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# The effects of ingested aqueous aluminum on floral fidelity and foraging strategy in honey bees (*Apis mellifera)*

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ABSTRACT

Pollinator decline is of international concern because of the economic services these organisms provide. Commonly cited sources of decline are toxicants, habitat fragmentation, and parasites. Toxicant exposure can occur through uptake and distribution from plant tissues and resources such as pollen and nectar. Metals such as aluminum can be distributed to pollinators and other herbivores through this route especially in acidified or mined areas. A free-flying artificial flower patch apparatus was used to understand how two concentrations of aluminum (2 mg/L and 20 mg/L) may affect the learning, orientation, and foraging behaviors of honey bees (*Apis mellifera*) in Turkey. The results show that a single dose of aluminum immediately affects the floral decision making of honey bees potentially by altering sucrose perception, increasing activity level, or reducing the likelihood of foraging on safer or uncontaminated resource patches. We conclude that aluminum exposure may be detrimental to foraging behaviors and potentially to other ecologically relevant behaviors.

## 1. Introduction

Secondary consequences of anthropogenic change can have important ecosystem effects. One example is substrate acidification through acid rain and carbon dioxide emission (Andrews and Schlesinger, 2001; Bonan, 2008). Acidification can ionize potentially harmful compounds and is of particular concern regarding uptake of metals by plants (Andrews and Schlesinger, 2001; Peralta-Videa et al., 2009; Pourrut et al., 2011). Uptake of potentially harmful species of metals such as aluminum can cause both direct damage to plants as well as ecosystem consequences through the food chain (Nagajyoti et al., 2010; Rout et al., 2009).

Heavy metals and excess intake of micronutrient metals can cause direct damage through protein modification, competition with essential micronutrients, and acute and chronic negative behavioral effects (Bouraoui et al., 2008; Leal et al., 2012; Needleman et al., 1990; Ragunathan et al., 2010; Rivera-Mancía et al., 2010). The micronutrient metals zinc and iron are known to contribute to neurodegeneration outside of their biologic range (Ayton et al., 2014; Leal et al., 2012). These metals may also work in tandem with other metals and increase toxicity (Mizuno and Kawahara, 2017). Metals that negatively interact with micronutrients may also cause damage on their own. For example, species of aluminum can be taken up and distributed through tissues causing food-web wide disturbance (Delhaize and Ryan, 1995; Kaizer et al., 2008). Despite this disturbance and a growing body of literature that aluminum is harmful, it has been classified as biologically unimportant (Exley and Mold, 2015; Mirza et al., 2017).

Aluminum (Al) occurs in variable concentrations in soils and may be increasingly bioavailable to organisms from mining activity, soil acidification, and carbon emissions (Andrews and Schlesinger, 2001; Bonan, 2008; Rabajczyk and Namieśnik, 2010). Bioavailable aluminum can then be absorbed through plant roots, stunting growth, and disrupting photosynthetic processes (Delhaize and Ryan, 1995; Tahara et al., 2008). The metal can then spread up the food chain through herbivory, pollen, and nectar collection (Delhaize and Ryan, 1995). Once ingested, aluminum cannot be excreted and builds up in cells (Exley and Mold, 2015). In animals, the effect of aluminum intoxication is conflictive and understudied, however literature suggests that this metal can affect the ecology of aquatic animals and is not a deterrent to pollinators (Alexopoulos et al., 2003; Meindl and Ashman, 2013; Sparling and Lowe, 1996). There is some evidence that aluminum contamination alters the cholinergic system, but the me-

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chanism and direction of such contamination is still unknown (Exley and Vickers, 2014; Mirza et al., 2017; Yellamma et al., 2010).

Aluminum contamination of the cholinergic system is expected to inhibit acetylcholinesterase the regulatory enzyme for the neurotransmitter acetylcholine (Jackson et al., 2011; Yellamma et al., 2010). The inhibition of this enzyme interferes with the regulatory breakdown of acetylcholine and causes overstimulation of the post-synaptic neuron, potentially resulting in memory deficits, hyperkinesia and an overactive autonomic nervous system (Čolović et al., 2013; Hasselmo, 2006; Williamson et al., 2013). Disruption of the cholinergic system in organisms that have direct interaction with aluminum contaminated food sources may suffer severe consequences (Williamson et al., 2013; Yellamma et al., 2010). Of particular concern when considering aluminum exposure are organisms that are already at risk, such as pollinators, which directly use pollen and nectar resources and are in decline partially as a result of known toxicants, pathogens, and habitat fragmentation/food stress (Bekić et al., 2014; Ellis et al., 2010; Potts et al., 2010).

For the purpose of this study we focused on honey bees as these organisms are easily reared, economically important, have been previously used for learning and toxicological study, but have not been investigated in terms of aluminum (Burden et al., 2016; Gallai et al., 2009; Williamson and Wright, 2013). One of the first concentrated research programs on learning in honey bees was started by Von Frisch (1919) with less organized work starting even earlier (Maeterlinck and Sutro, 2003). One learning methodology, the proboscis extension response, has been used to study the sub-lethal effects of toxicants specifically on learning (Abramson et al., 2012; Burden et al., 2016; Hladun et al., 2012). Similarly, free-flying experiments have been used to understand how honey bees behave under the influence of toxicants in more natural conditions (Craig et al., 2014; Karahan et al., 2015). Both free-flying and laboratory methods can be used to understand how toxicants may affect honey bee behavior (Burden et al., 2016; Karahan et al., 2015).

Foraging behaviors are integral to individual bee and hive success and will likely be affected by aluminum exposure. Patches must be found and effectively utilized, then bees must successfully return to the hive, expel their crop and communicate to other bees the location of the floral patch (Henry et al., 2012; Von Frisch, 1967). These behaviors are also required in other pollinators such as solitary bees and Lepidoptera in which successful forage is essential to survival (Badgett and Davis, 2015; Cameron et al., 2011). Chemical exposure can affect any foraging behavior and produce ecological effects as well as economic effects on humans. To lessen this risk we must attempt to understand the sub-lethal and ecologically relevant behavioral effects of chemical exposure to bees.

The purpose of this study is to determine how aluminum ingestion may sub-lethally affect honey bees. Specifically we use foraging choice as a measure of sub-lethal behavioral change using the research design of Karahan et al. (2015). We expect that foraging efficiency will be reduced by aluminum contamination resulting in reduced return-rate or feeding on low-carbohydrate quality resources.

## 2. Methods

### 2.1. Study species

*Apis mellifera* spp. were from the Namık Kemal Üniversitesi apiary in Tekirdağ, Turkey during the summer of 2016. Experimental bees were from two subspecies, *Apis mellifera caucasica* and *Apis mellifera carnica*, with a bias favoring *carnica* subspecies. All experimental bees were foragers and therefore assumed to be of approximately 3–4 weeks old (Huang and Robinson, 1996; Huang et al., 1994; Robinson, 1987). Colonies had equal access to food resources and contained ten hive-frames per super.

### 2.2. Flower Patch Construction

Flowers were constructed following Cakmak et al. (2009), Giray et al. (2015), and Karahan et al. (2015). The underside of Plexiglas flowers were painted with blue and white Testors enamel paint (Vernon Hills, IL, 1208C and 1245C, respectively). We used clear plastic dowels rather than wooden dowels for the stems. We assume that the stem change did not affect the apparatus as the stems are not visible from the top angle that the bees primarily see. During the experiment, flowers were placed so that they protruded from a large flat brown board approximately 0.5 m off the ground.

### 2.3. Pre-training

Before the experiment began, honey bees were trained to visit a scented 1 M sucrose solution feeder located approximately 2 m from the experimental setup. Scents were only used for pre-training and were removed for the experimental procedures. The olfactory stimulus provided a secondary cue for bees to find the flower patch while they established landmarks and flight patterns for quick returns. Several scents were used over the course of the experiment, including clove and peppermint. However, these scents did not present a competitive advantage over the local flora and were replaced mid-summer with distilled sunflower oil from locally acquired flowers. Approximately 1 mL of the sunflower solution was added to 500 mL of 1 M sucrose solution. The scented feeder was refilled before each experiment.

Once the feeder attracted approximately 50–100 bees, a petri dish filled with the same scented solution was placed in the center of an empty flower patch board to begin pre-training to the experimental patch. After consistent visitation (defined as approximately 5 bees simultaneously on the region being observed), the petri dish was exchanged for 4 artificial flowers (2 white and 2 blue, see Flower Patch Construction). Consistent visitation was defined after experimenters noted 5 simultaneously visiting bees created enough potential for additional recruitment to the patch. The 4 artificial flowers were manually filled with 10 μL of the scented solution using an Eppendorf Repeater Pipette (Hauppauge, NY). After consistent visitation to the scented flowers they were removed and 54 unscented flowers (27 white and 27 blue) were randomly placed equidistant on the board. Each of the flowers was then filled with 10 μL of unscented 1 M aqueous sucrose solution. Bees that visited unscented flowers were marked with enamel paint (Testors: 9115X) on the thorax, abdomen or a combination of the two. After approximately 10 bees were marked, the flowers were cleaned and refilled for phase one of the experiment.

### 2.4. Flower patch phases

Each experiment consisted of 3 phases loosely following Karahan et al. (2015). During each phase, flower color choice, and number of returning trips to the hive were recorded. Bees that did not visit a minimum of 10 flowers per phase were removed from the primary dataset and those that did not complete phase two (post-treatment) were analyzed in a drop-out dataset (n = 38). Visitation was defined as landing on a flower and extending the proboscis into the sucrose well. The first phase was 30 min with phases two and three each lasting 45 min following the procedure of Karahan et al. (2015) (Table 1). Phases were terminated when bees that had returned before the time period ended completed their visitation and left the flower patch area. During the first phase all 54 flowers, regardless of color, were filled with 4 μL of unscented 1 M aqueous sucrose solution. Bees that completed phase one were caught in matchboxes the next time that they landed on a flower after termination of the phase (see Aluminum Distribution). The flower patch was kept in phase one setup until the last bee was released from digestion holding to minimize drop-out due to empty flowers and maintain standard experimental phase (phases two and three) time lengths. Digestion holding was 15 min for each bee

**Table 1**
Time depiction of floral patch setup, flower color 1 could be blue or white and was randomly chosen via coin flip for each experimental trial.

| | Control Phase 1 (0–30 m) | Treatment administered and holding (30–45 m) | Experimental Phase 1 (45–90 m) | Experimental Phase 2 (90–135 m) |
|---|---|---|---|---|
| Flower Color 1 | 4 μL 1 M /flower | 4 μL of treatment | 4 μL 1.5 M/ flower | 4 μL 0.5 M/ flower |
| Flower Color 2 | 4 μL 1 M/ flower | 4 μL of treatment | 4 μL 0.5 M/ flower | 4 μL 1.5 M/ flower |

(for additional details see Aluminum Distribution). After the final digestion holding bee was released, flowers were cleaned and refilled for phase two (Table 1).

Phases two and three were administered following Karahan et al. (2015) without a secondary color control phase (Table 1). This format also resembles that of Giray et al. (2015) in which there was not a second control phase. Flowers were manually refilled with 1.5 M aqueous sucrose solution and 0.5 M solution depending on color (Table 1). Color 1 for phase two was determined via coin flip and reversed for phase three. After completion of each experiment, marked bees were eliminated to limit confusion for the following tests. During each experiment, unmarked bees were captured upon visiting a flower, held with access to aqueous sucrose, and released after completion of the experiment. These bees were considered naïve to future experimentation.

### 2.5. Aluminum solutions and distribution

Honey bees were randomly assigned to ingest a solution of 0 mg/L (0 mg/L Al) (Control, $n_{treated} = 53$, $n_{complete\ experiment} = 37$), 10 mg/L (2 mg/L Al) ($n_{treated} = 56$, $n_{complete\ experiment} = 41$) or 100 mg/L (20 mg/L Al) ($n_{treated} = 49$, $n_{complete\ experiment} = 42$) added aluminum chloride ($AlCl_3$) in 1 M aqueous sucrose solution. Concentrations of aluminum in pollen have been previously investigated but the bioavailable fraction was not differentiated. The concentrations used here were chosen assuming that bioavailable forms contributed at least 1–10% of the low-average aluminum concentrations (268 mg/kg) found in Brazil (Morgano et al., 2010). A stock solution of 200 mg/L (40 mg/L Al) of aqueous $AlCl_3$ was used throughout the experiments. The aluminum salt readily dissolved and did not precipitate out of solution. Using this stock, 1 M sucrose with aluminum salt solutions were made every other day to minimize bacterial growth. Aluminum chloride was chosen as it is a bioavailable form and a search of the literature revealed that chloride has not been implicated to cause behavioral change.

Honey bees that completed phase one and returned to the patch within 15 min of its conclusion were captured using small cardboard matchboxes placed over them then slid closed. Matchboxes were then opened approximately 2 mm to allow for proboscis extension. Honey bees drank 4 μL of a randomly selected treatment (0 mg/L, 2 mg/L, 20 mg/L) off of designated glass plates. Visual observation revealed that bees readily fed on all treatment solutions within approximately 30 s. Bees were considered dosed after the 4 μL drop was no longer visible on the glass plate. After the solution was consumed, the honey bee was immediately transferred to a labelled bee cup and held for 15 min to promote individual digestion and limit hive contamination (Cakmak et al., 2009; Evans et al., 2009; Karahan et al., 2015). No bees died during the holding portion of the experiment.

### 2.6. Analysis

Four dependent variables were analyzed, frequency of blue flowers visited per phase per treatment, average number of flowers visited per minute by treatment, average number of returns to the hive per minute by treatment, and the percentage of bees that participated in the control and treatment phases that did not return for phase two by treatment. Results for proportion of color visited within phase, number of flowers visited, and numbers of trips were analyzed using ANOVAs and MANOVAs in JMP Software (SAS, Cary, NC, V13). Percent return and mean frequency within phase was analyzed using χ-square tests.

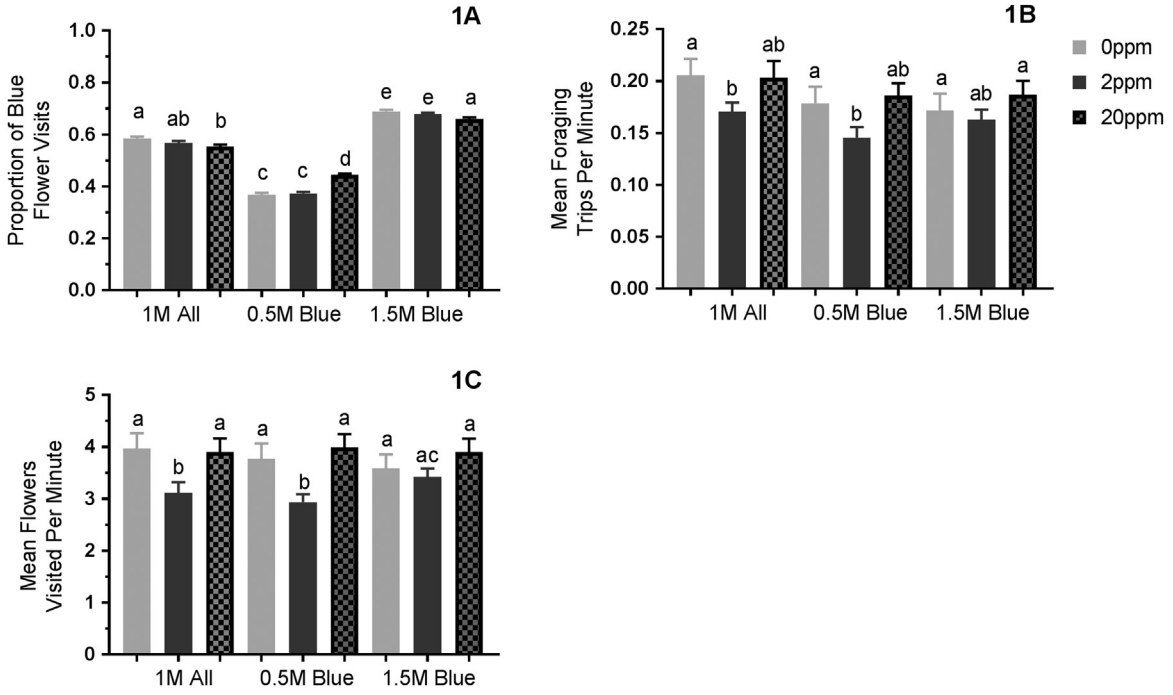


**Fig. 1.** Different letters represent significant differences within panels. All error bars represent ± 1 standard error of the mean. **1A:** The proportion of visits to blue flowers during each phase by treatment. **1B:** The mean number of trips per minute per phase by treatment. **1C:** The mean number of flowers visited per minute by treatment and separated by phase.

## 3. Results

MANOVAs showed that the blue 0.5 M sucrose content phase had significantly lower percent blue visitation than the first phase across all three treatments, meaning bees learned to visit the high sucrose flowers regardless of treatment as compared to phase 1 ($df_{0\ mg/L}$ =1, 36 $F_{0\ mg/L}$=1.0377 $p < 0.0001$, $df_{2\ mg/L}$ =1, 41 $F_{2\ mg/L}$ =1.152 $p < 0.0001$, $df_{20\ mg/L}$ =1, 41 $F_{20\ mg/L}$ =0.247 p=0.0028, Fig. 1A). Blue 1.5 M sucrose phase significantly increased blue visitation from the first phase in the control (df =1, 36 F=0.223 p=0.0075) and 2 mg/L aluminum treatments (df =1, 41 F=0.292 p=0.0013). However bees that were treated with high-dose aluminum did not follow the high-caloric value trend in this phase, demonstrating a reduction of quality assessment (df=1, 41, F=0.0475 p=0.1703, Fig. 1A).

Chi-square tests revealed that within phases there were significant differences between the control and the high-dose treatments with no variation between the control and the low-dose (Fig. 1A). The high-dose treatment bees were closer to a 50% white/blue visitation rate regardless of phase. In the 1 M All phase, control bees visited significantly more blue flowers than high dose (df=1, $\chi^2$=8.68 m p=0.0032), with no differences between control and low-dose or high-dose and low dose. For the 0.5 M blue phase, high dose had significantly lower mean frequency of blue flowers than the low dose (df=1 $\chi^2$: 67.4, $p < 0.0001$) and the control (df=1, $\chi^2$=12.61, p=0.0004). Similarly for the 1.5 M blue phase, both control (df=1, $\chi^2$=12.61, p=0.0004) and low-dose (df=1, $\chi^2$=5.17, p=0.023) visited significantly more blue flowers than the high-dose bees.

The number of trips indicated that the 2 mg/L exposed bees were slower than the control bees in the first phase (df=1, 69F=4.91, p=0.03, Fig. 1B) and slower than the high-dose in the 0.5 M blue phase (df=1, 78, F=6.62, p=0.012) when accounting for length of phase. There were no significant differences between the control and high-dose bees. The average number of flowers visited per minute varied significantly as a result of treatment (Fig. 1C). This is likely tied to the lower trip count seen in the low-dose bees. ANOVA revealed that the low-dose bees visited significantly fewer flowers than control in both the first phase (df=1, 78, F=5.71, p=0.019) and the 0.5 M blue phase (df=1, 78, F=6.65, p=0.012). The low-dose bees also visited fewer flowers during these phases than the high-dose bees (1 M all: df=1, 82, F=5.76, p=0.019, 0.5 M Blue: df=1, 81, F=12.43, p=0.0007).

Proportions of bees that returned after treatment were not significantly different ($\chi^2$=4.837, p=0.30). Nineteen sucrose treated bees did not return after treatment (38%) whereas 17 (29%) and 8 (17%) of treated bees (2 mg/L and 20 mg/L respectively) did not return after treatment. Although these values were not significant, they do represent a two-fold difference between control and 20 mg/L treatment drop-out proportions this may still present an ecologically relevant outcome.

## 4. Discussion

We expected to observe more low-molarity flower visits in the aluminum exposed bees compared to controls. A decline in flower choice was predicted to deviate from the control bees as has been shown when using this technique with pesticides and neurotransmitters (Giray et al., 2015; Karahan et al., 2015). Use of the flower patch in a previous publication has shown equal visitation of both colors in phase one and preference toward the high caloric color in phases two and three by control bees (Karahan et al., 2015). Based on the results in Karahan et al. (2015), we did not expect to see any differences between treatment groups for number of trips or number of flowers visited. As a result of anticipated disorientation from the consumption of aluminum, it was expected that fewer treated bees would return to the flower patch after treatment.

Based on previous literature, we expected to observe fewer correct high caloric value foraging choices in treated versus control bees. Although this was not the case for the low treatment bees, the high dose bees were statistically distinct from the control in each phase with a tendency to remain closer to a 50% white/blue visitation rate. This suggests that consumption of aluminum at even higher concentrations such as those found in contaminated pollen may negatively and drastically impact foraging decisions based on sucrose quality (Morgano et al., 2010).

Indiscriminate flower visitation at low concentrations of aluminum could have many ecologically significant ramifications. Poor foraging decisions may reduce the quality of winter stores and increase the number of energetically costly foraging trips to total the same caloric value. Color fidelity is only one ecologically important foraging trait required of bees and additional studies should take place to understand how aluminum may affect communication, return pace, and hive return to fully understand the ramifications of this toxicant. The combination of any of these factors with disrupted flower quality assessment compounds the toxicity of aluminum.

The bees did appear to have a slight preference for blue flowers regardless of sucrose content (Fig. 1A) which does not fit previous hypotheses of floral preference or previous uses of this methodology (Giurfa et al., 1995; Karahan et al., 2015; Raine and Chittka, 2007). Previous investigation of floral color preference suggests that it is determined by the pigment of the first flower visited by an individual bee (Giurfa et al., 1995). During this experiment, the local flora consisted of commercial or research-planted yellow sunflower (*Helianthus* spp.) fields and white morning glories (*Convolvulaceae*), both of which were visited by *Apis*. No blue flowers were observed outside of the experimental patch. The experimenters chose to use blue and white flowers for the artificial patch to most closely align with previous research that has used this behavioral apparatus (Cakmak et al., 2009; Giray et al., 2015; Karahan et al., 2015). Based on the natural flora in the area and the color preference literature, we expected the bees to show a slight preference toward white flowers. In the current experimental environment it would be more likely that the initial flight of a bee would include white flowers and create a general preference toward white. We do not expect that the blue preference is a product of poor visual perception as the flower patch was setup on a dark background and previous studies have used the same two colors (Hempel De Ibarra et al., 2000; Karahan et al., 2015). Rather, we interpret the results as an increased preference for blue flowers in the subspecies used (*carnica* and *caucasica)* as compared to previous studies that have used *Apis mellifera anatolica*.

Honey bee subspecies are highly variable in their behaviors from parasite hygiene to foraging strategies (Galindo-Cardona et al., 2013; vanEngelsdorp et al., 2013). The *carnica* and *caucasica* subspecies used here may have different baseline foraging strategies (e.g. preference for blue flowers and overall color fidelity) as compared to *anatolica* that have been used in similar previous studies (Cakmak et al., 2009; Giray et al., 2015; Karahan et al., 2015). This could partially explain why previous studies have seen very stark contrasts, nearly 50% differences across phases (Karahan et al., 2015), as compared to only a 35% difference (although significant) between the lowest and highest blue visitation frequencies as was seen here (Fig. 1A). In addition, previous work such as that by Karahan et al. (2015), did not observe significant differences between their control and blue 0.5 M sucrose phases whereas these frequencies were significantly lower across all treatments in this study. This may be because the previous study took place farther east in Turkey and may not have seen interference from the same natural floral resources.

Interference by natural floral resources was an unexpected concern while conducting this study. The flower patch setup was in the midst of many blooming commercial and research sunflower fields. Sunflowers were not expected to be preferred over the flower patch as research has shown a preference for pollen-free sunflowers over pollen carrying alternatives (Mallinger and Prasifka, 2017). In previous applications of this apparatus which took place outside of Bursa Turkey, the sunflower

industry is not as prevalent and therefore has not created a competitive aspect to the experiments. Our competitive disadvantage to the sunflower fields reduced the amount of time that experiments could be run. The alternative resources also drastically lengthened recruitment time and for these reasons the second control period used in Karahan et al. (2015) was no longer feasible. However, work using the flower patch has been completed without a post-treatment control previously by Giray et al. (2015). The methods used here were therefore a blend of two previous flower patch methods.

Sunflower presence may have also affected the proportion of bees that returned during the first phase. The pre-treatment drop-out rate was therefore excluded from the dataset as they were assumed to be foraging on sunflowers rather than the artificial flower patch. Several scents were used to attract bees in an attempt to compete with the sunflowers but were minimally successful. As a final effort to increase recruitment, we created a scent from distilled sunflower heads and had success attracting bees for the majority of the experiment. Further study is needed to understand how resources outside of free-flying experimental setup may interfere with this type of experiment as well as how various subspecies differ in baseline foraging behavior.

The number of trips per minute demonstrated that the low-dose bees were travelling slower than the control bees with high-dose bees showing no pace difference from controls. This may be a product of secondary factors such as randomly selecting slower bees for this treatment type or weather factors on the days that these bees were exposed. We did counterbalance for calendar variables between treatments so this should not have contributed to the dip seen with the low-dose bees. The mean number of flowers visited per minute closely matched the trends found in the trip data. This is expected as bees that did not return as often would logically visit fewer flowers than bees that spent more time at the patch. However, this measure was expected to be equal across treatment types. The lower number of flower visits and trips per minute within the 2 mg/L dose may be a consequence of the concentration not being high enough to interrupt the cholinergic system allowing other affected systems to dominate at this dose, or a result of variance between the bees randomly selected to each treatment. The low-dose bees may have also simply been slower than their counterparts or there may be an alternate mechanism of action that is affecting their behavior at this dose.

The primary mechanism of action for aluminum is through the cholinergic system, however this mechanism is not universally accepted (Kaizer et al., 2008; Meindl and Ashman, 2013; Yang et al., 2013). There is evidence that in addition to the cholinergic system, aluminum may affect insulin peptide generation (Cashion et al., 1996; Kaptanoglu et al., 2007). This mechanism may pose an alternative explanation for the differential number of floral visits by treatment type. Peptide modification by aluminum may affect the frequency of floral visitation by changing a bee's perception of sucrose rewards or their perception of “hunger” (Ihle et al., 2014; Pankiw et al., 2001).

A similar insulin-peptide response has been found in rats that were experimentally exposed to aluminum in which they consumed more calories after exposure to the metal (Cashion et al., 1996; Kaptanoglu et al., 2007). Similar to the rats, the low-dose bees may experience insulin peptide disruption that overpowers any immediate effect on the cholinergic system as the latter relies on the build-up of aluminum in ganglia cells (Kaizer et al., 2008). This may partially explain the unexpected reduction in visitation between the treatment types as low-dose bees are viewing the rewards differently than high-dose and control bees and may seek out additional resources outside of the artificial flower patch. This suggests that the insulin mechanism of action requires further study in combination with the cholinergic model to understand how dosages affect the dominant mechanism.

Aluminum is expected to accumulate within cells and bind to acetylcholinesterase causing overstimulation which is expected to manifest in increased number of individual flower visits (Kaizer et al., 2008; Yang et al., 2013). The dip in visitation at the 2 mg/L dose compared to the other two treatments may be the beginning of a trend toward higher floral visitation rates with increased exposure to aluminum as a result of a transfer in the dominant mechanism of action from insulin systems to the cholinergic. If the bee extended its proboscis but flew to another flower before finishing the 4 μL of sucrose solution or drinking at all this would be included as a visit. The return to normal visitation frequencies in the high-dose bees may be the combined effects of increased “hunger” from the insulin modification with hyperactivity from overstimulated cholinergic systems. This may be an ecological disadvantage to bees as they are not being as efficient at individual floral visits and may require more energetically costly stops before returning to the hive. Higher dosages will need to be tested in similar circumstances to understand the dip in floral visitation that is seen here.

The results of the drop-out dataset showed a 21% increase in the proportion of bees that returned after treatment between the control and 20 mg/L bees. We expected the opposite to be true as a result of aluminum induced disorientation. One potential explanation is a reduced ability to interpret novel stimuli as has been reported with other cholinergic toxicants in bees (Williamson and Wright, 2013). In this case the control bees may perceive alternative resource patches in which they have not been captured and held for 15 min and interpret the new location as a better alternative to the artificial patch whereas the exposed bees may not be able to differentiate the two stimuli and therefore return to the experimental patch (Williamson and Wright, 2013). Similarly, if the contaminant is affecting the memory of the bees they may not remember having been caught and held whereas the control bees may (Williamson et al., 2013). This could be an ecologically important yet unexpected memory response concerning foraging and predator avoidance in bees. For instance, if bees cannot locate new floral resources and learn to avoid potentially hazardous patches they may have an increased likelihood of toxicant exposure or predation.

The present study does not account for long-term effects of aluminum on the ecology of bees. The experiment began 15 min after treatment and totaled 105 min with only a single 4 μL dose. In a contaminated region such as Brazil, where average aluminum concentrations in pollen have been reported as high as 268 mg/kg, bees would be exposed during each flower visit and experience long-term and cumulative effects (Morgano et al., 2010). For free-flying studies, understanding the long-term exposure and collective effects is difficult because bees regurgitate their forage to create winter food stores. Their regurgitation creates potential for unwanted accumulation in the hive. To better understand long-term exposure a laboratory experiment or contaminated region experiment should take place.

The results of this study demonstrate that exposure to aluminum will adversely affect the foraging behavior of honey bees. The results are all the more significant as the bees were exposed to only a single dose. Given that their biology includes sharing food resources to maintain winter stores, aluminum can spread the throughout the hive, contaminate pollen stores, honey, and expose pupae (Exley et al., 2015; Maeterlinck and Sutro, 2003). In acidified or mining contaminated soils, a bee's food sources may contain unusually high bioavailable aluminum (Andrews and Schlesinger, 2001; Morgano et al., 2010; Pourrut et al., 2011). Exposure to aluminum contamination has been shown to decrease pupal weight and has been shown here to affect foraging decisions at high concentrations and increase return to an unsafe resource (Exley et al., 2015; Meindl and Ashman, 2013; van der Steen et al., 2012). A change in foraging strategy may reduce the amount of caloric value of food coming into the hive as well as the population dynamics of the hive (Huang and Robinson, 1996; Khoury et al., 2013; Russell et al., 2013). If aluminum limits a bee's ability to find quality patches or adapt to changing threat conditions in a previously explored patch they again run the risk of food limitation.

If sub-lethal effects are occurring as a result of metallic ingestion as is demonstrated here then it is possible that acidification and accidental metal contamination is as detrimental to pollinator populations as

harmful pesticides. Changes in short-term and long-term memory have been found in honey bees as a result of cholinergic pesticides such as the neonicotinoid imidacloprid and these chemicals have been banned in several countries (Alemanno, 2013; Boily et al., 2013; Karahan et al., 2015; Williamson and Wright, 2013). Given that the threat of metal contamination may be very harmful to pollinators it is necessary to explore the effects of these metals on population dynamics and other organisms. It is likely that if metals such as aluminum pose a risk to the insulin and cholinergic systems in bees that these conserved systems will also be affected in other organisms such as humans. Continued research into the sub-lethal effects of aluminum across taxa is needed to understand what risk this metal may pose.

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