served as an additional control to limit the influence of time-dependent factors, including the lifespan of worker bees, changes in the botanical composition of pollen and nectar sources, and climatic and weather conditions.

From each colony, samples of honey, wax, and bees were collected during the equalization period and at the end of the main period for laboratory analyses. Wax and honey samples were collected directly from the combs by cutting pieces measuring 5 × 5 cm. Wax cappings were then removed, and honey was separated by filtering through a metal mesh with a 0.5 mm pore size. For the analysis of the mineral composition of bee bodies, 5 individuals were used per sample. A total of 12 samples of honey, bees, and wax were examined.

**Table 1**
Scheme of the scientific experiment on feeding bee colonies with nano-$CeO_2$ in sugar syrup

| Period of the experiment | | | |
|---|---|---|---|
| Equalization period (26 April – 9 May 2023) | | Main period (10 May – 24 May 2023) | |
| Control group | Experimental group | Control group | Experimental group |
| Sugar syrup | Sugar syrup | Sugar syrup | Sugar syrup + nano-$CeO_2$ at a concentration of 1 mM, at a rate of 3 kg of syrup per colony |

Determination of honey quality indicators – mass fraction of water, diastase activity, and proline content – was carried out according to DSTU 4497:2005. The content of chemical elements (Ce, Mg, Zn, Se) in biological substrates (bees, honey, and wax) was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) on an Optima 2100 DV atomic emission spectrometer (Perkin-Elmer, Waltham, Massachusetts, USA, 2007). Analysis of Mg, Zn, Se, and Ce was performed in axial plasma viewing mode.

Metal content was assessed by ICP-OES after microwave digestion of samples. For this, a weighed sample was placed into an autoclave, 3.0 mL of 65% nitric acid (Merck, Germany) was added, the mixture was allowed to stand for 30 minutes, and then 2–3 drops of hydrogen peroxide (ChemLaborReaktiv LLC, Ukraine) were added. The sample was then digested in a MARS-one microwave system (CEM GmbH, Kamp-Lintfort, Germany, 2007). After cooling, the sample was transferred to a volumetric flask and brought to 10.0 mL with deionized water, after which it was analyzed for chemical element content. All analyses were performed in triplicate.

To construct the calibration curve, ICP multi-element standard solution No. 111355.0100 (Merck, Germany) with a concentration of 1000 mg/cm³, a standard cerium solution No. 170311.0100 (Merck, Germany) with a concentration of 1000 mg/cm³, and Se standard – State Standard Sample (DSSU) Se (IV) with a concentration of 1.0 mg/cm³ (ChemLaborReaktiv LLC, Ukraine) were used. Measurement accuracy was ensured by two parallel determinations of each element, with the relative standard deviation not exceeding 2.0%. The detection limits for Ce, Mg, Se, and Zn were 0.01, 0.05, 3.0, and 0.4 µg/L, respectively. External quality control of laboratory studies for determining Mg, Zn, and Se content in reference and test biological materials was performed according to the LAMP program of the Centers for Disease Control and Prevention (CDC, USA) and the SEQAS program (FORTREES, England). Mathematical processing of the obtained elemental analysis results was performed using the OEC-ICP WinLab32 software under the Windows XP Professional operating system.

To assess human Ce intake from honey, the Estimated Daily Intake (EDI) (Naccari et al., 2025) was calculated using the formula: $EDI = (C \times IR/BW)$, where C is the cerium concentration found in honey samples (mg/kg), IR is the daily honey intake by humans (1.92 g/day), and BW is body weight (70 kg for an adult).

Statistical processing of the test results was carried out using one-way analysis of variance (ANOVA). Comparisons of indicators in biological samples were made within the comparative and main periods of the experiment, as well as between periods. The dependence of the accumulation of specific mineral elements in bee products and bee bodies was determined using correlation and regression analysis. Data in the tables are presented as x ± SD. Differences between groups were considered statistically significant using Tukey's test at $P < 0.05$.

## Results

During the equalization period of the experiment, the quality of honey from the bee colonies met the current standards for water content, proline, and diastase activity (Table 2). No significant differences in honey quality indicators were observed between the experimental and control colonies during this period.

Feeding the colonies with sugar syrup supplemented with nano-$CeO_2$ did not significantly affect the mass fraction of water or proline content in the honey. However, the diastase activity of honey from bees fed with nano-$CeO_2$ increased by 32.8% compared to the corresponding values of the control group and by 18.6% compared to the experimental group during the equalization period.

The proline content in honey from the experimental and control colonies during the main period of the experiment exceeded the respective values recorded in the equalization period by 19.7% and 15.5%.

**Table 2**
Honey quality from bees fed with nanocerium dioxide (nano-$CeO_2$) in sugar syrup (x ± SD, n = 12)

| Indicator | Period of experiment | | | |
|---|---|---|---|---|
| | equalization | | main | |
| | control | experimental | control | experimental |
| Water content, % | 18.63 ± 0.35[a] | 18.26 ± 0.83[a] | 17.81 ± 0.56[a] | 18.31 ± 0.68[a] |
| Diastase activity, Gothe units | 9.28 ± 0.63[a] | 11.25 ± 0.96[a] | 10.71 ± 1.26[ab] | 13.82 ± 1.55[b] |
| Proline, mg/kg | 218 ± 12[a] | 223 ± 12[a] | 272 ± 35[b] | 264 ± 51[b] |

*Note*: different superscript letters indicate statistically significant differences between values in the same row of the table ($P < 0.05$) using Tukey's test.

Feeding bee colonies with nano-$CeO_2$ led to a 2.84-fold increase in Ce accumulation in the bees' bodies, accompanied by a 24.1% decrease in Se content, but did not affect the levels of Mg and Zn compared to the control (Table 3).

During the equalization period of the experiment, the mineral elements in the bees' bodies could be ranked in the following order: Mg > Zn > Ce = Se. Feeding with nano-$CeO_2$ changed the intensity of their accumulation in favor of Ce, resulting in the following order: Mg > Zn > Ce > Se. In the main period of the experiment, the magnesium content in the bodies of bees in both the control and experimental groups exceeded the corresponding values from the equalization period, while Zn concentration, on the contrary, decreased (Table 3).

**Table 3**
Mineral composition of bodies of bees fed with nanocerium dioxide (nano-$CeO_2$) in sugar syrup (µg/g, x ± SD, n = 12)

| Indicator | Period of experiment | | | |
|---|---|---|---|---|
| | equalization | | main | |
| | control | experimental | control | experimental |
| Ce | 0.33 ± 0.07[a] | 0.34 ± 0.08[a] | 0.31 ± 0.04[a] | 0.88 ± 0.12[b] |
| Mg | 421 ± 72[a] | 439 ± 73[a] | 505 ± 74[ab] | 509 ± 27[b] |
| Zn | 29.8 ± 6.4[a] | 26.1 ± 2.3[a] | 16.0 ± 2.2[b] | 16.3 ± 7.0[b] |
| Se | 0.34 ± 0.11[a] | 0.44 ± 0.12[ab] | 0.54 ± 0.05[b] | 0.41 ± 0.07[a] |

*Note*: see Table 2.

Analysis of the mineral composition of honey during the equalization period showed no differences in Ce, Mg, Zn, or Se content between the control and experimental bee colonies (Table 4). Notably, in honey, unlike in the bee bodies, all studied elements were distributed in the following order of concentration: Mg > Zn > Se > Ce. Feeding bee colonies with nano-CeO2 in sugar syrup did not affect the overall pattern of mineral component distribution in honey. However, the cerium content in honey increased 19.14-fold compared to the control (Table 4).

**Table 4**
Mineral composition of honey from bees fed with nanocerium dioxide (nano-$CeO_2$) in sugar syrup (µg/g, x ± SD, n = 12)

| Indicator | Period of experiment | | | |
|---|---|---|---|---|
| | equalization | | main | |
| | control | experimental | control | experimental |
| Ce | 0.013 ± 0.002[a] | 0.015 ± 0.004[a] | 0.014 ± 0.002[a] | 0.268±0.031[b] |
| Mg | 15.5 ± 3.2[ab] | 14.7 ± 1.2[a] | 21.3 ± 7.3[b] | 18.4 ± 1.4[b] |
| Zn | 0.60 ± 0.15[a] | 0.74 ± 0.14[a] | 1.00 ± 0.39[a] | 1.03 ± 0.24[a] |
| Se | 1.17 ± 0.10[a] | 1.80 ± 0.17[a] | 1.79 ± 0.14[a] | 1.51 ± 0.23[a] |

*Note*: see Table 2.

In honey from both the control and experimental groups, magnesium content slightly increased during the main period of the experiment compared to the corresponding values in the equalization period. Calculations showed a strong positive correlation between Ce content in bee bodies and in honey ($r = 0.925 \pm 0.096$, $P < 0.01$), which exhibited a linear relationship (Fig. 1).

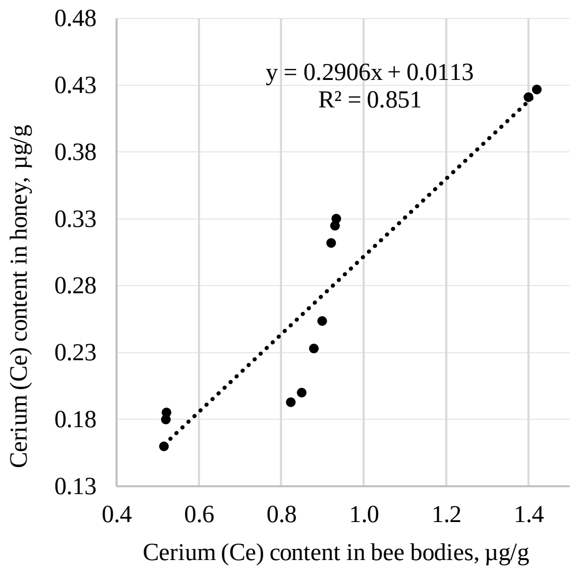


**Fig. 1.** Linear regression showing the relationship between Ce content (µg/g) in honey and its content in bodies of bees from colonies fed with sugar syrup supplemented with nano-$CeO_2$, n = 12

The estimated daily Ce intake from honey for a 70-kg human adult did not exceed 7.0 µg/day, compared with 0.4 µg/day in the control. The content of Ce, Mg, Zn, and Se in wax during the equalization period did not differ significantly between the control and experimental bee colonies (Table 5). It is also noteworthy that samples from the experimental group showed high Mg content in wax, honey, and bee bodies in compared to the control. In contrast, Zn content was lower in wax than in honey, while Se concentrations were similar in honey and wax. Finally, Ce concentration in wax were lower than those in bee bodies but higher than those measured in honey.

**Table 5**
Mineral composition of wax collected from colonies treated with nanocerium dioxide (nano-$CeO_2$) in sugar syrup (µg/g, x ± SD, n = 12 and the control)

| Indicator | Period of experiment | | | |
|---|---|---|---|---|
| | equalization | | main | |
| | control | experimental | control | experimental |
| Ce | 0.053 ± 0.003[a] | 0.064 ± 0.012[a] | 0.080 ± 0.017[b] | 0.124 ± 0.031[c] |
| Mg | 138.4 ± 20.7[a] | 146.1 ± 35.2[a] | 127.3 ± 7.6[a] | 117.1 ± 5.0[a] |
| Zn | 11.6 ± 3.2[a] | 13.5 ± 2.4[ab] | 14.3 ± 2.9[ab] | 18.7 ± 6.1[b] |
| Se | 1.52 ± 0.40[a] | 1.61 ± 0.42[a] | 0.95 ± 0.33[b] | 1.81 ± 0.15[a] |

*Note*: see Table 2.

Feeding bee colonies with sugar syrup supplemented with nano-$CeO_2$ did not significantly affect the Mg and Zn content in wax, but it increased cerium content by 1.51-fold and selenium content by 1.91-fold compared to the corresponding values in the control group (Table 5). A strong negative correlation was observed between selenium content in bee bodies and wax when bee colonies were fed sugar syrup with nano-$CeO_2$ ($r = -0.931 \pm 0.088$, $P < 0.01$). The regression line confirmed an inverse linear relationship between these parameters (Fig. 2).

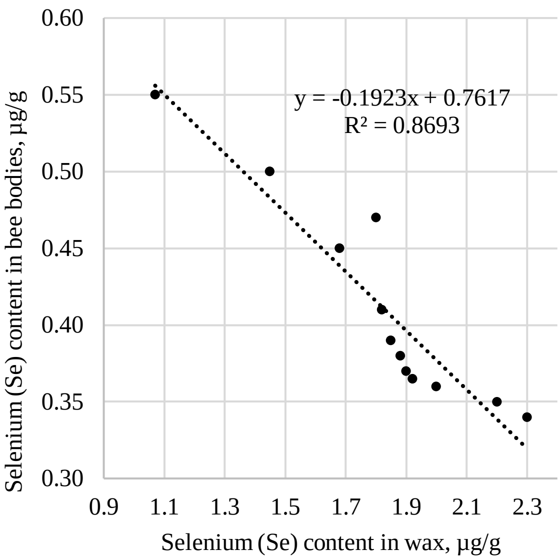


**Fig. 2.** Linear regression showing the relationship between Se content (µg/g) in wax and bodies of bees collected from colonies fed with sugar syrup supplemented with nano-$CeO_2$, n = 12

Compared to the equalization period, the mineral composition of wax changed during the main period of the experiment in both the control and experimental bee colonies. A trend toward increased cerium and zinc content in wax was observed in both groups. Thus, it has been demonstrated that feeding bee colonies with sugar syrup supplemented with nano-$CeO_2$ promotes its accumulation in bee bodies, honey, and wax.

## Discussion

Honey bees are considered economically beneficial insects, as they not only provide pollination for many agricultural crops but also produce a significant number of products used by humans for food and other purposes (Omelchun et al., 2022, 2023; Mair et al., 2023). Honey is of particular value, since its chemical composition reflects the quality, safety, and environmental status of the regions where entomophilous crops are grown (Voitsitskiy et al., 2019; Gałczyńska et al., 2021; Omelchun et al., 2025b), and it is also used as a source to synthesize nanoparticles, which are later utilized to produce nanomaterials for medical applications (Balasooriya et al., 2017; Khalifa et al., 2024).

The results of this study indicate that nano-$CeO_2$ had no effect on the main indicators of honey quality, including water content and proline concentration. At the same time, it should be noted that honey produced by bee colonies during the main experimental period exhibited higher diastase activity compared to honey obtained during the equalization period. This difference is most likely attributable to variations in the botanical composition of nectar sources flowering during these periods (Pasias et al., 2017). A potential effect of nano-$CeO_2$ on diastase activity in honey produced by colonies in the experimental group cannot be excluded; however, additional studies are required to substantiate this hypothesis.

The short life cycle of bees allows the assessment of the effects of dietary components over the honey flow season, thus confirming or excluding potential toxic effects of nano-$CeO_2$ on their metabolic and physiological processes (Tonk-Rügen et al., 2022). Although Ce is not an essential element, it is found in worker bees' bodies in background amounts. In bees, rare earth metals, including Ce, can enter the body through floral resources, such as pollen and nectar, as well as through dust particles from soil and water (Ma et al., 2018). At the same time, the content of rare earth metals, including Ce, in nectar, pollen, honey, and other bee products is generally low (<20 ng/g of Ce in honey) (Iskander, 1995).

Additional Ce intake by bees may also occur through the application of nano-$CeO_2$, fertilizers or dust contamination from mining activities (Zhou et al., 2025). Bees often forage in areas affected by wastewater discharges, like livestock farms or agricultural landscapes, potentially influencing Ce accumulation in their bodies (Doan, 2025). Furthermore, Ce accumulation in insects depends on their developmental stage and diet. For example, in a study on beetle larvae fed leaves treated with nano-$CeO_2$, low levels of Ce were detected, likely due to high excretion efficiency (~98%), whereas adult beetles exhibited higher Ce concentrations in their tissues compared to the amount excreted Ce (Majumdar et al., 2016). Similar results were obtained in crickets fed zucchini leaves treated with nano-$CeO_2$, where Ce content in their bodies was 0.0336 µg/g (Hawthorne et al., 2014). In contrast, our study found substantially higher Ce concentrations in bee bodies from the experimental group (0.88 ± 0.12 µg/g), which were significantly greater than those observed in the control group during the main experimental period (0.31 ± 0.04 µg/g). These findings indicate that feeding nano-$CeO_2$ in sugar syrup can increase Ce levels in honey bee tissues and highlight the need for further investigation into the potential health implications for worker bees.

Our study demonstrated that Ce nanocompounds, in addition to their high accumulation in worker bees' bodies, can influence the content of other essential elements, such as Se. Although the effect of nano-$CeO_2$ on Se concentration in worker bees has not been previously established, there is evidence that the trivalent cerium ion ($Ce^{3+}$) can compete with Ca (Constantin et al., 2025), which may indirectly affect the redistribution of other essential elements. Furthermore, studies on snails have shown that nano-$CeO_2$ can reduce arsenic (As) accumulation in their feet by 39%, thereby halving the potential risk of As toxicity to human consumers (Wang et al., 2024).

The positive effects of nano-$CeO_2$ on bees are possibly due to Ce's ability to shift its oxidation state between +3 and +4, which allows it to transport oxygen, absorb ultraviolet radiation, and undergo biodegradation (Filho et al., 2023). Considering its low toxicity (Tourinho et al., 2015), nano-$CeO_2$ can be regarded as a promising material for the development and design of drug carriers (Parimi et al., 2019) intended for use in humans and animals, including honey bees.

The entry of metal nanoparticles into honey bees' bodies could lead to their transfer into bee products, such as honey and wax; however, this process remains poorly understood (Abou-Shaara et al., 2021; Matuszewska et al., 2021). Typically, the mineral content in honey is low, ranging from 0.02–0.30%. It is influenced by the chemical composition of nectar, which depends on soil composition, botanical diversity, and the flowering period of nectar- and pollen- producing plants (Magdas et al., 2021). Soil characteristics, climate, and weather conditions can also affect the mineral composition of honey (Flamminii et al., 2024; Vit et al., 2024). Our study does not directly demonstrate the transfer of nanoparticles from bee bodies to hive products. Instead, it shows changes in mineral composition in bees, honey, and wax after nano-$CeO_2$ feeding. These changes are likely related to normal physiological and metabolic processes involved in honey processing and wax production, rather than to direct nanoparticle movement.

The total number of mineral elements detected in honey reaches 54 components, which are divided into several groups, including macroelements (Na, K, Ca, Mg, P, S, and Cl), microelements (Cu, Fe, Zn, Mn, Co, Mo, Se, etc.), and heavy metals (Pb, Cd, Sr, Al, Hg, etc.) (Ligor et al., 2022; Zavrtnik et al., 2024). Potassium is considered the main mineral component of honey, accounting for up to 33% of the total mass of all elements. Depending on geographic location and the botanical composition of nectar and pollen sources, honey has been found to contain barium (0.01–0.08 mg/100 g), boron (0.05–0.30 mg/100 g), chlorine (0.4–56 mg/100 g), cobalt (0.1–0.35 mg/100 g), fluorine (0.4–1.34 mg/100 g), iodine (10–100 mg/100 g), lithium (0.225–1.56 mg/100 g), molybdenum (up to 0.004 mg/100 g), nickel (up to 0.051 mg/100 g), rubidium (0.04–3.5 mg/100 g), silicon (0.05–24 mg/100 g), strontium (0.04–0.35 mg/100 g), sulfur (0.7–26 mg/100 g), vanadium (up to 0.013 mg/100 g), and zirconium (0.05–0.08 mg/100 g) (Puscion-Jakubik et al., 2020).

In honey from Spain, the main mineral elements were K, Ca, and Mg, present in ranges of 2.7–530.0 mg/kg, 23.0–387.0 mg/kg, and 41.0–331.0 mg/kg, respectively, while sodium content ranged from 9.2–1321.4 mg/kg. Honey produced in Portugal contained a high amount of potassium (1150.1 mg/kg), accounting for 76% of the total mineral mass. In honey from Morocco, K predominated, followed by Na, Ca, and Mg (Bouhlali et al., 2019). The mineral composition of honey also depends on the species diversity of nectar sources. Monofloral honey contained, on average, Ca – 20.1 mg/L, K – 407.5 mg/L, P – 20.6 mg/L, Mg – 10.7 mg/L, Na – 1.8 mg/L, and Zn – 13.9 mg/L (Mărgăoan et al., 2021), whereas polyfloral honeys differed in elemental content depending on the harvesting area (Labsvards et al., 2023). In this study, the full spectrum of mineral composition was not analyzed, but the content of Mg, Zn and Se in honey from the nano-$CeO_2$ treatment group did not differ significantly from the controls (18.4 ± 1.4 and 21.3 ± 7.3; 1.03 ± 0.24 and 1.00 ± 0.39; 1.51 ± 0.23 and 1.79 ± 0.14, respectively), and the concentrations were lower than in the above-mentioned honeys. Only Ce was significantly higher in treated colonies during the main period compared to the control during the equalization period (0.268 ± 0.031 and 0.013 ± 0.002 µg/g, respectively).

It should also be noted that most studies on Ce content in honey have been carried out only in the context of environmental contamination assessment, which does not reflect the effects of feed additives. Nevertheless, assessing its safety for consumers remains necessary. Studies on Ce intake in humans indicate that the average oral intake is 5.6–8.6 µg/day. Slightly higher levels, 83–145 µg/day, can occur in populations living in areas of rare earth metal extraction (Höllriegl et al., 2010). In our study, the daily intake of cerium from honey for humans is comparable to estimates from other researchers and does not exceed the estimated oral intake of this element.

The content of Mg and Zn in beeswax when bee colonies were fed nano-$CeO_2$ approached similar values found in beeswax collected from various apiaries in Slovakia (Zafeiraki et al., 2022). Most studies on beeswax, as well as other bee products, have focused on toxic metal content, which reflects the ecological state of the ecosystem. Therefore, among essential elements, Zn content was considered for this study, which in beeswax depended on its age and ranged from 1.0–81.2 µg/g (Formicki et al., 2013), 5.7 µg/g (Ullah et al., 2022), and 780–19,700 µg/g (Aljedani, 2020). The levels found in this study are within the reported levels, and levels of Zn in wax extracted from experimental colonies during the main period were significantly higher compared to the control during the equalization period (18.7 ± 6.1 and 11.6 ± 3.2 µg/g, respectively). There is very little information in the literature regarding Se and Ce concentrations in beeswax. Here we found significantly higher levels of Se in treated colonies compared to the control during the main phase of the experiment (0.124 ± 0.031 and 0.080 ± 0.017 µg/g, respectively). It is possible that the increase in Se content in beeswax under the influence of nano-$CeO_2$ is associated with its enhanced excretion from the bees' bodies, not only via feces but also through the secretion of the wax glands. This conclusion is supported by the observed inverse correlation between the Ce content in the bodies of bees and in their wax.

## Conclusions

The addition of nano-$CeO_2$ e at a concentration of 1 mM to the sugar syrup used for feeding bee colonies did not significantly alter the chemical composition of honey, particularly the water and proline content, but increased its diastase activity by 32.8%. The intake of nano-$CeO_2$ with the sugar syrup by bees did not affect the levels of Mg and Zn, but stimulated the accumulation of Ce in the bee bodies by 2.84 times and in honey by 19.14 times, while reducing Se content in their bodies by 24.1%.

Feeding bee colonies with nano-$CeO_2$ did not cause redistribution of mineral components in honey, but such changes occurred in the bee bodies, with the mineral composition following the order: Mg > Zn > Ce > Se. A strong direct correlation was observed between Ce content in bee bodies and in honey ($r = 0.925 \pm 0.096$, $P < 0.01$). The accumulation of Ce in honey did not exceed the human daily intake limit and reached 7.0 µg.

Supplementing bee colonies with nano-$CeO_2$ in sugar syrup promoted its accumulation in wax by 1.51 times, with a simultaneous increase in Se content by 1.91 times, without affecting the levels of other mineral components. A strong inverse correlation was observed between Se content in bee bodies and wax under nano-$CeO_2$ supplementation ($r = –0.931 \pm 0.088$, $P < 0.01$).

These results indicate a high capacity of nano-$CeO_2$ to be absorbed by bees and accumulate in bee products such as honey and wax. Combined with its low toxicity, this makes nano-$CeO_2$ a promising candidate for the production of functional bee products, as well as for the development of preventive agents in veterinary and human medicine.

The authors declare that they have no potential conflict of interest.

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