JOURNAL OF
EMERGING INVESTIGATORS

Article

# Beeing sustainable: Honey as a bioindicator for pollution

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## SUMMARY

**Honey is a bioindicator for the environment, meaning that the pollution within honey mirrors that of its local environment. Therefore, honey can be analyzed to better understand local pollution levels. This research study investigated how Chicago's industrialized environment impacts the traces of heavy metals found within local honey samples and the extent to which neighborhoods differ in the pollutants they contain. ICP-MS analysis was used to determine the concentrations of heavy metals within honey samples, signifying pollution levels of the neighborhoods from which the corresponding apiaries were located. There was no significant correlation between heavy metal concentrations in honey samples and their corresponding apiaries' distance from industrial corridors. Similarly, there was no significant correlation between heavy metal concentrations in honey samples for the heavy metals tested and the number of environmental cleanup sites located within a 4 km radius of their corresponding apiaries. Although further research and action will be necessary to protect both the environment and inhabitants of Chicago, this research study has led to a better understanding of industrial pollution within Chicago that can inspire future environmental action.**

## INTRODUCTION

Current pollution levels in many environments are rising. A study performed in Bangalore, India noted that there are two primary causes of pollution to their local ecosystem, sewage infiltration and industrial contaminants, both of which involve human interference with the natural environment (1). Similarly, a study conducted in Chile found large traces of copper in honey and plants due to copper mining in an environment (2). This research demonstrates that humans have a large influence on the spread of pollutants that are devastating ecosystems and disrupting species. While the FAO writes that improved pollution management would increase crop yields, the statistical evidence of where exactly the dangerous pollutants are coming from and how they can be managed is lacking (3). Although there are many types of pollutants that may disrupt ecosystems, heavy metals are not only a health threat to both humans and the environment, but also can also be quantitatively measured in a lab, and that same feasibility cannot be said for other atmospheric pollutants, such as carbon dioxide. Therefore, it is important to study heavy metals as symptoms of pollution that are present throughout various environments to prevent further ecological collapse.

According to the Food and Agricultural Organization (FAO) of the United Nations, bees are hardworking, vital animals, pollinating around 35% of the food we eat (3). They are a keystone species, playing an essential role in several ecosystems. Additionally, an environmental study conducted in Italy found that bees are unique in that their byproducts, such as honey, act as bioindicators for the natural environment, mirroring the chemical makeup of the air, soil, and water (4).

Chicago is an important area of study because no experiments analyzing honey as a bioindicator for pollution have been conducted in the United States, and Chicago's diverse urban environment is a prime location to change that. Chicago is also an ideal location for this bioindicator experiment due to the availability of honey samples from around the city and accessible information on the locations of industrial corridors. In order to gain a better understanding of harmful pollution in Chicago, we researched the impact of industrialization in Chicago on the traces of heavy metals found across various neighborhoods. For the purposes of this study, industrialization is defined as the process by which an agriculture-based economy develops to become manufacturing-based, leading to the development of environmental cleanup sites and greater strips of manufacturing sites, or industrial corridors. To carry out our study, we used honey as a medium to reveal heavy metals in the environment, simultaneously bringing attention to the overall importance of bees. We used inductively coupled plasma mass spectrometry (ICP-MS), a type of elemental analysis technology, to determine the relative quantities of specific heavy metals within neighborhoods of Chicago and the Chicagoland area.

We sought to determine how Chicago's industrialization impacts the traces of heavy metals found within local honey samples and to what extent various neighborhoods differ in the pollutants they contain. We hypothesized that Chicago's industrialization plays a large role in the concentration of pollutants within honey samples. Specifically, we predicted that areas located closer to industrial corridors or located near a greater number of environmental cleanup sites will contain greater levels of pollutants because of the high industrial capacities of those regions (5). After statistical analysis, the study did not reveal a clear relationship between the proximity

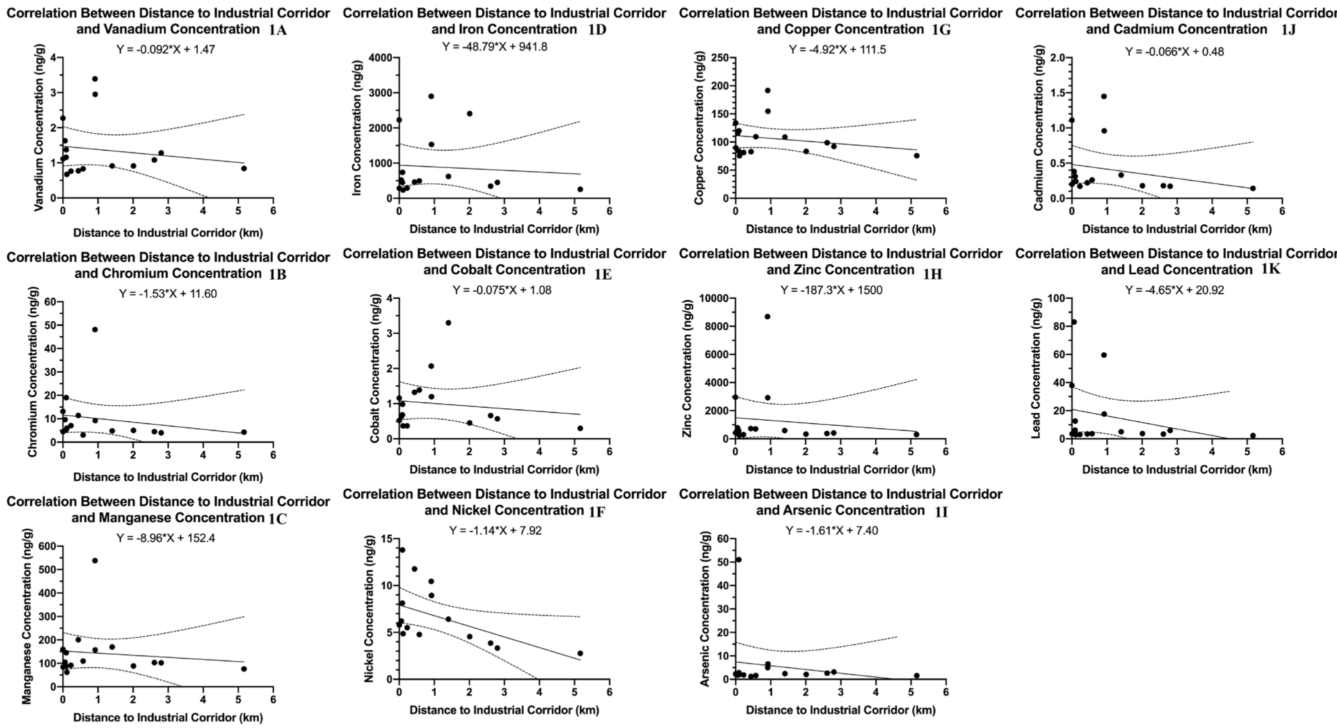


**Figure 1:** Correlations between the distance from the hive to the industrial corridor. The concentrations of heavy metals A) Vanadium, B) Chromium, C) Manganese, D) Iron, E) Cobalt, F) Nickel, G) Copper, H) Zinc, I) Arsenic, J) Cadmium, and K) Lead as a function of hive distance from industrial corridors.

of industrial corridors or environmental cleanup sites and the concentration of heavy metals in honey. Therefore, while industrial corridors and environmental cleanup sites may still play a large role in polluting the larger Chicago environment, that pollution was not locally contained as was hypothesized. Moving forward, more research should be conducted on how heavy metal pollutants are dispersed and how heavy metal pollution in Chicago contributes to environmental racism.

## RESULTS

We first analyzed heavy metal concentration in the city honey samples as a function of distance from the nearest industrial corridor. To do this, we selected samples of honey and analyzed them for heavy metals Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Cadmium (Cd), and Lead (Pb). We found that there are negative-sloped lines of best fit for every heavy metal analyzed, including V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb, demonstrating a negative correlation between the concentration of these heavy metals in honey and the distance from their corresponding apiaries to the nearest industrial corridor (**Figure 1**). After a linear regression statistical analysis was conducted with 95% confidence intervals, we found that the deviation from zero was not significant for all heavy metals tested except for Ni. Because the *p*-value for Ni was less than 0.05, this test initially rejected the null hypothesis, indicating that the relationship between the concentration of Ni in honey samples and the distance from their corresponding apiaries to the nearest industrial corridor was significant (**Figure 1F**). However, following the application of multiple hypotheses Bonferroni correction to account for error, the Ni test accepted the null hypothesis. Therefore, correcting for multiple hypotheses revealed that none of the comparisons were statistically significant, and the null hypothesis was accepted for each test.

We analyzed heavy metal concentration in the suburban honey samples as a function of the number of IEPA cleanup sites within a 4 km radius. The ICP-MS process and metals tested on these samples were identical to that of the city samples. Regarding the suburban data (**Figure 2),** there are negative lines of best fit for heavy metals Cr, Mn, Fe, Cd, and Pb, demonstrating a negative correlation between the concentration of these heavy metals in honey and the number of Illinois Environmental Protection Agency (IEPA) designated environmental cleanup sites within 4 km. We also found positive lines of best fit for heavy metals V, Co, Ni, Cu, Zn, and As, demonstrating a positive correlation between the concentration of these heavy metals in honey and the number of IEPA designated environmental cleanup sites within 4 km. After a linear regression statistical analysis test was conducted with 95% confidence intervals, we found that the deviation from zero was not significant for any heavy metals tested.

## DISCUSSION

Our data supports the conclusion that there is no significant correlation between concentrations of the heavy metals tested in this experiment in honey samples and their

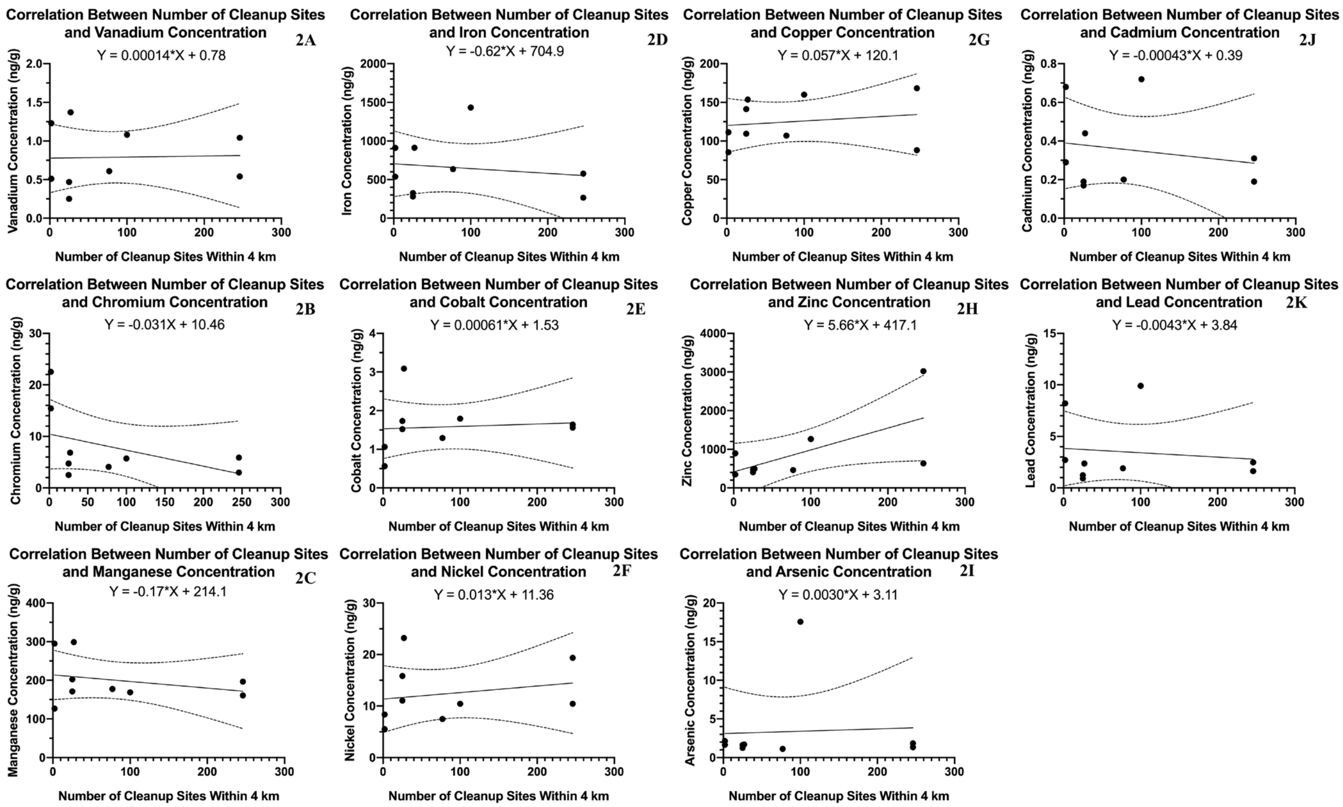


**Figure 2:** The concentrations of heavy metals A) Vanadium, B) Chromium, C) Manganese, D) Iron, E) Cobalt, F) Nickel, G) Copper, H) Zinc, I) Arsenic, J) Cadmium, and K) Lead as a function of the number of IEPA cleanup sites within a 4 km radius of hive.

corresponding apiaries' distance from industrial corridors. Similarly, there is no significant correlation between heavy metal concentrations in honey samples for the tested metals and the number of IEPA environmental cleanup sites located within a 4 km radius of their corresponding apiaries.

Through analysis of our data, it is clear that no graph expresses a statistically significant trend. One explanation for the lack of statistical significance is air pollution, which can cause pollutants to travel up to 25 km from their place of origin (6). Because of this, pollutants from industrial sites may be more widely dispersed throughout the city than was hypothesized, and therefore were not found at significantly higher levels in honey samples closer to the industrial corridors or environmental cleanup sites. Additionally, because bees can fly up to 4 km away from their hive, the honey samples from hives within 0 km to 4 km away from an industrial site could end up containing the same pollutant traces. While it should be noted that all of the city data followed negative correlations, which supports the idea that hives closer to industrial corridors encounter greater amounts of heavy metal pollution, the lack of statistical support signifies that these trends could be a product of error and confounding factors. In addition, the data indicates that metals Fe, As, and Pb (**Figure 1D, I, K**) exceed their limit values in water from certain honey samples, which raises concerns regarding human and environmental health. The maximum concentration limit (MCL) of As is 0.01 mg/L, the MCL of Pb is 0.5 mg/L, and the MCL of Fe is 0.3 mg/L (7, 8, 9, 10). It will be important for research to investigate the source(s) of these pollutants, which may be done through further honey bioindicator research on Fe, As, and Pb specifically or through direct collaboration with the Chicago government to investigate sites that may produce traces of those metals.

An important consideration is the errors and limitations of our study. The lack of a reliable control sample makes comparison of urban and suburban data to areas without industrialization data unfeasible. Confounding variables for this study included air pollution and exposure to excess metals. As pollution can travel through the air, the honey samples may have elements of pollution not directly derived from industrial corridors within their neighborhoods. That said, air pollution is a significant factor in human health, and the honey samples were intended to be bioindicators for all of the pollution within Chicago's neighborhoods. In fact, honey is a great bioindicator in part due to the hyper-interaction of bees with their environment, collecting heavy metal pollutants from water, plants, air, and soil. Although honey samples may have gathered traces of heavy metals from minimal contact with metals inside of honey jars, these traces would have been negligible compared to the honey itself and would not have interfered with the experiment.

In all settings, it is important for industrial corridors to be located in areas that will mitigate harm to both humans and the environment. Additionally, the study did not address

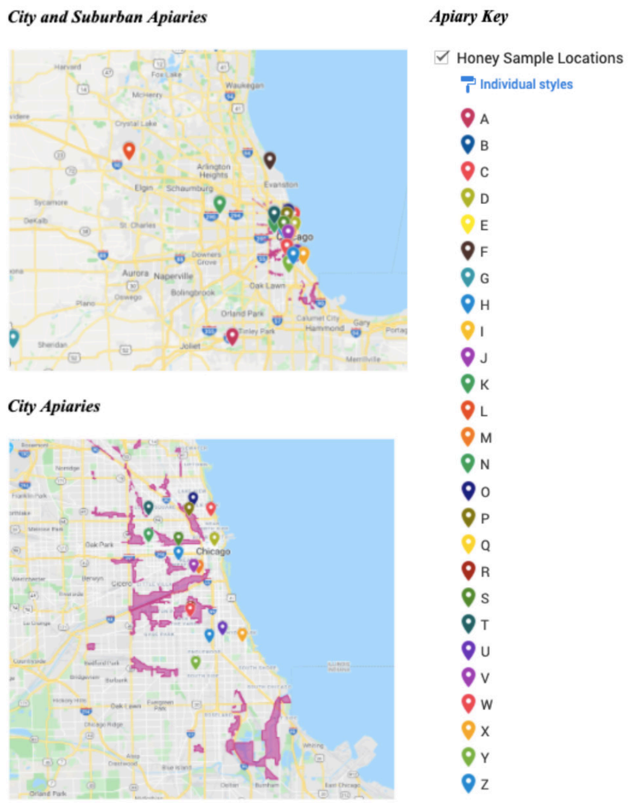


**Figure 3:** Apiary Maps with Industrial Corridor Layover and Key. Because some colors in the key occur twice, the following distinctions are necessary with relation to the previous maps: apiary C is north of apiary W, apiary H is north of apiary Z, apiary K is west of apiary N, and apiary J is west of apiary V. It should also be noted that in both maps, the apiary labels (A-Z) correspond to the honey sample labels

environmental racism in Chicago due to its scope. Research has demonstrated that Chicago's South and West side minority neighborhoods have the most exposure to toxic air pollutants, posing threats to human health (11). The environmentally disenfranchised South and West sides of Chicago predominantly consist of immigrant communities and people of color, as opposed to the white communities on the North side of the city. These clear divides within Chicago demonstrate the racism and inequity that environmental challenges coincide with. Although this study lacked the population demographics to investigate the impacts of environmental racism in Chicago, it is important that future bioindicator research strives to do so.

This study successfully investigated the impact of industrialization on heavy metal concentrations within the city of Chicago. While many similar studies have been conducted in different locations around the world, this was the first documented analysis of honey as a bioindicator for pollution in Chicago. Although this study was unable to draw significant trends and results from the collected data, replication of this research in Chicago and other cities is necessary to solidify the findings and understand the full effect of industrialization. In addition, it is important for future research to investigate the biomagnification of heavy metals in bees and the consequences for humans who consume contaminated bee products. As bees and other organisms that provide essential resources to the scientific community dwindle in numbers, it is crucial for research to be conducted that will bring a greater awareness to these issues and sustainable solutions.

## MATERIALS AND METHODS

To collect our data, we used inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is capable of detecting concentrations of heavy metals in "parts per billion," equivalent to nanograms per gram, through sensing the mass of specific heavy metal isotopes and the frequency of their interaction with the instrument. This technology is considered one of the few instruments with "high instrument sensitivities" for trace metal analysis and its high sensitivity is beneficial when studying elements in low concentrations (2).

Known beekeepers in Chicago were contacted through email. The beehive sites were selected for a variety of locations and convenience (i.e. responsiveness and known contacts). They were asked to voluntarily provide an approximately one ounce sample of honey. All beekeepers who agreed to send a sample were given information on the purpose of the study and were guaranteed access to the study after completion. In addition, all beekeepers were asked to fill out a brief questionnaire detailing the conditions of the beehive from which the samples were taken. Beekeepers' names and personal information were not used within the study, and all apiary locations were abstracted. All IRB procedures were followed and no personally identifying information was collected.

After the locations of beehives were determined, they were input into a Google Maps layer, so the spread of locations could be assessed. Each sample was labeled with a corresponding letter of the alphabet. Layers of Chicago Industrial Corridors (12) were applied to the map for a better visual representation of Chicago's city-wide pollution hotspots and later analysis (**Figure 3).**

Samples were stored in containers provided by beekeepers up until the experiment. All samples were kept at room temperature and none were stored for longer than a month after collection. All 26 samples underwent the process of wet digestion. Wet digestion "applies strong acids to digest organic material in honey" which in turn "remove[s] the predominant sugars from honey, thereby allowing for the collection of concentrated metal extracts that can then be analyzed through…inductively coupled plasma mass spectrometry (ICP-MS)" (2). To complete this process, each sample was added to a pre-weighed, certified metal-free falcon tube (VWR International, Radnor, PA, USA) with a plastic spatula. Then, the new mass was recorded. Because the mass of samples before and after dilutions was recorded, the masses of individual samples within test tubes could vary, as the concentrations, rather than numerical amount, of metals in the honey samples was the final dependent variable. Next,

| Honey Sample Label | Heavy Metal Concentration (ng/g) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | V | Cr | Mn | Fe | Co | Ni | Cu | Zn | As | Cd | Pb |
| A | 0.61 | 4.08 | 177.51 | 636.23 | 1.29 | 7.48 | 106.87 | 460.03 | 1.13 | 0.2 | 1.91 |
| B | 1.37 | 6.84 | 298.75 | 912.55 | 3.09 | 23.22 | 153.65 | 494.78 | 1.69 | 0.44 | 2.37 |
| C | 0.91 | 4.83 | 169.83 | 621.41 | 3.3 | 6.4 | 108.93 | 586.18 | 2.44 | 0.33 | 5.07 |
| D | 0.83 | 3 | 109.57 | 495.15 | 1.39 | 4.78 | 109.41 | 704.99 | 1.61 | 0.26 | 3.66 |
| E | 1.08 | 5.7 | 168.93 | 1432.76 | 1.79 | 10.44 | 159.87 | 1266.07 | 17.57 | 0.72 | 9.91 |
| F | 0.51 | 15.45 | 126.87 | 537.69 | 0.56 | 5.5 | 85.44 | 347.21 | 1.65 | 0.29 | 2.71 |
| G | 1.23 | 22.53 | 294.69 | 910.1 | 1.06 | 8.37 | 111.21 | 894.26 | 2.14 | 0.68 | 8.21 |
| H | 2.95 | 9.23 | 157.47 | 1530.48 | 1.2 | 8.94 | 154.63 | 2920.04 | 6.57 | 0.96 | 17.63 |
| I | 0.54 | 2.98 | 196.46 | 266.21 | 1.64 | 10.44 | 88.07 | 635.13 | 1.34 | 0.19 | 1.63 |
| J | 0.25 | 2.51 | 171.21 | 280.04 | 1.52 | 15.84 | 141.27 | 403.61 | 1.63 | 0.17 | 0.92 |
| K | 1.04 | 5.89 | 160.94 | 577.58 | 1.56 | 19.35 | 168.17 | 3021.23 | 1.84 | 0.31 | 2.48 |
| L | 0.47 | 4.73 | 202.65 | 326.06 | 1.73 | 11.05 | 109.45 | 477.16 | 1.26 | 0.19 | 1.23 |
| M | 1.63 | 9.64 | 104.99 | 518.83 | 0.63 | 6.22 | 115.5 | 783.28 | 1.84 | 0.38 | 83.07 |
| N | 0.77 | 11.44 | 200.56 | 462.81 | 1.32 | 11.77 | 82.91 | 729.16 | 1.21 | 0.22 | 3.48 |
| O | 3.39 | 48.12 | 538.14 | 2903.16 | 2.07 | 10.44 | 191.64 | 8690.38 | 4.98 | 1.45 | 59.52 |
| P | 2.27 | 13.13 | 159.65 | 2227.37 | 1.16 | 5.86 | 133.95 | 2957.53 | 2.35 | 1.11 | 37.86 |
| Q | 0.47 | 4.02 | 7139.19 | 485.74 | 2.85 | 24.38 | 325.77 | 588.62 | 1.71 | 2.48 | 10.53 |
| R | 1.11 | 4.45 | 84.09 | 284.17 | 0.52 | 5.79 | 89.75 | 425.45 | 2.15 | 0.2 | 3.51 |
| S | 0.76 | 7.07 | 91.91 | 290.62 | 0.37 | 5.51 | 81.29 | 292.86 | 1.86 | 0.17 | 3 |
| T | 0.67 | 5.98 | 62.31 | 233.61 | 0.37 | 4.87 | 75.76 | 248.04 | 2.12 | 0.24 | 2.84 |
| U | 1.28 | 3.97 | 102.41 | 452.88 | 0.57 | 3.33 | 92.24 | 408.77 | 3.1 | 0.17 | 5.94 |
| V | 1.37 | 19.02 | 145.44 | 739.23 | 0.98 | 13.79 | 119.94 | 581.63 | 2.81 | 0.31 | 12.61 |
| W | 1.16 | 5.22 | 87.92 | 448.76 | 0.68 | 8.12 | 83.67 | 325.18 | 51.04 | 0.24 | 6.13 |
| X | 0.84 | 4.26 | 76.36 | 255.85 | 0.3 | 2.77 | 75.75 | 303.18 | 1.58 | 0.14 | 2.27 |
| Y | 0.91 | 5.04 | 88.75 | 2407.62 | 0.45 | 4.56 | 83.5 | 336.81 | 2.07 | 0.18 | 3.71 |
| Z | 1.08 | 4.51 | 103.25 | 345.57 | 0.66 | 3.85 | 98.86 | 376.2 | 2.65 | 0.18 | 3.4 |

**Table 1:** Heavy metal concentrations in honey determined by ICP-MS.

3:1 trace-grade nitric acid (> 69%, Thermo Fisher Scientific, Waltham, MA, USA) and trace-grade hydrogen peroxide (> 30%, GFS Chemicals, Columbus, OH, USA) were added to each tube via pipet, then heated at 65 degrees Celsius for at least four hours until completely digested. Next, samples were diluted with ultra-pure $H_2O$ (18.2 MΩ·cm) to a final acid matrix of 5% acid (v/v), and the final weight was recorded. A quantitative standard was made to generate a calibration curve using a 100 µg/mL mixed element standard containing V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb (Inorganic Ventures, Christiansburg, VA, USA) to create a 100 ng/mL mixed element standard in 5.0% nitric acid (v/v) in a total sample volume of 50 mL.

Before the ICP-MS analysis, the instrument had to be tuned to ensure optimal results. First, it was established that all necessary tubes, computer interactions, and explicit qualities of the instrument were functioning properly. Then, the instrument was turned on and time was given for the plasma to reach the appropriate temperature. To achieve reliable results, a process called optimization must take place, "including calibration with standard solutions, fortification of samples, and the use of a reference material" (2). In this case, instrument performance was optimized through autotuning followed by verification via a performance report, passing manufacturer specifications.

ICP-MS was performed on a computer-controlled (QTEGRA software) Thermo iCapQ ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) operating in kinetic energy discrimination (KED) mode and equipped with an ESI SC-2DX PrepFAST autosampler (Omaha, NE, USA). Internal standard was added in-line using the prepFAST system and consisted of 1 ng/mL of a mixed element solution containing Bi, In, $^{6}Li$, Sc, Tb, Y (IV-ICPMS-71D from Inorganic Ventures). This solution had known concentrations of its elements such that we could monitor recovery during sample analysis to ensure the ICP-MS technology was functioning properly. Online dilution was also carried out by the prepFAST system and used to generate a calibration curve consisting of 100, 50, 20, 10, 2, 1, 0.2 and 0.1 ppb. Each sample was acquired using 1 survey run (10 sweeps) and 3 main (peak jumping) runs (40 sweeps). The isotopes selected for analysis were $^{51}V$, $^{52}Cr$, $^{55}Mn$, $^{57}Fe$, $^{59}Co$, $^{60}Ni$, $^{63,65}Cu$, $^{64,66,68}Zn$, $^{75}As$, $^{111}Cd$, $^{206,207,208}Pb$, and $^{89}Y$, $^{115}In$, $^{159}Tb$ (chosen as internal standards for data interpolation and machine stability). Check standard solutions were analyzed during the beginning, middle, and end of honey sample runs. The validity of the samples' data was ensured, as the known check standard concentration was measured accurately throughout the experiment. Lastly, legitimate honey sample analyses were carried out. Digested honey sample tubes were placed in the autosampler trays, and the instrument's connected computer software compiled each corresponding sample's data into a spreadsheet (**Table 1**).

Visual data was created by organizing the locations of Chicago honey samples' corresponding apiaries by their proximity to industrial corridors, which included samples P, R, M, V, W, T, S, N, D, O, H, C, Y, Z, U, and X. Suburban samples, which came from apiaries located outside of Chicago's geographical boundaries, included samples A, B, E, F, G, I, J, K, and L. Because there is no comprehensive industrial corridor data available for the suburban apiaries, they were categorized by the number of environmental cleanup sites, as determined by the IEPA, located within a four mile radius from the apiary. These sites cause varying types of pollution, including air pollution, water pollution, and underground leakage (13). Finally, the control sample, sample Q, was not accounted for on the figures because of its lack of reliability as a control. Sample Q was chosen as a control initially because it was located outside of the Chicagoland area, but ultimately it was more polluted than many of the Chicago samples, possibly due to a nearby industrial site noted by the beekeeper. Scatterplot figures were then created for each heavy metal, with metal concentration on the y-axis and distance from the industrial corridor or number of cleanup sites within a 4 km radius for city and suburban apiaries, respectively, on the x-axis. In order to determine the statistical significance of these results a P-test was conducted using Graphpad Prism Software Version 9.0.0 for linear regression statistical analysis. Bonferroni correction was also used to account for further error due to the large number of metals tested.

We gained access to the lab at Northwestern University through contacting the manager of the ICP-MS technologies, who helped in assisting with and overseeing the conduction of the laboratory procedures. Metal analysis was performed at the Northwestern University Quantitative Bio-element Imaging Center, generously supported by NASA Ames Research Center Grant NNA04CC36G.

**Received:** June 10, 2020
**Accepted:** June 24, 2021
**Published:** October 6, 2021

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