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Honey Bees (Hymenoptera: Apidae) as Indicators of Radionuclide Contamination: Investigating Contaminant Redistribution Using Concentrations in Water, Flowers, and Honey Bees

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Honey Bees (Hymenoptera: Apidae) as Indicators of Radionuclide Contamination: Investigating Contaminant Redistribution Using Concentrations in Water, Flowers, and Honey Bees

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ABSTRACT As part of ongoing research concerning the use of honey bees, *Apis mellifera* L., as indicators of environmental radionuclide contamination, samples of water, flowers, and honey bees were collected for 2 consecutive years. The samples were collected within a study site containing radionuclide contamination above background levels. The samples were analyzed for concentrations of radionuclides, and the results were compared using rank sum, correlation, and trend analysis. Results were then used to assess the redistribution pathway of radionuclides within the study site. Results indicate that honey bees receive the majority of their contamination directly from the source, a radioactive waste lagoon. The amount of contamination the honey bees receive from flowers during nectar collection appears to be insignificant compared to the amount received during water collection. Results did not demonstrate significant patterns of correlation or trend between the lagoon, bees, or flowers. Sample results showed a significant bioaccumulation of cobalt-60 and sodium-22 within the honey bees but no significant bioaccumulation within the flowers.

KEY WORDS *Apis mellifera*, honey bees, contamination, bioaccumulation, ecotoxicology, radionuclides

LOS ALAMOS NATIONAL Laboratory (LANL) has been involved in the research and development of nuclear related materials for the last 5 decades. A consequence of this type of research is the production of radioisotopes, some of which have been released into several areas within LANL boundaries. As part of ongoing research at LANL concerning the use of honey bees as indicators of bioavailable radionuclide contamination (Haarmann 1997), I conducted experiments to investigate the redistribution pathway of radionuclide contaminants.

Past research has demonstrated that honey bees are useful indicators of environmental contamination (Cesco et al. 1994, Bromenshenk 1988, Konopacka et al. 1993). Honey bees have been referred to as mobile samplers that efficiently cover a sample area during their foraging flights, then return to a central location (Bromenshenk 1990). Honey bees forage in an area with a radius as large as 6 km and often cover a total area up to 100 square km (Leita et al. 1996, Visscher and Seeley 1982). During their foraging flights, the bees collect nectar, water, pollen, and plant resins. In addition, bees inadvertently contact and accumulate a wide array of environmental pollutants that are often returned to the colony (Crane 1984, Bromenshenk et al. 1985).

Honey bees have been used in the past to monitor the presence and distribution of pesticides (Anderson and Wojtas 1986), trace elements including fluoride (Bromenshenk et al. 1988a, Mayer et al. 1988), lead (Migula et al. 1989), zinc (Bromenshenk et al. 1988b), nickel (Balestra et al. 1992), potassium (Barbattini et al. 1991), and the bioavailability of radionuclides (Gilbert and Lisk 1978, Morse et al. 1980, Wallwork-Barber et al. 1982), including tritium (White et al. 1983, Fresquez et al. 1997), cesium (Bettoli et al. 1987, Tonelli et al. 1990), plutonium (Hakonson and Bostick 1976) cobalt, and sodium (Haarmann 1997).

Besides being indicators of bioavailable contaminants, honey bees may prove to be a good model from which to explore the redistribution of contaminants from abiotic components (such as water and soil) into biological systems. However, before honey bees can be fully used in this context, more information is needed concerning the dynamics of radionuclide contaminant uptake in honey bees and the factors, such as biomagnification and bioaccumulation, that ultimately determine levels of radionuclide contamination in the bees (Gladney et al. 1983).

The purpose of my study was to investigate the redistribution of contaminants within a study site as the contaminants move from the source, in this case, a radioactive waste lagoon, to the honey bees. The area adjacent to the lagoon provides a habitat for many flowering plants that are used by honey bees as forage. Samples were collected from beehives, flowering plants, and the lagoon in order to assess the dynamics of the contaminant pathway. My experiments were designed to investigate several questions: (1) From what source do the bees uptake the majority of con-

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taminants—the lagoon or nearby flowers? (2) Are the levels of contaminants in the bees, flowers, and water correlated or do they demonstrate similar trends? and (3) Is there an observable bioaccumulation of contaminants within the bees or flowers?

Materials and Methods

Study Site. The study area is located in the Los Alamos National Environmental Research Park within the boundaries of Department of Energy property in Los Alamos County, NM. The study site is located on the eastern part of LANL property in Technical Area 53 near the Los Alamos Meson Physics Facility. I chose the site because it contains levels of radionuclide contamination that are well above background levels. Vegetation, soil, water, bee, and honey samples collected from the study site exceed background levels (LANL 1996a). The study site is located adjacent to a 7 million-liter, radioactive waste lagoon that contains known bioavailable contamination including tritium, cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181. The levels of contaminants within the lagoon fluctuated throughout the study period. The lagoon was the nearest source of water for the colonies in the experiment.

Bee Samples. I conducted experiments over a 2-yr period using beehives consisting of a standard Langstroth hive stocked with Italian honey bees *(Apis mellifera ligustica)*. During 1995, 2 colonies were established at the study site. These colonies were brought into the study site from an uncontaminated area. After 3 mo, bee tissue samples were collected from both of the colonies. Fifteen separate samples (8 samples from 1 colony and 7 from the other), each containing 100 g of bees, were collected from the 2 colonies. Each individual 100-g sample consisted of approximately 1,000 bees. Bees were collected using a swarm retriever manufactured by the Southern Ohio Hive Parts Company, Centerville, OH. The swarm retriever consists of a vacuum motor mounted on top of a collection box. Bees were vacuumed off frames that were removed from the honey supers into disposable vacuum bags, weighed, transferred, and double bagged into plastic resealable bags. Bees were collected from the honey frames in order to stay consistent with the methodology used in past LANL experiments. All samples were kept in a cooler and frozen on returning to the laboratory. With each sample collected, a new disposable vacuum bag was used to avoid cross-contamination of samples.

During 1996, the experiment was repeated with 2 new colonies. These colonies were brought into the study site from an uncontaminated area. However, in 1996, instead of collecting the samples at the end of the season, I sampled the bees periodically throughout a 4-mo period from each of the 2 colonies. Eight separate samples (4 samples from each colony), each containing 100 g of bees, were collected. Each of the 8 samples was analyzed separately, but the results from the 2 samples collected on the same day (1 from each hive) were averaged and statistically treated as 1 sample. During 1996, because the swarm retriever required electricity that was difficult to provide in the remote study site, bee samples were collected using a small, rechargeable vacuum. Bees were vacuumed off frames that were removed from the honey supers, transferred to a plastic resealable bag, weighed, and double bagged into plastic resealable bags. All samples were kept in a cooler and frozen on returning to the laboratory. With each sample collected, the vacuum collection area was thoroughly cleaned to avoid cross-contamination of samples.

Lagoon Water Samples. In 1995 and 1996, water samples were collected from the radioactive cooling lagoon. These samples were collected on separate occasions, each coinciding with the collection of the bee samples. All water samples were placed in a 125-ml high-density polyethylene (HDPE) bottle which, as a safety precaution, was subsequently placed in a 1,000-ml HDPE bottle containing absorbent material. All samples were kept in a cooler and immediately returned to the analytical laboratory. Thirteen samples were collected in 1995, and 4 samples were collected in 1996.

Floral Samples. During 1995 and 1996, floral samples were collected from the area adjacent to the cooling lagoon during the same period the water and bee samples were being collected. Floral samples were taken from all plant species present in the area that are foraged by honey bees. Within each sample, a proportional amount of flowers was included relative to the abundance of the plant species found in the area. For example, if the sample was collected from a location that contained 60% sweet clover *(Melilotus albus)*, the sample itself would also contain 60% sweet clover. All samples were double bagged into 1-gal. plastic resealable bags. Samples were kept in a cooler and frozen on returning to the laboratory. Ten samples were collected in 1995, and 5 samples were collected in 1996.

Radiochemical Analytical Methods. All 1995 and 1996 samples were analyzed by the LANL Environmental Chemistry Group. The 1995 samples were analyzed for tritium, whereas the 1996 samples were analyzed for tritium and gamma-emitting nuclides. All gamma-emitting radionuclide concentrations were determined using high-resolution germanium detector gamma-ray spectrometry according to LANL standard operating procedure ER 140 (LANL 1996b). The tritium samples were analyzed using liquid scintillation counting. Five milliliters of moisture were distilled from each sample, mixed with 15 ml of a scintillation solution, and counted on a scintillation counter for 50 min according to LANL standard operating procedure ER210 (LANL 1996b). All laboratory quality control parameters were met.

Statistical Methods. A nonparametric rank sum statistical test (Kruskal–Wallis, Wilcoxon) was used to compare the levels of contaminants within each of the 3 groups; that is, the lagoon, the bees, and the flowers. These tests were applied to the 1995 data, which included tritium, and to the 1996 data, which included

tritium, cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181.

A nonparametric Spearman rank correlation test was used to compare the radionuclide concentrations of the 3 groups. The 1995 lagoon and floral tritium samples were compared. However, I could not use the Spearman rank correlation test for trend on the 1995 bee samples because all the bees were collected on the same day. The 1996 bee, floral, and lagoon samples were all individually compared against each other for all tritium, cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181 samples.

The Mann–Kendall test was used to analyze significant trends within each of the 3 groups (Gilbert 1987). These tests were applied to the 1995 tritium data and to the 1996 data which included tritium, cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181. I analyzed the 1995 lagoon and flowers and the 1996 lagoon, flowers, and bees. I could not use the Mann–Kendall test for trend on the 1995 bee samples because all the bee samples were collected on the same day.

Results

Individual sample results for both experiments are listed in Tables 1 and 2. Several data points used in the statistical analysis were below detection limits (designated as less than [<] in Table 2). The actual concentration of these samples is unknown but is somewhere between 0 and the detection limit. I assigned a value of ½ detection limit for these data points (Gilbert 1987). For example, when the amount was <2.0 pCi/g, I assigned a value of 1.0 pCi/g. This substitution had no impact on the statistical test results because the detection limit was less than the lowest detected value, so the rank could not change by the substitution.

The lagoon samples contained the highest levels of tritium in 1995 and 1996, followed by the bees and flowers, respectively (Table 3). The rank sum tests indicate that there was a significant difference in the levels of tritium within each of the 3 groups (Table 4). The remainder of radionuclides were highest in the bees, followed by the lagoon and the flowers, respectively (Table 3). The 1996 rank sum test results (Table 5) indicate a significant difference between the contaminant levels in the bees and the flowers. The lagoon and flower samples are significantly different for tritium, cobalt-60, sodium-22, and tungsten-181. Lastly, the bee and lagoon samples are significantly different for cobalt-60 and sodium-22.

The Spearman rank correlation analysis indicated no significant correlations between any of the 3 groups during 1995 and 1996. While not significant, the lagoon and bees shared the greatest number of positive correlations.

Trend analysis (Table 6) indicates significant trends in the lagoon samples during 1995 for tritium, and during 1996 for cobalt-60, manganese-54, and sodium-22. Significant trends were observed in the floral samples during 1995 for tritium. No significant trends were observed in the bee samples. The only shared, significant upward trends were seen in the 1995 lagoon and floral tritium samples. Although not all significant, the lagoon and bee samples showed similar upward trends for cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181, whereas the floral samples demonstrated an upward trend during this same time period for tungsten-181.

Table 1. Levels of tritium in samples collected near a LANL radioactive waste lagoon in 1995

Sample type	Sample no.	Tritium, pCi/ml	Analytical uncertainty
Lagoon	1	3,150.0	90.0
	2	2,770.0	80.0
	3	2,670.0	80.0
	4	510.0	20.0
	5	530.0	20.0
	6	960.0	300.0
	7	2,100.0	100.0
	8	2,800.0	100.0
	9	3,500.0	100.0
	10	4,100.0	100.0
	11	4,600.0	100.0
	12	5,800.0	200.0
	13	7,100.0	200.0
Floral	1	8.1	0.9
	2	17.7	1.4
	3	4.9	0.7
	4	1.9	0.7
	5	28.0	1.6
	6	26.2	2.5
	7	10.4	1.0
	8	11.8	1.0
	9	33.7	1.8
	10	40.5	2.0
Bees	1	1,100.0	100.0
	2	1,100.0	100.0
	3	1,100.0	100.0
	4	1,100.0	100.0
	5	1,200.0	100.0
	6	1,200.0	100.0
	7	1,100.0	100.0
	8	1,100.0	100.0
	9	100.0	10.0
	10	100.0	10.0
	11	100.0	10.0
	12	100.0	10.0
	13	100.0	10.0
	14	100.0	10.0
	15	100.0	10.0

Discussion

Previous studies at LANL have investigated the redistribution of radionuclide contaminants within the environment. Hakonson and Bostick (1976) measured the contaminant levels of tritium, cesium-137, and plutonium in bees, honey, surface water, and vegetation. The authors concluded that tritium levels in bees appear to equilibrate with the source. Cesium-137 and plutonium concentrations were low or undetectable in the bees during this study and, therefore, difficult to use in analysis. The authors suggest that because there appeared to be several locations from which the bees received the radionuclides, it was difficult to interpret the data and understand patterns of redistribution.

Table 2. Levels of radionuclides in samples collected near a radioactive waste lagoon in 1996

Sample type	Sample No.	Tritium, pCi/ml	Analytical uncertainty	Co-56, pCi/g	Analytical uncertainty	Co-60, pCi/g	Analytical uncertainty	Mn-54, pCi/g	Analytical uncertainty	Na-22, pCi/g	Analytical uncertainty	W-181, pCi/g	Analytical uncertainty
Lagoon	1	4,849.00	2.00	0.03[a]	0.1	0.3[a]	0.03	0.2[a]	0.02	101.5[a]	8.4	71.2[a]	6.8
	2	3,740.00	132.00	<6.6[a]	NA	5.2[a]	0.6	4.4[a]	0.8	122.0[a]	11.0	67.0[a]	17.0
	3	2,546.00	101.00	<4.1[a]	NA	6.0[a]	0.7	11.0[a]	1.0	132.0[a]	12.0	82.0[a]	18.0
	4	2,555.00	102.00	9.5[a]	3.2	21.0[a]	2.0	76.0[a]	7.0	170.0[a]	16.0	215.0[a]	34.0
Floral	1	12.04	246.00	1.3	0.7	<0.3	NA	0.2	NA	1.7	1.3	5.0	1.8
	2	67.55	338.00	4.2	0.9	1.5	0.5	1.5	0.5	1.8	0.6	8.4	3.3
	3	26.54	0.25	<0.4	NA	<0.3	NA	0.8	0.2	<0.1	NA	8.1	1.5
	4	13.01	0.20	<1.4	NA	<0.3	NA	<0.3	NA	<0.4	NA	9.8	3.6
	5	29.28	0.25	<0.5	NA	<0.3	NA	1.0	0.2	<0.1	NA	8.6	1.6
Bees (1)	1	0.14	0.14	14.6	5.5	48.6	5.4	62.0	7.7	2,031.0	181.0	183.0	50.0
	2	171.82	0.49	<14.1	NA	62.3	7.0	37.7	6.5	2,722.0	242.0	164.0	51.0
	3	480.38	0.77	27.0	11.4	163.0	17.0	53.7	8.4	4,392.0	389.0	335.0	73.0
	4	77.90	0.36	<13.2	NA	115.0	12.0	64.1	9.8	3,158.0	2,832.0	242.0	68.0
Bees (2)	1	445.90	0.74	23.9	8.7	154.0	16.0	383.0	38.0	2,489.0	223.0	311.0	67.0
	2	164.38	0.41	<11.0	NA	78.0	9.4	39.0	7.3	2,815.0	251.0	267.0	56.0
	3	318.64	0.64	<17.9	NA	340.0	35.0	154.0	19.0	5,253.0	466.0	1,046.0	159.0
	4	629.14	0.87	36.8	14.1	553.0	53.0	523.0	51.0	4,559.0	403.0	849.0	125.0

Measurements of picocuries per gram are ash weight. These numbers were converted to wet weight when appropriate for certain statistical tests. NA, not applicable.

[a] Values are given in picocuries per milliliter (pCi/ml).

In my study, because the lagoon was the only major source of tritium, it is easier to understand the redistribution of tritium within the study site. Because the levels detected in the flowers are consistently less than those present in the bees, and because the lagoon levels are consistently higher than the levels in the bees, it appears that within the study plot, the bees are receiving the majority of their tritium from the lagoon, with much less being contributed by the flowers. In areas with lower source levels, the redistribution patterns would certainly be different, including the possibility that the flowers would be a significant contributor of tritium to the bees.

Consistently, the floral samples contained the lowest levels of all contaminants. The levels were all significantly lower than those observed in either the lagoon or the bees. These results are to be expected because the majority of plants in the study site are not uptaking the contaminants directly from the lagoon water; therefore, the redistribution of contaminants to the plants in the area is somewhat limited.

The levels of cobalt-60 and sodium-22 detected in the 1996 bee samples were significantly higher than the levels in the lagoon samples. As part of an ongoing LANL surveillance program, air, water, soil, and foodstuffs are monitored in the study site (LANL 1996c). These studies indicate that the only major source of cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181 near the study site is the waste lagoon. Because the bees are receiving cobalt-60 and sodium-22 only from the lagoon and because the levels found in the bees are significantly higher than those at the source, it is apparent that bioaccumulation of sodium-22 and cobalt-60 is occurring within the honey bees. There is not a significant bioaccumulation of radionuclides within the floral samples.

Although correlation analysis did not detect significance, I would not rule out the fact that a relationship may exist between the levels of contaminants in the lagoon and those in the flowers and bees. Analysis indicating "no significant correlations" in the contaminant levels may simply be a result of the small sample size and the difficulties associated with detecting correlations of data sets with small sample sizes. There appears to be a positive correlation between the levels of contaminants in the lagoon and the bees. This is in agreement with the findings of the rank sum tests indicating the lagoon as the primary source of bee contamination. Similarly, Fresquez et al. (1997) examined 17 yr of data on the tritium levels in honey and bees and found no significant correlation between the levels in the bees and the honey.

With the exception of 1996 tritium, upward trends were seen in the lagoon and the bees for all the con-

Table 3. Table of 1995 and 1996 mean (median) values for the 3 groups

	Tritium 1995	Tritium 1996	Co-56	Co-60	Mn-54	Na-22	W-181
Lagoon	3,122.31	3,422.50	3.72	8.12	22.90	131.38	108.8
	(2,800.00)	(3,147.50)	(2.67)	(5.60)	(7.70)	(127.00)	(76.60)
Bee	646.67	286.01	4.78	55.69	48.33	996.86	124.79
	(1,100.00)	(288.27)	(5.56)	(53.12)	(48.50)	(928.49)	(116.50)
Flower	18.32	29.68	0.05	0.17	0.27	0.28	2.75
	(14.75)	(26.54)	(0.22)	(0.05)	(0.23)	(0.06)	(3.09)

Results are given in wet weight picocuries per gram (pCi/g).

Table 4. Nonparametric statistical comparisons made on the 1995 and 1996 tritium sample results

Yr	Sample compared	Statistical test	P
1995	Lagoon/floral/bees	Kruskal–Wallis	<0.001*
	Lagoon/floral	Wilcoxon	<0.001*
	Floral/bees	Wilcoxon	<0.001*
	Bees/lagoon	Wilcoxon	0.001*
1996	Lagoon/floral/bees	Kruskal–Wallis	0.005*
	Lagoon/floral	Wilcoxon	0.016*
	Floral/bees	Wilcoxon	0.016*
	Bees/lagoon	Wilcoxon	0.029*

*, Significant at $P = 0.05$.

taminants. This further supports the hypothesis that the bees are likely receiving the majority of their contamination from the lagoon. The floral samples showed a variety of trends. For example, for the 1st yr tritium lagoon and flower trends showed upward trends, whereas for the next year they showed opposite trends. In fact, for most cases the flowers and lagoon showed opposite trends.

In conclusion, although trend and correlation analysis did not result in statistically significant findings, the bioaccumulation of certain radionuclides within the honey bees is apparent. As for understanding the dynamics of contaminant redistribution, perhaps larger sample sizes will be needed in future studies so that correlation and trend analysis may prove more statistically powerful. Additionally, it would be useful in future studies to analyze nectar itself rather than the whole flower. Samples could also be collected from air and soil within the study plot to assess further other potential pathways. Nonetheless, this study is helpful by improving our understanding of which point sources significantly contribute to the levels of contamination within the bees, as well as by underscoring the issue of bioaccumulation of certain radionuclides within the honey bees.

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Table 5. P values of Wilcoxon rank sum test (1-sided) comparing the contaminant levels in the lagoon, flowers, and bees during 1996

Radionuclide	Bees/flower	Lagoon/flower	Bees/lagoon
Tritium	0.008*	0.008*	0.90
Cobalt-56	0.008*	0.145	0.34
Cobalt-60	0.008*	0.020*	0.03*
Manganese-54	0.008*	0.055	0.17
Sodium-22	0.010*	0.010*	0.01*
Tungsten-181	0.008*	0.008*	0.56

In the "Bees/flower" column, the bees were compared against the flowers in a 1-sided analysis; in the "Lagoon/flower" column, the lagoon was compared with the flowers in a 1-sided analysis, and so on.
*, Significant at $P = 0.05$.

Table 6. Summary of trend analysis comparing the 3 separate groups of samples

Year	Radionuclide	Lagoon	Flowers	Bees
1995	Tritium	Upward*	Upward*	Not analyzed
1996	Tritium	Downward	Upward	Upward
	Cobalt-56	Upward	Downward	Upward
	Cobalt-60	Upward*	Downward	Upward
	Manganese-54	Upward*	No trend	Upward
	Sodium-22	Upward*	Downward	Upward
	Tungsten-181	Upward	Upward	Upward

*, Significant at $P = 0.05$.

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