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Effects of different environmental conditions on the cognitive function of honeybee (*Apis mellifera* L.) and mineral content of honey

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Summary: The aim of the present study was to determine the effect of local area inhabited by bee colonies on regional efficiency, foraging behavior and the content of certain metal elements in honey. Bee colonies from the same genetic source in different regions demonstrated significant variation (P<0.001) in behavior and performance. Initially, the number of forager worker bees exiting and entering the hive was approximately equal to each other. However, over time a significant difference (P<0.001) occurred between regions. Varying regional conditions caused considerable difference (P<0.001) in the average honey yields of colonies (between 28.60 ± 3.27 and 0.571 ± 2.76 kg/colony). Significant differences (P<0.01) in the amount of wax produced were also observed between regions. These regional differences were further reflected in concentrations of certain heavy metals in centrifugal honey samples. Environmental effects were determined to be the most important reason for the differences in all phenotypes, such as behavior, honey yield and heavy metal concentrations in honey. Those colonies inhabiting industrial or polluted areas died before the winter. Therefore, colonies are only productive when provided with appropriate environments or conditions.

Keywords: Animal health, behavior, environment, honey, honeybee.

Farklı çevre şartlarının bal arısı (*Apis mellifera* L.) bilişsel fonksiyonu ve bal mineral içeriği üzerine etkileri

Özet: Bu çalışmada kolonilerin arıcılık sezonunu geçirdikleri bölgenin veya lokal alanın koloni verimine, işçi arıların tarlacılık davranışlarına, koloni yaşama gücüne ve üretilen balın bazı metal element içeriğine etkisi araştırılmıştır. Aynı genetik kaynaktan gelen arı kolonileri farklı bölgelerde önemli düzeyde (P<0.001) farklı davranış ve performans göstermişlerdir. İşçi arıların kovandan uçuş ve kovana girişleri başlangıçta tüm bölgelerde benzer düzeylerde iken geçen zaman içerisinde bölgeler arasında önemli düzeyde (P<0.001) farklılık oluşmuştur. Farklı bölgesel koşullar kolonilerin bal veriminde ortalama 28.60±3.27 ile 0.571±2.76 kg/koloni gibi önemli (P<0.01) farklılığa neden olmuştur. Bal mumu üretiminde de bölgeler arasında önemli farklılık belirlenmiştir (P<0.01). Kolonilerde bölgeler arası meydana gelen bu olumsuzluk bal örneklerinde belirlenen ağır metal miktarlarına da yansımıştır. Davranış, verim ve balın metal içeriği gibi tüm fenotiplerde belirlenen farklılıkların en önemli kaynağının çevre olduğu tahmin edilmiştir. Sanayi ve aşırı hayvan otlatma bölgelerinde tutulan kolonilerin bir süre sonra söndükleri görülmüştür. Bu nedenle kolonilerin verimli olmaları ancak kendilerine uygun çevre sağlandığında mümkün olmaktadır.

Anahtar sözcükler: Bal, balarısı, çevre, davranış, hayvan sağlığı.

Introduction

The honeybee (*Apis mellifera* L.) is highly adaptive and is found throughout the world with the exception of the polar region. This adaptive strength is the result of genetic diversity. The ability of colony worker bees to monitor the environment, their resistance to pathogens and tolerance of environmental stress varies as a result of genetic diversity, and this increases over time (15). Although bees have the ability to modify their environment according to their needs in order to protect themselves against temperature and humidity changes, bacteria, viruses and fungi, honey yields and colony vigour vary between regions (8). Pollutants such as chemical drugs, heavy metals and radioactivity is limiting productivity and adaptation of honey bees (23). Over the last 100 years, the development of industry, chemical pollutants, excessive pesticide and fertilizer use, excessive soil loss and heavy traffic have all had a negative impact on nature resulting in the significant pollution of, and reductions in, bee food resources. It has been suggested that mass bee deaths are the result of this environmental degradation. These environmental changes are associated with altered gene expression within the brain which may alter phenotypic behaviour (19, 21). The biochemical properties of honeybee products are directly related to environmental quality, since the bees transfer all potential soil, water and air contaminants to their honey and other products (10, 20). Giurfa (12) emphasised that there is a significant relationship between the environment and olfactory, learning, and foraging activities of bees. This heightened sensitivity in bees, indicated by the presence of stress in colonies, gives us important clues regarding changes (mutations) in other biological systems (2, 20, 30). Studies in Ukraine between 1986 and 1989, after the Chernobyl nuclear plant accident, observed the continued presence and influence of radioactive isotope contaminants in the region (6). However, there is insufficient data on the negative effects of residue levels in honey within the polluted region, or on colony efficiency and forager behaviour, compared with unpolluted regions. Similarly, we do not have sufficient information regarding the interaction between bee colonies and dense human settlements.

In this study, we aimed to determine the effects of metal pollutants, excessive use of fertilizers and pesticides, over-grazing, busy traffic routes and the natural environment on the foraging behaviour of worker bees, colony productivity and heavy metal contents in the honey produced.

Materials and Methods

Sampling bee materials and colonies: Queen bees were reared from the same genetic source by larval transfer and colonies were controlled in terms of hive type and construction, number of frames and food stocks. The locations in which each colony spent the beekeeping season were selected from different areas of the Black Sea Region. For the purpose, in addition to areas in proximity to industrial area, heavy traffic, intensive agricultural activity, excessive use of pesticides and chemical fertilizers, and over-grazing, remote sites were also chosen far from human habitation and in unpolluted natural areas. Four areas were identified, which differed significantly from each other. A total of 23 honey bee colonies were used in this study.

The properties of the selected regions were as follows (Figure 1);

R_1: The place is located 400 m to the forest land and 3 km to the highway. There are low-level environmental pollution and medium-level pollen and nectar-producing plants. Five colonies were located in this region.

R_2: The place with an altitude of 50 m is approximately five hundred metres far away from the Black Sea coast road having heavy traffic. This region is close to a copper factory and a thermal power station. In the place there is small-scale cultivation of tobacco, vegetables and fruit. Five colonies were located in this region.

R_3: The place with an altitude of 70 m is three kilometres from the road. There is intensive field agriculture and livestock (sheep and cattle farming). Seven colonies were located in this region.

R_4: The place with an altitude of 2000 m was selected as a control group region. It has low intensity of agricultural activities necessitating low or no use of pesticide and artificial fertilizers. In addition it rich in terms of plant species and subspecies as a source of pollen and nectar (13). Six colonies were located in this region (Figure 1).

Figure 1. Regions where the experimental colonies were placed during the summer.
Şekil 1. Deneme kolonilerinin yaz sezonunu geçirdiği bölgeler.

Behavioral evaluation: The total number of frames covered with adult bees in each colony (frames/colony) was recorded every month between May and October. The number of frames covered with open (egg and larvae) and closed (pupae) brood in each colony (frames/colony) were recorded in May, June, July, August and September. The number of worker bees was determined by counting the number of bees flying to the hive and returning to the hive for 60 seconds every 15 days. During the study period, counts were made six times. The honey from the colonies was harvested in the third week of August. Frames were harvested when two thirds of the frame contained glazed honey. Firstly, the number of frames with honey was determined for each colony. The honey required by the colony was left and the remainder was recorded as honey yield. Frames containing honey from each colony were weighed before and after centrifuging. Thus the amount of honey produced by each colony (kg/colony) was determined. Colonies were examined every 5–6 days in May, June, July and August which is the comb construction period. Standard foundation combs were provided when needed and recorded on the colony chart. The total number of foundation combs (number/colony) for each colony was counted during the honey harvest. The average amount of wax produced per colony (g/colony) was calculated by multiplying the basic honeycomb produced separately in each colony by 79.089 g, which represents the average amount required to produce the main honeycomb according to the Langstroth scale, as determined by Guler (13). The number of colonies lost within one year of the start of the experiment was determined.

Honey samples and biochemical analysis: Random samples from each group were taken from five colonies during centrifugal honey harvesting. Amounts of Fe, Ni, Cu, Zn, Cd and Pb metals in these samples were analysed. From each colony, 300 g harvested honey was sterilized and collected in jars. These were then placed in a water bath at 90ºC for homogenisation, in order to remove any crystals. Following homogenisation, 100g sub-samples were turned to ash by placing them in an autoclave at 450ºC for 24 hours. These ash samples were then dissolved in nitric acid, allowing analysis of heavy metals, such as cadmium, nickel, lead, iron, zinc and copper, by an atomic absorption spectrophotometer (Shimadzu, AA 6701F) (27).

Statistical evaluation: Brood production, yield of honey, wax production, number of forager worker bees entering and leaving the hive and honey samples were analysed using one-way multivariate general linear model (completely randomised) analysis of variance using the SPSS (26) package program. Turkey's multiple comparison tests were employed for comparison of means.

Results

Adult bee populations: There were large differences in the size of adult bee populations between colonies in different regions ($P<0.001$). The largest adult bee population was in the fourth region (R_4), whilst the smallest were in the second and third regions (R_2 and R_3). The adult bee population showed significant differences between colonies ($P<0.001$), with respect to the region and the period (Figure 2).

Brood production: Brood production between colonies from the various regions was significantly ($P<0.01$) different. Whilst the largest brood production was in the fourth region (R_4), the smallest were in the third and second regions (R_3 and R_2). Effectiveness of hatchery production determined the size of the hive at the end of the season. Regional and temporal interactions were significantly different ($P<0.01$) (Figure 3).

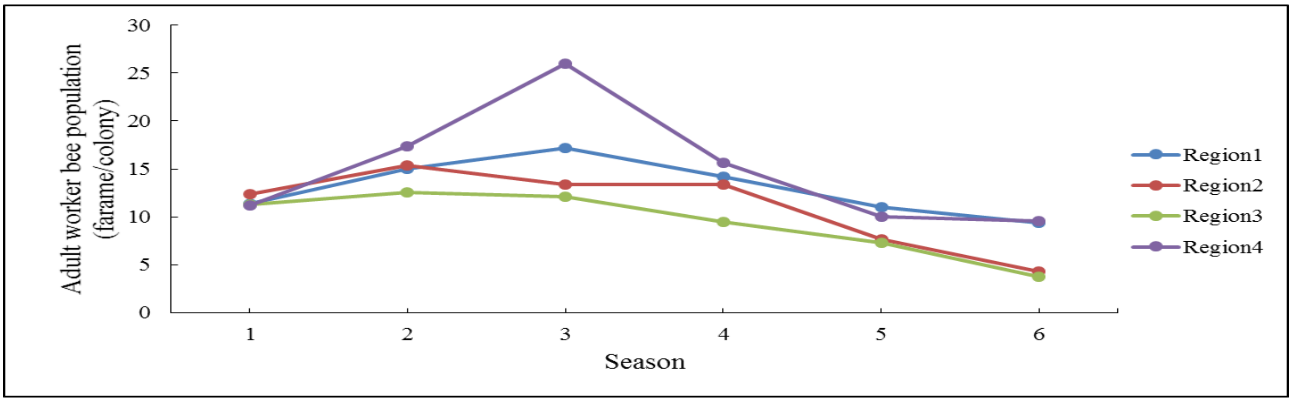

Figure 2. Mean number of frames/colony measured in six periods throughout the season.
Şekil 2. Sezon boyunca altı periyotta ölçülen ortalama adet/koloni sayısı.

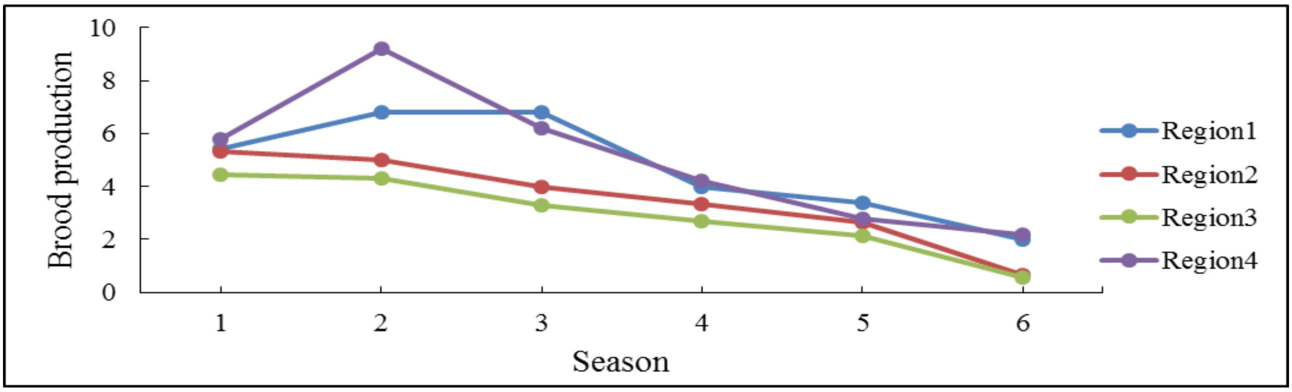

Figure 3. Brood production (frames/colony) measured in six periods throughout the season.
Şekil 3. Sezon boyunca altı periyotta yavrulu çerçeve (adet/koloni) sayılarına ilişkin değişim düzeyi.

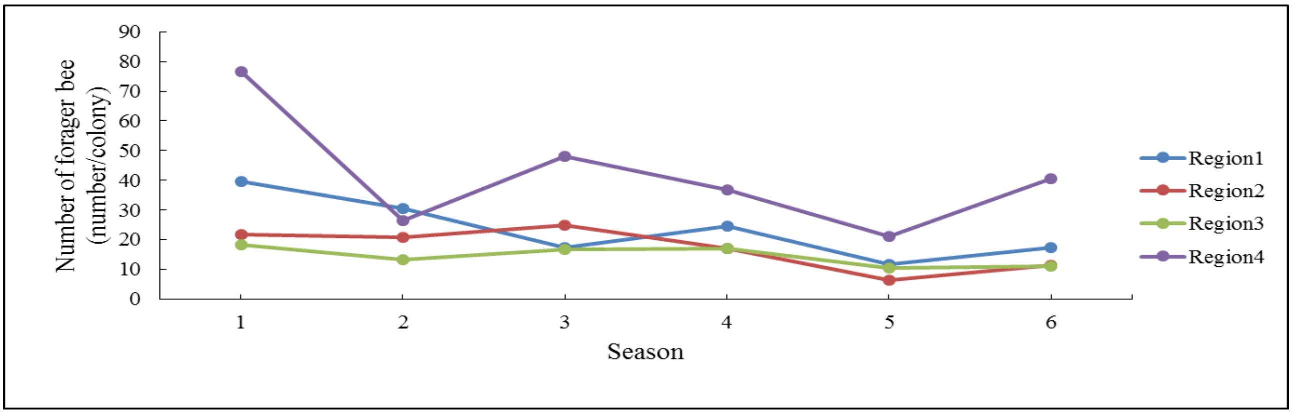

Figure 4. Number of forager worker bees (number/colony) leaving hives, measured for 60 second intervals throughout the season.
Şekil 4. Kolonilerin 60 saniyede kovandan uçuş yapan ortalama işçi arı değişimi.

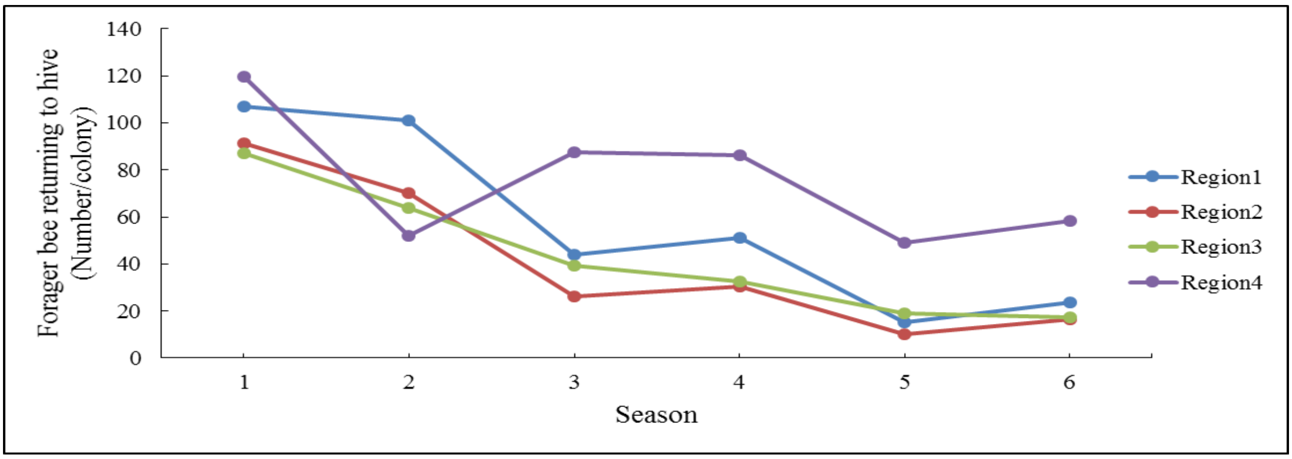

Figure 5. Number of forager worker bees (number/colony) returning to hives, measured for 60 second intervals throughout the season.
Şekil 5. Kolonilerin ortalama 60 saniyede tarladan dönüş yapan işçi arı sayısı ilişkin değişim.

Table 1. Means and standard errors of heavy metals in honey samples produced from different regions (mg/kg) and honey yield (kg/colony) and wax production (g/colony) to experiments colonies in different regions.
Tablo 1. Gruplara ait bal örneklerinde belirlenen ortalama ağır metal düzeylerine ilişkin değerler ve kolonilerin farklı alanlardaki bal verimleri, balmumu üretimleri ile ilişkin ortalama ve standart hata değerleri.

	Region			
	R_1	R_2	R_3	R_4
Honey Yield	9.00±3.27 [b]	2.67±4.22 [c]	0.57±2.76 [c]	28.60±3.27 [a]
Wax production	632.00±125.81 [b**]	579.33±162.42 [b]	417.57±106.33 [b]	1406.20±125.81[a]
Fe	0..49±0..22 [a]	0..92±0..22 [a]	0.60±0.15 [a]	0.30±0.14 [b]
Ni	0..39±0..08 [a]	0.32±0.08 [a]	0.15±0.08 [b]	ND
Cu	0..19±0..04 [a]	0.17±0.05 [a]	0.17±0.02 [a]	0.04±0.01[b]
Zn	0..15± 0..06 [b]	0.32±0.06 [a]	0.31±0.10 [a]	0.20±0.03 [c]
Cd	0..09±0..02 [b]	0.18±0.02 [a]	0.17±0.01 [a]	ND
Pb	0..02±0..01 [b]	0.43±0.12 [a]	0.51±0.03 [a]	ND

Forager worker bees leaving hives: The number of forager worker bees leaving hives varied (P<0.001) among regions. More bees left hives in the fourth region (R_4), while the lowest numbers of foragers were seen in the third and second regions (R_3 and R_2). There were significant differences between number of forager worker bees leaving hives with respect to region and period (Figure 4).

Forager worker bee returning to hives: The average number of forager worker bees returning to hives showed significant variability (P<0.001) between the different regions. The largest numbers of forager bees returning to hives was 75.37 ± 4.13 bees/colony in the fourth region (R_4), the smallest was 40.78 ± 5.33 and 43.12 ± 3.49 bees/colony in the second and third regions (R_3 and R_2), respectively (Figure 5).

Honey yield: Honey yields, wax production and the number of surviving colonies, together with mean and standard error values, are given in Table 1. Honey yields were significantly different between regions (P<0.01). The maximum average yield of 28.60 ± 3.27 kg/colony was observed in the fourth region (R_4). Honey yields lost of colony in R2 and R3 but not in others groups (Table 1).

Wax production: Wax was produced in varying amounts by colonies in the different regions (P<0.01). In the fourth region (R_4), the average amount of wax produced (1406.20±125.81 g/colony) was higher than in the other experimental three groups (Table 1).

Metal contents of honey samples: The Fe, Ni, Cu, Zn, Cd and Pb contents of honey samples from the various regions were significantly (P<0.05) different. Lead levels, an important indicator of environmental pollution, were compared to inter-regional averages. They were determined to be highest in the third region (R_3) and lowest in the fourth region (R_4). The amount of cadmium was particularly high in the second (R_2) and third regions (R_3). The highest concentrations of Fe, Ni, Zn, Cd and Pb were in honey from the second region (R_2), while the lowest concentrations of Fe, Ni, Cd and Pb were in honey from the fourth region (R_4) (Table 1).

Discussion and Conclusion

The region into which a colony settled in the main nectar and pollen flow period, was determined to be important for size of adult bee populations, brood production, honey and wax production and the content of the honey produced. The behaviours of forager bees were also impacted by location of the hive. Previous studies (1, 28) identified significant differences in the performance and behaviour of various honey bee subspecies within the same environment. In this study we demonstrated different levels of performance and foraging behaviour in bee colonies from the same genetic source in different regions. The differences reported in previous studies result from the genetic make-up of the bees, whereas in this study we have seen that these differences are the result of the environment. Kravitz (16), Schulz et al. (25) and Eckholm et al. (8) reported that environmental factors affected the behaviour and productivity of colonies. Furthermore, it is believed that heavy metal pollution in this area may have negatively influenced brood rearing. However, any assumptions or hypothesis concerning the adverse effects of heavy metals on brood rearing activity must be verified by studies in which heavy metals are introduced into brood food or royal jelly. Nonetheless, several studies have reported an association between environmental conditions and behaviour in honeybees and some chemicals are known to reduce these senses (3, 4, 9). Environmental conditions are also known to have significant impacts on metabolic and physiological processes (2, 5, 14). The results of our study support many aspects of these hypotheses. Indeed, the colonies in this study were adversely affected by the copper plants, mobile power plant, traffic routes and excessive livestock grazing areas. Once colonies were settled in the second (R_2; heavy industry) and the third (R_3; excessive livestock grazing) regions, bee brood production and adult growth first increased, then paused, before finally decreasing dramatically. Similarly, while the number of worker bees entering and leaving the hives was initially similar in R1 and R2 region, over the course of time foraging activity of worker bees in the second (R_2) and the third (R_3) regions decreased significantly (Figure 3, Table 3). These results were confirmed by interactions between colony character, behaviour and the periods of the colonies that were subject to this study. In addition, the heavy metal analysis of the honey samples taken from regions was also confirmed (Table 1). In this study, the colonies that were settled in the third region (R3) were most adversely affected. In these colonies, honey production, wax productions were inefficient and adult bee populations were reduce (Table 1). Stress was therefore determined to negatively affect (25) the learning behaviour and resistance of the colonies, ultimately leading to the death of bees (2). In this study, the amount of honey produced by colonies was an important indicator of they were being influenced, by their host region. The average honey yield in the fourth region (R_4) was 28.60 ± 3.27 kg/colony while in the third region (R_3; overgrazing area) it was only 0.571 ± 2.76 kg/colony. This is despite the fact that all the colonies were given the same amount of syrup in spring, were hosted in similar hives, had basic honeycombs produced by the same company, and had queen bees of the same age and from the same genetic source. Similarly, wax production also significantly different among regions. Interestingly, professional beekeepers favour different regions during nectar flow periods and operate a migratory beekeeping system to make use of the greater efficiency of hives in optimal areas. It is clear, therefore, that local floras and environmental pollution must be considered when identifying regions for efficient apiculture (11, 24).

In our study, heavy metal levels in honey samples were found to be a good indicator of environmental conditions. In fact, heavy metal pollution of the honey produced in the second and third regions (R_2 and R_3) was shown to have negative impacts on the performance of the colonies, as well as clearly indicating environmental contamination levels and this supports the findings of previous studies (7, 17, 20, 22, 28).

Consequently our observations, different environmental conditions was determined to be the main cause of all the phenotypic differences such as honey yield, foraging behaviour, colony survivability, products quality and amounts, in colonies produced from the same genetic source. This results indicates that honey bee colonies can only produce high quality products efficiently if they are hosted in the appropriate conditions.

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